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Chemical composition and antibacterial activity of some herbal essential oils against *Streptococcus mutans*

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ABSTRACT

Background and aims: One of the most common chronic diseases in the world is tooth decay. A variety of bacteria are involved in this disorder of which *Streptococcus mutants* is the most common. Essential oils are considered as new natural compounds for use in combating drug-resistant bacteria. This study was aimed to evaluate the antibacterial activity of some essential oils prepared from *Eucalyptus caesia* Benth, *Cuminum cyminum* L. and *Satureja hortensis* L. on S. *mutants*.

Methods: In this study, essential oils were extracted by hydrodistillation method. *E. caesia* Benth, *C. cyminum* L. and *S. hortensis* L. were characterized by using gas chromatography–mass spectrophotometry (GC–MS). Antibacterial activity indices including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and zone of inhibition for the above essential oils against *Streptococcus mutans* were determined using broth macro-dilution and disk diffusion methods. Data analysis was performed using one-way ANOVA and Tukey test.

Results: Results showed that all three extracts had antibacterial activity against *S. mutants*. *S. hortensis* L. essential oil with the lowest MIC and MBC value (13.2 and 18.4 µg/ml, respectively) and the biggest inhibition zone showed the strongest antibacterial effect against S. *mutants* in all exposure times and at all concentrations, compared with two other essential oils. Furthermore, *C. cyminum* L. essential oil had higher anti-bacterial activity against *S. mutant* than *E. caesia* Benth essential oil.

Conclusions: The essential oils used in the present study with different components showed antibacterial activity (especially *S. hortensis* L essential oil), and therefore they can be used as a new antibacterial substance.

Keywords: Dental caries, *Streptococcus mutans*, Essential oils, Antimicrobial.

Original article

INTRODUCTION

One of the most common chronic diseases in the world is dental caries.[1] *Streptococcus mutans* is a bacterial species that plays a major role in dental caries.[2,3] This bacteria is a facultative anaerobic, gram-positive cocci which generally found in the

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human oral cavity.[4] *S. mutans* is regarded as the main cause of tooth decay because of its ability to bind to tooth surfaces and produce lactic acid in the presence of fermentable sugars, which contributes to corrosion of enamel.[2,3,5] Numerous new antimicrobial agents have been produced in the last decade resulting in the evolution of drug resistance by microorganisms.[6,7] The increase of drug resistant pathogens produces challenges to the successful treatment of microbial diseases, including tooth decay. To combat disease caused by antimicrobial resistant microorganisms, there is an urgent need to discover novel products, which have antimicrobial properties. Recently, there has been much interest in novel biological active and anti-microbial active extracts of natural products, particularly those found in medicinal plants.[8-12] Plants may yield antimicrobial therapeutic agents in the form of extracts, latex, alkaloids, phenolics, flavones, tannins, flavonoids, and essential oils, which are the fraction recovered after hydrodistillation of entire tissues or seeds.[13,14] *S. mutans* are multiply resistant to many antibiotics commonly used to treat infections and are thus prime candidates for targeted investigations of natural products which are suspected to have antimicrobial properties. In the present study, the effects of three plant extracts (Eucalyptus, *Cuminum cyminum* L., *Satureja hortensis* L.) were evaluated on *S. mutans*. *Satureja* (savory, saturei) is a genus belonging to the aromatic plants of the Lamiaceae family. These aromatic plants are employed as natural food preservatives and spices, as well as for different medical purposes due to their aromatic and antiseptic properties. Plants of the Satureja genus are widely distributed over regions of southern and south-eastern Europe, Asia Minor, and northern Africa, with the most abundant populations found in the Mediterranean.[15] Savory species produce secondary metabolites, such as carvacrol, during to the normal growth and development in response to pathogens and as a result of stress, and apart from its use in cooking and food preservation, may have antimicrobial effects on many anaerobic microorganisms.[15,16] *C. cyminum* L. is a flowering plant belonging to the Apiaceae family, and is native from the east Mediterranean to India. The aromatic compounds present in the plants have attracted the attention of researchers to test its use as a therapeutic agent.[17] Iranian traditional medicine uses cumin as an agent for the treatment of gastrointestinal, gynecological, respiratory disorders as well as diarrhea, epilepsy, and toothache.[18] Studies have shown that extracts of the cumin seed may prevent biofilm formation in *S. mutans* and *S. pyogenes*.[19] *E. caesia* is traditionally used in the treatment of diabetes.[20] Leaf extracts and essential oils of *E. caesia* Benth have been found to have antibacterial, antifungal, antioxidant properties.[21] Research suggests the fruit of the plant contains the majority of the compounds, which convey antimicrobial activity.[22]

