doi: 10.17221/180/2016-VETMED

# Antibacterial properties of *Carum copticum* essential oil against *Streptococcus mutans* and *Streptococcus sobrinus* isolated from canine dental plaque

S.N. Gharajalar*, M. Hassanzade

Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

*Corresponding author: saharnouri@hahoo.com

**ABSTRACT**: Dental caries is amongst the most prevalent oral diseases in both humans and dogs. *Streptococcus mutans* and *Streptococcus sobrinus* (mutans streptococci) are the major cariogenic bacteria isolated from dental caries. Since these bacteria generally show resistance to common antibiotics, natural products such as plant essential oils could be a good substitute. For this study, we aimed to evaluate the antibacterial activity of *Carum copticum* essential oil against *Streptococcus mutans* and *Streptococcus sobrinus*. Twenty canine dental plaque samples were collected and the presence of *S. mutans* and *S. sobrinus* in the samples was confirmed using biochemical, culture and polymerase chain reaction (PCR) assays. The resistance patterns of isolates were determined using a disc diffusion method according to the Clinical Laboratory Standards Institute protocol against the following antimicrobials: chloramphenicol, tetracycline, penicillin, erythromycin, ceftriaxone, cefotaxime, vancomycin and azithromycin. The antibacterial activities of *Carum copticum* essential oil were based on the disc diffusion method as well on a determination of the minimum inhibitory ($MIC_{50}$) and minimum bactericidal concentration values. *S. mutans* and *S. sobrinus* were isolated in 8 (40%) and 2 (10%), respectively, of plaque samples. Most of these isolates were determined to display multidrug resistance patterns to the eight antibiotics evaluated. Screening of the antibacterial activity of the essential oil indicated that $MIC_{50}$ and minimum bactericidal concentration values were 20 µg/ml and 80 µg/ml, respectively, and that the zone of inhibition in the disc diffusion method ranged from 2 to 5 mm for serial concentrations of the essential oil. Based on our results, we suggest that *Carum copticum* essential oil exerts antibacterial effects against *Streptococcus mutans* and *Streptococcus sobrinus* and may be a useful treatment for carious lesions with bacterial aetiologies.

**Keywords**: dog; dental caries; biofilm; cariogenic bacteria; PCR; *Trachyspermum ammi*

Tooth decay caused by dental plaque is a common dental disease in both humans and dogs (Galvao et al. 2012). Dental plaques are adherent bacterial communities that form on tooth surfaces, particularly in places where mechanical salivary removal is reduced (Zambori et al. 2012). Many investigations have revealed the significant role of human dental plaque in dental caries (e.g., Zomorodian et al. 2015). The prevalence of dental caries among adults in industrialised countries reaches almost 100% of the population. This problem is seen with lower frequency (60–80%) in children (Maripandi et al. 2011). Similar studies in animals are very few in number, although similar infections do occur in animals (Percival et al. 2011). Amongst the bacterial pathogens associated with the formation of plaques that can lead to dental caries, *Streptococcus mutans* and *Lactobacillus* species are the main culprits (Fayaz et al. 2014). Mutans streptococci (MS; *Streptococcus mutans* and *Streptococcus sobrinus*), the most common bacteria isolated from dental plaques, are the main aetiological agents in the pathogenesis of dental caries (Al-Mudallal et al. 2008).

Mutans streptococci are facultative anaerobic, Gram-positive cocci, and are a part of the normal bacterial microflora in the mouths of dogs and cats

doi: 10.17221/180/2016-VETMED

(Golestannejad et al. 2015). Since these species are highly acidogenic and aciduric and can break down several salivary glycoproteins, they are generally responsible for biofilm formation and dental caries (Galvao et al. 2012). *Streptococcus* species are often identified on the basis of their colony morphology, Gram staining, catalase and biochemical tests such as the hydrolysis of adonitol, starch, arabinose, cellobiose, dextrin, dextrose, dulcitol, galactose, glucose, inulin, lactose, maltose, mannose, mannitol, melibiose and arginine (Amoroso et al. 2003). Methods commonly used for the detection of pathogenic bacteria in the oral cavity, such as culture methods or biochemical tests, are less sensitive and less specific than molecular tests. Therefore, molecular methods such as PCR are now used for bacterial identification (Rawashdeh et al. 2008).

