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The influence of different solvent ratios on the antimicrobial activity of essential oils against *Streptococcus mutans*

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ABSTRACT

Aims: This study aimed to evaluate and compare the antibacterial activity (AA) of *Origanum dubium* and *Cinnamomum cassia* essential oils dissolved in different ratios of three different solvents against *Streptococcus mutans*.
Methodology and results: The essential oils were obtained from *O. dubium* leaves and *C. cassia* barks using hydro-distillation method and gas chromatography/mass spectrometry (GC/MS) analyses were performed to identify the compositions. The obtained essential oils were diluted in dimethylsulphoxide (DMSO), Tween 20 and ethanol with concentrations of 10, 20 and 60% respectively. Each oil mixture was further diluted with distilled water to provide different ratios. Then, the AA of the dilutions were evaluated. Comparison of AA of essential oils showed that *C. cassia* had higher AA than *O. dubium* for each dilution ratio for Tween and ethanol.
Conclusion, significance and impact of this study: The results obtained in our study lead us to affirm that the AA of both oils are dependent on dilution ratios of the solvents and the AA of *C. cassia* is higher than that of *O. dubium* except for the 1:8 and 1:16 dilution ratios of DMSO. Increasing ratios of solvents used to dilute the *O. dubium* and *C. cassia* essential oils resulted in a decrease in the antimicrobial activity against *S. mutans*.

Keywords: Essential oils, *Streptococcus mutans*, antibacterial, carvacrol, cinnamaldehyde

INTRODUCTION

A great majority of the evidence for epidemiology of dental caries suggests that *Streptococcus mutans* one of the most effective cariogenic bacteria in the initial formation of caries (Van Houte, 1994). The use of antibiotics in traditional treatment modalities are proven methods to prevent dental caries (Chen and Wang, 2010). Extensive use of synthetic chemicals; namely antibiotics and antivirals resulted in the emergence of antibiotic-resistant bacteria. As synthetic chemicals are more toxic than natural chemicals; studies are being carried out on alternative products against them (Prabu *et al.*, 2006). Phytochemicals isolated from plants and used in traditional medicine seems to be a good alternative (Chitme *et al.*, 2004).

Natural products obtained from herbs have been used in traditional medicine since ancient times. In recent years, the pharmaceutical companies have tried to develop new therapeutic agents using plants due to their reliability, high quality and proven effectiveness (Calixto, 2000). In dentistry, herbal products have been used as antibiotic, analgesic, anti-inflammatory, sedative agents and also endodontic irrigants. The essential oils obtained from plants can be used as antibacterial agents in dentistry. Due to their antimicrobial effects, *Origanum dubium* and *Cinnamomum cassia* essential oils obtained from oregano and cinnamon plants may be used in preventing dental caries. The pure form of essential oils have high volatile character and undesirable organoleptic properties that cause several side effects; therefore, their use is limited (Karadağlıoğlu *et al.*, 2019). Some solvents are used to eliminate the harmful effects of pure essential oils. The aim of this study was to evaluate and compare the antibacterial activity of *O. dubium* and *C. cassia* essential oils in different dilutions of dimethylsulphoxide (DMSO), Tween and ethanol solvents against *S. mutans.*

MATERIALS AND METHODS

Extraction of essential oils

Origanum dubium leaves were collected from Yeşilırmak, Turkish Republic of Northern Cyprus in the season and air dried in a cool area. *Cinnamomum cassia* oil was obtained from *C. cassia* barks (Senfoni, Nicosia, TRNC) which were purchased from the local market. *O. dubium* and *C. cassia* oils were obtained by using a Clevenger

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apparatus (Ildam, Ankara, Turkey) for 3 h with water distillation method. Each essential oil in the organic layer oil was dried by anhydrous sodium sulphate and stored in 0.45 µm sterile filtered volatile flasks (Miller-HV, Merck KGaA, Darmstadt, Germany) at 4 °C until used. Pure *O. dubium* and *C. cassia* essential oils were obtained as a result of this process.

Gas chromatography/mass spectrometry (GC/MS) analysis of essential oils

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD (Gas Chromatography Mass Selective Detector). Innowax FSC column with dimensions of 60 mm × 0.25 mm and a 0.25 mm film thickness, and helium (0.8 mL/min) was used as carrier gas during the analysis. The GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. The split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was determined between 35-450 m/z.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. Temperature of the flame ionization detector (FID) was adjusted to 300 °C. Simultaneous auto-injection was applied on a duplicate of the column with the same operational conditions in order to obtain the same elution order with GC/MS. The relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to series of n-alkanes. Computer matching against commercial Wiley GC/MS Library, MassFinder 3 Library (Johnston, 1989; Koenig *et al.*, 2004) and in-house "Başer Library of Essential Oil Constituents" was built up by genuine compounds and components of known oils, as well as MS literature data (Joulain and Koenig, 1998; Boelens, 1999) and used for characterization of the components.