METHODS

Plant materials and isolation of essential oils: Fresh aerial parts of *E. caesia* Benth, *C. cyminum* L. and *S. hortensis* L were collected from the Lorestan and Chaharmahal and Bakhtiari provinces, Iran in 2012. The herbs were dried at room temperature for 3 days. The dried herb samples were grounded and 500 g were subjected to hydro distillation using a Clevenger-type apparatus. The oils were dried over anhydrous Na_2SO_4 and stored at 4 °C in a sealed amber vials.

In order to analyze the volatile compounds in the essential oils, gas chromatography/mass spectrometry (GC/MS) was used. Analytical GC was performed with an Agilent 7890 A (Made in USA) with 5% HP-5 MS column (column length: 30 m, internal diameter column: 0.25 μm, outer diameter column: 0.25 mm). Helium gas

(99.999% purity) was used as a carrier at 0.8 ml/min. The initial temperature of the column was 280 °C and final temperature of column was 60 °C. The temperature was programmed to increase by 4 °C/min. The separation ratio was set on 40:1, and the injector temperature was set on 300 °C. A Hamilton syringe was used to inject 0.1 ml of essential oil. Agilent 5975 C was used for GC/MS analysis. Ionization energy in the mass spectrometer was 70 eV and mass spectrum was from 50 to 550 m/z. Inhibition index (IR) for all components was calculated using a homologous series of n-alkanes (C5–C25), which were injected using the same procedure as the samples. Identification of essential oil compounds was accomplished by comparing their retention times with the retention times of authentic standards and with mass spectral analysis patterns, as described by Adams.[23]

Preparing bacterial strain: The bacterial strain S. *mutans* PTCC 1683 was acquired from the Iranian Research Organization for Science and Technology (IROST). In this experiment tripticase soy broth (TSB) and blood agar (BA) were acquired from Merck Company. In order to determine the antimicrobial effect of the plant extracts, the disk diffusion method was used. After 18 h of incubation, 500 µl of bacterial broth of standard density (1×10^8 CFU ml^{-1}) of 0.5 McFarland in TSB, was transferred to BA. The liquid was gently distributed on the surface of BA using a sterile loop. Essential oils (30 µl) of concentration 0.625, 1.25, 2.5, 5, 10, 20, 40, 60, and 80 µg/µl were transferred to disks of 6 mm in diameter, and these disks were placed on the inoculated BA. A disk containing 30 µl of water was used as negative control. The diameter of the IZ (Inhibition Zone) was measured after 24 h of incubation at 37 °C at 24, 48, and 72 h in triplicate.[8]

Macrodilution method was used to determine the MIC value (the lowest concentration of an antibacterial agent that prevents visible bacterial growth after 24 hours of incubation at 37◦C) and the MBC value (the lowest concentration required to kill certain bacteria). For determination of MIC, dilution of wells was used. A bacterial suspension of S. *mutans* was prepared from liquid culture with a standard darkness of 0.5 McFarland. The essential oil was diluted to 10% with water from an initial concentration of 500 µg/µl, and six different dilutions were added to the pipes containing 10 ml of liquid culture medium. MIC of essential oils against each strain was determined using the Microwell method. In this method, 95 µl of TSB and 5 µl of microbial suspension were added to a 96-well plate. 100 µl of the essential oil with concentration of 500 µg/µl was added to the first well then 100 µl was taken from the first well and transferred to the next well. This process was continued until the 6th well. The last well was contained 195 µl of TSB culture medium and 5 µl of microbial suspension without any essential oil. This well was considered a negative control for this text. Each well was mixed using a Rotary Shaker for 20 min. Then the plate was put in an incubator for 24 h at 37 °. The microbial growth was quantified by measuring the optical density at 600nm. The effect of each essential oil was determined on S. *mutans* separately with 3 replicates.[8,24] Data were analyzed using SPSS software (version 20) via one way ANOVA method and mean comparison was done through Tukey method.