Antibiotics are very useful in treating infectious diseases caused by bacteria. However, constant use of antibiotics over a long period of time leads to the selection and dissemination of antibiotic-resistant pathogens (Rasheed et al. 2014), which represents a major challenge for the successful treatment of bacterial diseases such as dental caries (Golestannejad et al. 2015). As a result, there is a current need to identify new antimicrobial products such as plant extracts or their essential oils (Golestannejad et al. 2015). *Trachyspermum ammi* (*Carum copticum*) is a plant that grows in Iran, India, Pakistan and Egypt. The essential oil of this plant contains monoterepenes such as thymol, p-cymene and γ-terpinene. Since this essential oil has antibacterial properties, it has been used by some researchers against foodborne pathogens (Rabiey et al. 2014). We hypothesised that *Carum copticum* essential oil may exert antimicrobial activity against cariogenic bacteria. Thus, the goal of this work was to study the antibacterial effect of *Carum copticum* essential oil against MS isolated from canine dental plaque.

## MATERIAL AND METHODS

**Sample collection**. Twenty dental plaque samples were collected from dogs referred to Dr. Onsori's pet clinic in Urmia, Iran. Collected plaque samples were then placed in tubes containing sterile phosphate-buffered saline (pH = 7). Following a homogenisation process that consisted of 30 s of vortexing at the maximal setting, 0.1 ml of homogenates were spread on mitis salivarius agar (MS agar; Oxoid, UK) with 0.2 units of bacitracin per ml and 1.5% (W/V) sucrose (MSB agar). Plates were incubated in a 5% $CO_2$-enriched atmosphere at 37 °C in anaerobic jars (Beighton et al. 1981).

**Phenotypic identification of *S. mutans* and *S. sobrinus***. Three colonies from each plate were used for Gram staining to reveal bacterial morphology. Colonies with characteristic morphologies for *S. mutans* and *S. sobrinus* were then subjected to biochemical identification using the catalase test and the utilisation of different carbohydrate sources. Brain heart infusion broth (Sigma Aldrich, USA) supplemented with phenol red (0.02%) and 10% of each carbohydrate (manitol, inulin, salicin and raffinose) was inoculated with the bacterial isolates. Cultures were incubated anaerobically for 72 h at 37 °C. A change in the colour of the media to yellow is indicative of carbohydrate utilisation.

**Genotypic detection of *S. mutans* and *S. sobrinus***. Chromosomal DNA from the isolated bacteria was extracted using a DNA extraction kit (Cinna gene, Iran).

A 517-bp DNA fragment of the *gtfB* gene from *S. mutans* and a 712-bp DNA fragment of the *gtfI* gene from *S. sobrinus* were isolated using PCR. The PCR reaction was performed in a volume of 25 µl under the following conditions: initial denaturation at 95 °C for 4 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 5 min. PCR-amplified products were then analysed by horizontal electrophoresis using a 1.5% agarose gel (Franco e Franco et al. 2007). The primer sequences used in this study are provided in Table 1.

**Antibacterial susceptibility testing**. Antibacterial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. A volume of 100 µl of an overnight culture of each of the *Streptococcus* isolates in nutrient broth with 0.5 McFarland standard turbidity was spread on Mueller-Hinton agar plates (Sigma Aldrich, USA) (Momtaz et al. 2012). The antimicrobial agents were tested at the following concentrations: chloramphenicol (30 µg), tetracycline

Table 1. Primer sequences used for amplification of *gtfB* and *gtfI* genes

| | |
|---|---|
| *GtfB F* | 5'-ACTACACTTTCGGGTGGCTTGG-3' |
| *GtfB R* | 5'-CAGTATAAGCGCCAGTTTCATC-3' |
| *GtfI F* | 5'-GATAACTACCTGACAGCTGACT-3' |
| *GtfI R* | 5'-AAGCTGCCTTAAGGTAATCACT-3' |

doi: 10.17221/180/2016-VETMED

(30 µg), penicillin (10 units), erythromycin (15 µg), ceftriaxone (30 µg), cefotaxime (30 µg), vancomycin (30 µg) and azithromycin (15 µg; Padtan Teb, Iran).

After incubating the inoculated plates anaerobically at 37 °C for 18–24 h, we determined the susceptibility of *Streptococcus* isolates to each antimicrobial agent.

***Carum copticum* collection**. Seeds of *Carum copticum* were collected from Tabriz, in the East Azerbaijan province of Iran. Plants were identified by the herbarium maintained by the Faculty of Pharmacy at the University of Tabriz.

**Essential oil extraction**. Dried seeds of the plant were powdered and subjected to hydrodistillation for 3 h using a Clevenger-type system. *Carum copticum* essential oil was dried over anhydrous sodium sulphate and then stored at 4 °C in sealed glass vials (Mahmoudi et al. 2013a).