Determination of antibacterial activities of essential oils

Before the determination of the antibacterial activity, pure essential oils were applied to agar plates to ensure that they were not contaminated. For this purpose, the agar diffusion method was used in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendation (EUCAST, 2013). To test the pure oils, the oils were spread to the plates. The plates were inoculated with *Streptococcus mutans* ATCC 35668 (ATCC, Manassas, USA). Bacteria were cultured on Columbia agar with 5% sheep blood (BioMerieux SA, Marcy- l'Etolle, France) and were grown anaerobically at 37 °C for 24 h (EC 160 CO_2 Incubator, Nuve, Ankara, Turkey). Several colonies of cultured bacteria were transferred in inoculum saline (MicroScan Inoculum Saline, Beckman Coulter, Inc., Brea, CA, USA). Inoculum saline was used for adjusting the turbidity of bacterial suspensions in order to help maintaining cell integrity and viability according to Clinical and Laboratory Standards Instute (CLSI, 2015; CLSI, 2018). The density was adjusted to McFarland Standard 0.5 (MicroScan Turbidity Meter, Siemens, Deerfield, IL, USA). A total of 0.1 mL of bacterial suspension in inoculum saline was taken by sterile ecuvion and spread on Mueller-Hinton agar plates (90 mm in diameter) with 5% defibrinated horse blood and 20 mg/L β-NAD (BioMerieux SA, Marcy- l'Etolle, France) to be tested essential oils and they were grown at 37 °C for 24 h (EC 160 CO_2 Incubator, Nuve, Ankara, Turkey).

Preparation of essential oil-solvent mixtures

Each essential oil was diluted with, 10% DMSO (VWR Chemical, Paris, France), 20% Tween (Tween 20, Fischer Scientific, USA) and 60% ethanol (Ethanol absolute for analysis, Emsure, Darmstadt, Germany) for obtaining homogeneous mixtures containing 50% solvent and 50% pure essential oil combination. The ratio of this undiluted mixture represented as 1:1. Subsequently, solvents in the homogenous mixtures were further diluted with distilled water. The percentage of solvent obtained in the mixtures after dilution had been 25%, 12.5%, 6.25% and 3.12% and the solvent: essential oil ratios had been 1:2, 1:4, 1:8, 1:16 respectively. The represented ratios and proportions of essential oils, solvents and distilled water in diluted mixtures were shown in Table 1.

Table 1: The represented ratios and proportions of essential oils, solvents and distilled water in diluted mixtures.

Ratios	Essential oil (%)	Solvents (%)	Distilled water (%)
Pure essential oil	100.00	0.00	0.00
1:1	50.00	50.00	0.00
1:2	50.00	25.00	25.00
1:4	50.00	12.50	37.50
1:8	50.00	6.25	43.75
1:16	50.00	3.12	46.88

Determination of antibacterial effect of essential oils-solvent mixtures

The agar-disc diffusion method was chosen to determine the antibacterial activity of essential oils-solvent mixtures. Sterile filter paper discs, 6 mm in diameter were impregnated with 45 µL of each pure essential oil. After the discs were left to dry for 15 min, they were placed in the plates and the plates were inoculated with *S. mutans* ATCC 35668 (ATCC, Manassas, USA). For essential oil-solvent mixture, sterile paper filter discs were impregnated with 20 µL of each dilution. Then, the paper discs were placed on inoculated agar plates and were

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grown at 37 °C for 24 h (EC 160 CO_2 Incubator, Nuve, Ankara, Turkey). Antibacterial activity of both pure essential oils and essential oils-solvents mixtures were evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. The process was repeated triplicate for each pure essential oil and essential oil-solvent mixtures.