RESULTS

Data were analyzed using SPSS software (version 20) via one way ANOVA method and mean comparison was done through Tukey method. The effects of different concentrations of *E. caesi*, *C. cyminum* L. and *S. hortensis* L. on *S. mutans* extracts were determined using disk diffusion method after 24, 48, and 72 h of

incubation. An essential oil concentration of 1200 (µg/µl) caused the largest zone of inhibition compared to the lower concentrations (P<0.001).

Anti-bacterial activity of *Saturej hortensis* L. against *S. mutants* was higher at all incubation times than two other essential oils (Fig 1). The inhibition zone was bigger at higher concentration.

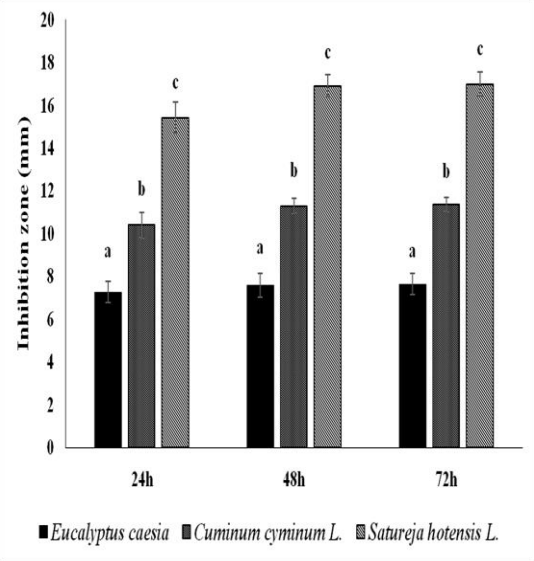

Fig 1: Comparison of the inhibition zone from the most effective concentration (80 µg/ml) of the three essential oils on *S. mutants* after 24, 48, and 72 h incubation.

Antibacterial activity of three essential oils against *S. mutants* was demonstrated by MIC and MBC evaluation. The *E. caesia* Benth essential oil had MIC and MBC values of 34.8 µg/ml and 47 µg/ml, respectively. The *C. cyminum* L. essential oil had MIC value of 19.5 µg/ml and MBC value of 25.3 µg/ml. The *S. hortensis* L. essential oil had MIC and MBC values 13.2 µg/ml and 18.4 µg/ml, respectively. These results indicated that the essential oil of *S. hortensis*, with lower MIC and MBC values than two other essential oils, had higher antimicrobial activity against *S. mutants*. Furthermore, the MIC and MBC values of the *C. cyminum* L. essential oil were lower than MIC and MBC of *E. caesia* Benth (Table 1).

Table 1: In vitro anti-microbial activity (MIC and MBC) of three essential oils against *S. mutants*

NO	Essential oils	MIC (µg/ml) Mean ± SD	MBC (µg/ml) Mean ± SD
1	*E. globulus*	34.8±1.15	47.0±1.53
2	*C. cyminum*	19.5±0.41	25.3±0.56
3	*S. hortensis*	13.2±0.32	18.4±0.47

Present compounds in the essential oils were identified using GC/MS analysis. The analysis of *S. hortensis* L essential oil resulted in identification of 20 distinct compounds. Carvacrol was the highest in concentration.

The analysis of *E. caesia* essential oil with GC/MS resulted in the identification of 27 distinct compounds, with 1, 8-cineol having the highest proportion (40.18%). The analysis of *C. cyminum* L. essential oil by GC/MS revealed the presence of 24 different compounds, and the highest components were Cuminic alcohol and γ-Terpinene (30.32% and 25.32%, respectively).