**GC/MS analyses**. Components of the essential oil were analysed using volatile gas chromatography/mass spectrometry (GC/MS). The system consisted of an Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass-selective detector (Agilent, USA). The GC was equipped with a HP-5MS capillary column (30 × 0.2 mm ID × 0.2 µm film thickness, Hewlett Packard, USA). The initial temperature of the column was 50 °C and the final temperature of the column was 300 °C. The temperature was programed to increase by 15 °C/min. The injector temperature was set to 290 °C. Helium was used as a carrier at 0.8 ml/min (Mahmoudi et al. 2013a). Table 2 provides the determined composition of the *Carum copticum* essential oil.

**Antibacterial activity of the essential oil**. Bacterial sensitivity to the essential oil was studied in a broth microdilution assay that employed 96-well plates (Sigma Aldrich, USA). An overnight culture of each bacterium in nutrient broth media was adjusted to 0.5 McFarland standard turbidity. The essential oil was dissolved in 10% dimethyl sulfoxide (DMSO) (Sigma Aldrich, USA). Then, serial dilutions were made over a concentration range of 0.31–80 µg/ml (Goudarzi et al. 2011). A 95-µl sample of sterile nutrient broth (Sigma Aldrich, USA) medium and 5 µl of bacterial suspension were added to each well of the plates. Then, 100 µl of essential oil containing the prepared dilutions were added to individual wells. Plates were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 37 °C for 24 h. As a negative control, the last well of each plate contained 195 µl of nutrient broth medium and 5 µl of bacterial suspension but no essential oil (Mahmoudi et al. 2013b).

Following the incubation period, bacterial growth was determined by measuring absorbance at 600 nm using the EL 800 Universal Microplate Reader (Biotech Instruments, Highland Park, Vermont, USA). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the essential oil that inhibited bacterial growth. To determine the minimal bactericidal concentration (MBC), 10 µl from each clear well were spread on nutrient agar medium. Plates were then incubated for 24 h at 37 °C. The MBC was determined as the lowest concentration of essential oil that had bactericidal effects on the tested bacteria.

**Disc diffusion method**. The agar disc diffusion method was used to screen the antibacterial activities of the essential oil. Ten µl of each dilution of essential oil were coated on separate sterile filter paper discs of 6 mm in diameter (Padtan Teb, Iran). Bacterial inoculum, adjusted to 0.5 McFarland standard turbidity, was spread onto Mueller-Hinton agar plates (Sigma Aldrich, USA) using a sterile cotton swab. Coated discs were then placed on the inoculated agar plates (Upadhyat et al. 2010). The experiment was performed for all of the tested bacteria and the mean values of the diameters of the inhibition zones ± SD were calculated.

Table 2. Antimicrobial susceptibility patterns of mutans streptococci isolated from dog dental plaque

| Antimicrobials | Bacterial species | Isolates | Strains |
|---|---|---|---|
| Chloramphenicol (30 µg) | *Streptococcus mutans* | 8 | 87.5 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Ceftriaxone (30 µg) | *Streptococcus mutans* | 8 | 75 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Cefotaxime (30 µg) | *Streptococcus mutans* | 8 | 87.5 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Tetracycline (30 µg) | *Streptococcus mutans* | 8 | 50 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Erythromycin (15 µg) | *Streptococcus mutans* | 8 | 87.5 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Penicillin (10 U) | *Streptococcus mutans* | 8 | 100 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Vancomycin (30 µg) | *Streptococcus mutans* | 8 | 87.5 |
| | *Streptococcus sobrinus* | 2 | 100 |
| Azithromycin (15 µg) | *Streptococcus mutans* | 8 | 87.5 |
| | *Streptococcus sobrinus* | 2 | 50 |

Isolates = total number of isolates tested, strains = percentage of resistant strains

doi: 10.17221/180/2016-VETMED

## RESULTS

Nineteen out of the 20 canine dental plaque samples were grown on MSB agar plates. The results of Gram staining, the catalase test, and carbohydrate fermentations indicated that eight (40%) of the isolates exhibited *S. mutans* characteristics, i.e., Gram-positive cocci with a negative catalase test and the ability to utilise mannitol, raffinose, inulin and salicin in growth media containing each of these carbohydrates. Also, two (10%) of the isolates had *S. sobrinus* properties, i.e., Gram-positive cocci, a negative catalase test, the ability to utilise mannitol, inability to ferment salicin, and variable reactions for raffinose and inulin. Following the phenotypic fermentation of isolates, the presence of *S. mutans* and *S. sobrinus* was also confirmed using the PCR method. According to the analysis of PCR results on a 1.5% agarose gel, all of the bacteria identified as *S. mutans* had a 517-bp DNA fragment of the *gtfB* gene and all of the microorganisms identified as *S. sobrinus* harboured a 712-bp DNA fragment of the *gtfI* gene (Figure 1).