Statistical analysis

Descriptive statistics were performed for each group and the distributions of the essential oils were checked by normality tests (Shapiro-Wilk). Three-way analysis of variance ANOVA was performed to determine the statistical difference between all groups in evaluating the antibacterial activities of essential oils. The Tukey test was applied for pairwise comparison with 95% confidence intervals, where $p < 0.05$ (Bonferroni adjusted alpha= 0.05) was accepted as statistically significant. All analysis were performed the SPSS Version 18 (SPSS Inc., Chicago, IL, USA) software. Tests of between-subject effects were performed to find out which factors and interactions (partial eta squared values of solvent type and essential oil type) were affecting the results and to find out the effect size (R Squared) of the ANOVA tests. p <0.001 indicated that the effect on the result of that parameter was high. R Squared ≥ 80 indicated that the total effect of the parameters included in the measurement was about 80% and the reliability of the statistical analysis and the results were too high.

RESULTS

GC/MS analysis revealed that the major component of the essential oils were carvacrol (88.30%) and cinnamaldehyde (91.79%) for *O. dubium* and *C. cassia* respectively. The results including the components and relative percentage amounts of *O. dubium* oil and *C. cassia* are shown in Table 2 and 3.

Pure forms of the two tested essential oils showed hemolysis zones that were observed in the contamination test performed. This test is done to ensure that the essential oils were not contaminated with another bacterium. The border of the hemolysis zone was not very clear especially for the *O. dubium* oil on the agar plate. Thus, the first clear line was considered to border the hemolysis zone of the pure forms of essential oils (Figures 1 and 2).

The observed changes in inhibition zone diameters according to pure essential oils and different ratios were shown in Table 4. It was observed that the diameter of inhibition zones of both essential oils decreased from pure essential oil to 1:16 dilution ratio. The highest antibacterial activity was observed in pure form of *C. cassia* in agar plates including essential oil-solvent mixtures with ethanol 60%. Overall, *C. cassia* showed higher antibacterial activity than *O. dubium* for both pure forms and for each dilution of essential oil-solvent mixtures of 20% Tween and 60% ethanol. *O. debium* and *C. cassia* showed antibacterial effect only between pure form to 1:2 dilution ratio (Figure 3) and pure form to 1:4 (Figure 4) dilution ratio of 10% DMSO respectively.

Table 2: Essential oil composition and relative percentage amounts (%) of *O. dubium*.

RRI	Compound name	Relative percentage amounts (%)
1020	α-Pinene	0.30
1024	α-Thujene	0.50
1172	Myrcene	0.40
1177	α-Phellandrene	0.20
1192	α-Terpinene	0.90
1211	Limonene	0.10
1223	β-Phellandrene	0.10
1260	γ-Terpinene	2.70
1288	*p*-Cymene	3.80
1299	Terpinolene	0.10
1478	Trans-sabinene hydrate	0.40
1556	Linalool	0.10
1565	cis-sabinene hydrate	0.10
1625	Terpinene-4-ol	0.70
1629	β-Caryophyllene	0.10
1639	trans-dihydrocarvone	Tr
1718	α-Terpineol	0.50
1728	Borneol	0.10
1771	Carvone	Tr
2108	Elemol	0.10
2159	Spathunelol	0.10
2210	Thymol	0.20
2243	Carvacrol	88.30
2273	β-Eudesmol	0.10
	Total	100.00

Relative percentage amount (%) was calculated from FID data. FID: Flame ionization detector; RRI: Relative retention indices calculated against n-alkenes; Tr: Trace (< 0.1).

It was observed that the inhibition zone diameters decreased to one-third of its original size, from 30.67 mm to 9.67 mm and 35.33 mm to 9.33 mm as the concentration of *O. dubium* essential oil showed no antibacterial effect in 1:8 and 1:16 dilutions in Tween and ethanol respectively.

The interaction between the solvents and the solvents and essential oils were determined by the "Test of Between Subjects Effect" test (Table 5). It was observed that the interactions between these compounds had a significant effect on the results of this study (p <0.001).

DISCUSSION

Some plants are being used with varying degrees of success on showing antibacterial activity on *S. mutans* and other microorganisms in dentistry (Van der Weijden *et al.*, 1998). Phytochemical and biological studies on *Origanum* species have proved that these are rich source of compounds with insecticidal, antibacterial, antifungal,

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Table 3: Essential oil composition and relative percentage amounts (%) of *C. cassia*.