DISCUSSION

Despite successful treatment and control of tooth decay with fluoride, the disease remains the most widespread oral health problem in many countries.[24] Essential oils with antimicrobial properties have been described for many years,[25] and the antimicrobial effect of essential oils on *S. mutans* was previously discovered. The mechanism of action appears to be mainly on the cell membrane, disrupting its structure and causing cytoplasmic leakage

leading to cell death. Other mechanisms of action may exist, such as disrupting membrane synthesis, and inhibiting cellular respiration.[26] Essential oils impair biofilm formation and may delay the progression of *S. mutans* infections, thus they may potentially be useful for the treatment and prevention of tooth decay and periodontal disease.[25,27] It is suggested that brushing with toothpastes containing essential oils twice a day may have clinical benefit.[28] Essential oil-based mouth washes may also be effective against oral microorganisms, and may be considered for daily use in oral health. Eucalyptus is a potentially beneficial herb used for food preservation and as a pharmaceutical, due to its anti-microbial properties.[29] The antibacterial activity of Eucalyptus essential oil was evaluated by Khan,[30] who showed that eucalyptus extracts had antibacterial activity against gram positive tested bacteria such as of *S. mutans*. Fani and Kohanteb[31] demonstrated that eucalyptus essential oils administered *in vitro* had inhibitory activity on all tested microorganisms, including *S. mutans*. In another study, eucalyptus extract did not result in antibacterial activity against *S. mutans* and *Lactobacillus acidophilus* even at high concentrations. In the present study, eucalyptus essential oil produced antimicrobial activity against *S. mutans* (MIC value: 34.8 µg/ml, MBC value: 47 µg/ml). Cumin extract is known as a traditional medicinal plant with pharmaceutical properties.[32] Antibacterial activity of its essential oil, against a wide range of gram-positive and gram-negative bacteria, was evaluated in previous work.[33] Shayegh and colleagues reported the antimicrobial activity of cumin essential oil on *S. mutans* and some other bacteria included the prevention of biofilm formation. Our results demonstrate the anti-microbial properties of cumin essential oil against *S. mutans* (MIC value: 19.5 µg/ml, MBC value: 25.3 µg/ml). *S. hortensis* L. has been used to treat many diseases in some traditional medicinal practices. Özkalp and Özcan showed that it has anti-microbial activity against *S. mutans* and a few other bacteria. Out results also demonstrated that the *S. hortensis* L. essential oil had anti-microbial properties.[34] In fact; it had the lowest MIC and MBC values, 13.2 µg/ml and 18.4 µg/ml, respectively among the three tested oils. The results of the disk diffusion method, consistent with the results of MIC test, also demonstrate the anti-microbial properties of the three oils tested. The *S. hortensis* L. essential oil had the strongest anti-microbial properties against *S. mutants* compared two other essential oils. Disk diffusion and MIC results showed that the *C. cyminum* L. essential oil had the second strongest anti-microbial effects against *S. mutants*. In this study, the essential oils were analyzed by GC/MS and several compounds were identified in each essential oil. In the *S. hortensis* L. essential oil, carvacrol had the highest percentage compared to other compounds (32.38%). Carvacrol is a phenolic compound and has known anti-microbial effects. It has been shown to act on the cell membrane, altering its function. In some cases, the cell membrane swells causing the loss of the pH gradient and proton motive force, leading to decreased ATP levels and finally cell death.[35] GC results from *E. caesia* Benth essential oil indicated that 27 components were present, comprising 91.6% of the essence. The major component of this oil is 1, 8-cineol, at 40.18%. Cineole is an important substance in *Eucalyptus* plant.[36] The amount of Cineole essential oil extracted from leaves of *Eucalyptus* species varies by countries.[37,38] The overall quality and quantity of the essential oil vary according to season and geographical location of the plants. Climate and soil conditions can also affect the composition of the oil.

CONCLUSIONS

The results of this study demonstrated that all three essential oils (*Eucalyptus*, *C. cyminum L.*, *S. hortensis* L.) have anti-microbial activity against *S. mutans*. *S. hortensis* L. essential oil had strong antimicrobial effect against *S. mutans* even in comparison with the other two essential oils, was more effective. Finally, according to acceptability of these essential oils, it can be used these essential oils for the treatment of *S. mutans* infections, but after toxicological tests and clinical trials of the oil.

CONFLICT OF INTEREST

There is no conflict of interest associated with this study.

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