RRI	Compound name	Relative percentage amounts (%)
1021	α-pinene	0.61
1073	Camphene	0.67
1119	β-pinene	0.17
1212	Limonene	0.23
1221	1,8-cineole	1.18
1288	*p*-cymene	0.08
1515	α-cubebene	0.07
1556	Benzaldehyde	0.46
1605	Bornyl acetate	0.54
1625	Terpinen-4-ol	0.48
1629	β-caryophyllene	0.05
1718	α-terpineol	0.83
1728	Borneol	0.19
1787	δ-cadinene	0.03
1818	Benzenepropanal (=phenylpropyl aldehyde)	0.34
1940	(*Z*)-cinnamaldehyde	0.06
2091	(*E*)-cinnamaldehyde	91.79
2104	1-epi-cubenol	1.16
2188	Cinnamyl acetate	0.72
2242	Carvacrol	0.01
2514	Coumarin	0.19
	Total	99.84

Relative percentage amount (%) was calculated from FID data. FID: Flame ionization detector; RRI: Relative retention indices calculated against n-alkenes.

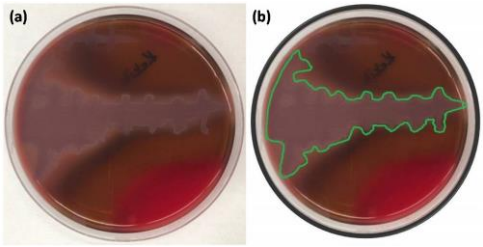

Figure 1: (a) Contamination test for the pure form of *O.dubium* essential oil. (b) The green line shows the first clear line bordering the hemolysis zone.

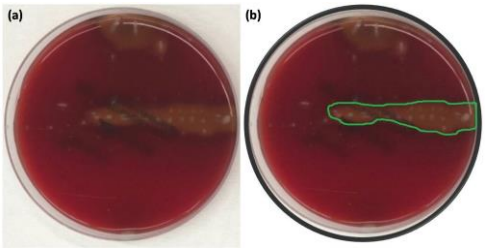

Figure 2: (a) Contamination test for the pure form of *C. cassia* essential oil. (b) Green line shows the first clear line bordering the hemolysis zone.

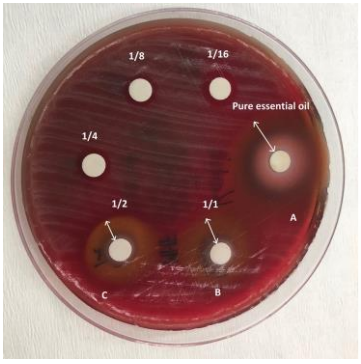

Figure 3: Diameter of inhibition zones (DIZ) for different dilution ratios of *O.dubium* essential oil in DMSO. A: DIZ of pure oil; B: DIZ of 1:1 ratio; C: DIZ of 1:2 ratio. Ratio 1:4, 1:8 and 1:16 did not show any antibacterial effect.

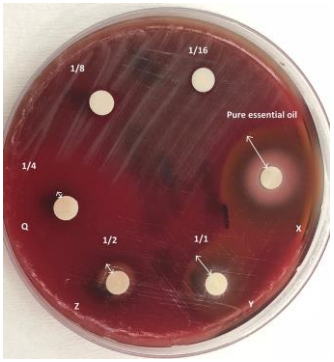

Figure 4: Diameter of inhibition zones (DIZ) for different dilution ratios of *C..cassia* essential oil in DMSO. X: DIZ of pure oil; Y: DIZ of 1:1 ratio; Z: DIZ of 1:2 ratio. Q: DIZ of 1:4., 1:8 and 1:16 did not show any antibacterial effect.

antioxidant and anti-carcinogenic activities (Burt, 2004; Oke and Aslim, 2010). Although there are a number of studies on the antibacterial activity of different oregano species on *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and also *S. mutans* (Oke and Aslim, 2010*;* Özkalp *et al.*, 2010*;* Brierley and Kelber, 2011; Karadağlıoğlu *et al.*, 2019), there is very little information about the antibacterial activity of *O. dubium* essential oil on *S. mutans* (Karadağlıoğlu *et al.*, 2019) in the literature.

According to the GC/MS analysis of the present study, carvacrol was found as the major component (88.3%) of *O. dubium* essential oil suggesting that the antibacterial effect of oregano oil may be related to the carvacrol in its content. This finding is parallel with two previous researches which reported that antimicrobial and antioxidant activity of *O. dubium* is related to its high carvacrol ingredient (Özkalp *et al.*, 2010; Brierley and Kelber, 2011).

Wiwattanarattanabut *et al.* (2017) reported that cinnamon bark had medicinal properties such as

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Table 4: Diameter of inhibition zone (mm) of essential oils diluted in different solvents.

Solvents	Essential oils	Diameter of inhibition zone (mm) (Mean ± SD)					
		Pure essential oil	1:1	1:2	1:4	1:8	1:16
DMSO	*O. dubium*	30.33 ± 0.58^{Aa}	29.33 ± 1.15^{Aab}	20.67 ± 0.58^{Ba}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}
	C. cassia	$39.67 \pm 0.58^{A*}$	$33.33 \pm 0.58^{A*}$	$31.67 \pm 0.58^{A*}$	$19.67 \pm 0.58^{B*}$	$0.00 \pm 0.00^{C*}$	$0.00 \pm 0.00^{C*}$
Tween	*O. dubium*	30.67 ± 0.58^{Aa}	24.00 ± 0.00^{Ba}	11.00 ± 1.00^{Cb}	9.67 ± 0.58^{Cb}	0.00 ± 0.00^{Da}	0.00 ± 0.00^{Da}
	C. cassia	$39.67 \pm 0.58^{A*}$	$35.67 \pm 0.58^{A*}$	$33.00 \pm 1.00^{AB*}$	$27.67 \pm 0.58^{B°}$	$21.00 \pm 1.00^{C°}$	$15.67 \pm 0.58^{C°}$
Ethanol	*O. dubium*	35.33 ± 1.15^{Aa}	32.00 ± 2.00^{ABb}	26.67 ± 4.16^{Ba}	9.33 ± 8.33^{Cb}	0.00 ± 0.00^{Da}	0.00 ± 0.00^{Da}
	C. cassia	$45.33 \pm 1.15^{A*}$	$42.67 \pm 1.15^{A°}$	$41.33 \pm 1.15^{A°}$	$32.67 \pm 1.15^{B°}$	$13.33 \pm 5.77^{C®}$	$8.00 \pm 0.00^{C°}$
Distilled water	-	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}	0.00 ± 0.00^{Ca}

Means sharing same superscript letters and superscript symbols are not significantly different (p >0.05). Uppercase letters compare means in each row. Lowercase letters compare means in each column for *O. dubium*-solvent mixtures. Symbols compare means in each column for *C. cassia*-solvent mixtures.

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Table 5: "Test of Between Subject Effect" showing the interactions between the solvents and the solvents and essential oils combinations (p <0.001).

Group	Type III total square	Sig.
Solvents	83.815*	0.000
Solvents+ essential oils	242.259*	0.000

* R Squared: 0.999

antiplaque, anticarcinogenic and antimicrobial effects. Furthermore, there were also studies related with the high antibacterial properties of cinnamaldehyde (Choi *et al.*, 2016; Ribeiro *et al*., 2018). The amount of cinnamaldehyde for different species of essential oils varied (50-88%) in the literature (Wang *et al.*, 2003; Ooi *et al.*, 2006). Different extraction methods, the use of different solvents and the yield of the plant from which the oil is obtained, may affect the composition of the essential oil and the concentration of the major component (Ooi *et al.*, 2006). The major component of *C. cassia* was found to be cinnamaldehyde with the concentration of 91.79% when hydro distillation method was used for extraction of *C. cassia* essential oil in the present study, in accordance with previous researchers (Ooi *et al.*, 2006; Singh *et al.*, 2007) reporting 85% and 99.4% concentrations.

Disc-agar diffusion method is one of the most commonly used methods for the evaluation of antibacterial activity of plants and plant extracts (EUCAST, 2013) and it was used in this study for the determination of antibacterial activity of the pure essential oils. Since the essential oils are highly viscous and have hydrophobic structure; disc-agar diffusion method was preferred in this study as it eliminates the disadvantages of other methods including homogenous mixing problems or volatilization of essential oils into agar.

During the procedures performed to determine the antibacterial activity of essential oils, it was observed that especially pure *O. dubium* essential oil showed a high degree of hemolysis when added on agar plates (Figure 1). The high hemolysis of oregano oil in the agar suggested that the use of pure oregano oil could produce cytotoxic effects. Previous studies on the antibacterial mechanism of plant essential oils including oregano essential oil, have shown that hydrophobic bioactive compounds may cause cell damage, increase cell membrane permeability, cytoplasmic changes, cellular pH deterioration and affect adenosine triphosphate (ATP) production and protein synthesis (deSouza *et al.*, 2010; Hyldgaard *et al.*, 2012). For this reason, the essential oils were not used in their pure form and were diluted in different solvents in this study. During the addition of solvents to oils, care was taken not to exceed the rates used in the literature.

DMSO was used to prepare the stock solution in a study investigating the sensitivity of essential oil obtained from *Origanum vulgare* plant and the effect of carvacrol and thymol against methicilin-resistant bacteria were analyzed (Nostro *et al.*, 2004). DMSO has an expanded use as a solvent and the effects of it on the microorganisms with which it interacts is great importance in the therapeutic and pharmacological studies. DMSO may interact with thecell membrane of *S. mutans* and alter the pH gradient of the membrane, which explains why they have bactericidal effects (Nascimento *et al.*, 2000). DMSO use was shown to be safe at concentrations not greater than 80%. 10% DMSO was used in the stock solution preparation in a study that investigated the antifungal, herbicidal, phytotoxic and insecticidal properties of *Origanum* species grown in Turkey (Kordali *et al.*, 2008). The same ratio of DMSO solvent was used in our study.

Tween is a non-ionic solvent and it is relatively inactive than the other emulsifiers (Kim *et al.*, 1995; Harkenthal *et al.*, 1999; Delespaul *et al.*, 2000; Cleff *et al.*, 2010). It is assumed that Tween increases the solubility of essential oils and thus reduces the extent of solubility in the bacterial cell membrane (Juven *et al*., 1994). As a consequence, it is frequently applied as an emulsifying agent. In a study evaluating the efficacy of *O. vulgare* on 16 different *Candida* species; Tween was used to dilute the essential oil (Van Haute *et al.*, 2016). A 20% ratio of Tween was used in the present study. Research on essential oils diluted with Tween showed that antibacterial effect of essential oils decreased when the dilution ratio of Tween increased (Juven *et al.*, 1994; Carson and Riley, 1995; Sedlaříková *et al.*, 2017). In accordance with these studies, our study also showed lower inhibiton zones with DMSO, Tween and ethanol solvents when their dilution ratios increased.The results of our study agree with these previous studies for all the three solvents used.

Ethanol altering the pH of the cell membrane of *S. mutans* due to its bactericidal effects. Ethanol extracts from 70% of the plants were found toxic to cell (Nascimento *et al.*, 2000). Ethanol is a solvent with antibacterial activity on its own. Studies have shown that ethanol can cause a change in the antibacterial activity of the tested materials (Chaudhari *et al.*, 2012). Ethanol was used at 60% ratio in this study to stay within 50% to 75% confidence interval (Valgas *et al.*, 2007; Habibi *et al*., 2015).

Except for the dilution ratio of 1:8 and 1:16 of DMSO, comparison of the activities of both pure essential oils and essential oils-solvent mixtures showed that *C. cassia* essential oil had higher antibacterial activity than *O. dubium* essential oil for three types of solvents tested. Different DIZ were obtained in the dilutions of essential oils in the same proportions obtained using different solvents. When the results obtained for *O. dubium* were evaluated, it was observed that DMSO did not show any antibacterial effect when the samples were diluted at 1:4, 1:8 and 1:16 ratios. Similarly, *O. dubium* diluted at 1:8 and 1:16 ratios with Tween and ethanol did not show any antibacterial effect. In case of the results obtained for *C. cassia*, the samples diluted with DMSO in 1:8 and 1:16 ratios showed no antibacterial effect, however the samples diluted with Tween and ethanol had antibacterial activity at each concentration. The highest inhibition zone

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diameter was observed in pure *C. cassia* essential oil for ethanol group. In order to determine the safety of use, the toxicity of each oil mixture diluted with different ratios of the solvents should be tested before initiation of clinical trials.

CONCLUSION

In the light of the results of this study, it can be concluded that *O. dubium* and *C. cassia* essential oils show antibacterial effect on *S. mutans,* the bacteria responsible for caries. The highest antibacterial activity was observed in pure form of *C. cassia* in agar plates including essential oil-solvent mixtures with ethanol 60%. However, pure forms of solvent-free essential oil used in the plaques create a high rate of hemolysis. This suggests that their pure form may be cytotoxic. For this reason, essential oils should not be used in pure form and they should be diluted in several solvents to prevent damage in live tissues. Paralled to this, the antibacterial effect of *O. dubium* and *C. cassia* essential oils decreased as the dilution ratios of DMSO, Tween and ethanol increased. The effectiveness of *C.cassia* essential oil was higher than *O. dubium* essential oil either in pure form or when diluted in three types of solvents except for the 1:8 and 1:16 dilution ratios of DMSO.

This study provided some important insights and shows the antibacterial effects of essential oils against *S. mutans.* The interpretation of the results and conclusions drawn should be based on biocompatibility and safety of use *in vivo* with further studies.

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