### Antibiotic susceptibility results

The results of the susceptibility tests to the eight antibiotics used in this study are provided in Table 2. Amongst the antibiotics tested, the highest resistance was towards penicillin (100% of tested strains), followed by cefotaxime (90%), erythromycin (90%) and vancomycin (90%). The highest susceptibility patterns were toward azithromycin (70% of tested strains) and chloramphenicol (60%).

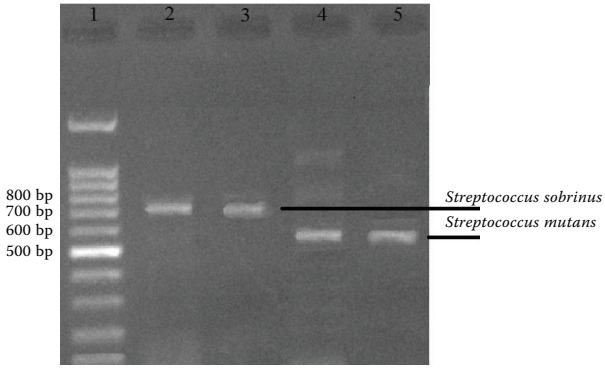


Figure 1. Gel electrophoresis of the PCR products of *gtfB* and *gtfI* determinants

Column 1 = 100 bp ladder marker; columns 2 and 3 = 712 bp DNA fragment of the *gtfI* gene from *S. sobrinus*; columns 4 and 5 = 517 bp DNA fragment of the *gtfB* gene from *S. mutans*

### Essential oil and fraction yields

The components of the essential oil were identified using GC/MS analyses. The results are provided in Table 3. The analysis of *Carum copticum* essential oil resulted in the identification of eight different compounds. Phenol was the predominant component (42.26%), followed by benzene methyl (23.11%) and γ-terpinene (19.69%).

### Antimicrobial activity

The antibacterial activity of the essential oil against mutans streptococci was determined on the basis of MIC and MBC evaluation (Table 4). As shown in Table 4, the $MIC_{50}$ value was 20 µg/ml, and the MBC value was 80 µg/ml.

### Inhibition zone diameter

Serial concentrations of essential oil (10–80 µg/ml) were used in a disc diffusion assay. The results are provided in Table 5 and show that inhibitory zones significantly increased in a dose-dependent manner.

## DISCUSSION

Problems related to oral cavities such as periodontal disease, teeth loss, attrition and abrasion, caries and tumours are significant diseases in dogs. These disorders are usually related to quality of

Table 3. Phytochemical composition of *Carum copticum* essential oil

| No. | Phytochemicals | Formula | RT (min) | PROB (%) | COMP (%) |
|---|---|---|---|---|---|
| 1 | 2-β-pinene | $C_{10}H_{16}$ | 5.68 | 97 | 3.88 |
| 2 | 2-β-pinene | $C_{10}H_{16}$ | 5.79 | 96 | 2.53 |
| 3 | decane | $C_{10}H_{22}$ | 5.99 | 95 | 1.34 |
| 4 | benzene methyl | $C_6H_5$-$CH_3$ | 6.72 | 97 | 23.11 |
| 5 | γ-terpinene | $C_{10}H_{16}$ | 7.31 | 97 | 19.69 |
| 6 | ethanone | $C_2H_4O$ | 10.26 | 46 | 1.67 |
| 7 | phenol | $C_6H_6O$ | 12.14 | 97 | 37.28 |
| 8 | phenol | $C_6H_6O$ | 12.39 | 97 | 4.98 |

COMP = percentage of compound, PROB = probability, RT = retention time (the time taken for a solute to pass through a chromatography column)

doi: 10.17221/180/2016-VETMED

Table 4. Antimicrobial activity ($MIC_{50}$ and MBC) of *Carum copticum* essential oil against mutans streptococci

| Isolate | MIC (µg/ml) | MBC (µg/ml) |
|---|---|---|
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus sobrinus* | 20 | 80 |
| *Streptococcus sobrinus* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |
| *Streptococcus mutans* | 20 | 80 |

Table 5. The quantification of the antimicrobial activity of *Carum copticum* essential oil was measured using the agar disc diffusion assay. The effectiveness of the essential oil is demonstrated by the size of the bacterial growth inhibition zone around the paper disc, which is typically expressed as the diameter of the zone in mm. A mixture of strains is used for the test

| Essential oil concentrations (µg/ml) | Mean ± SD of zone inhibition (mm) |
|---|---|
| 80 | 5.31 |
| 40 | 3.90 |
| 20 | 3.20 |
| 10 | 2.10 |

diet, inappropriate oral hygiene and interaction of bacteria. A diet based on soft foods and also a lack of oral hygiene, leads to dental plaque accumulation. Among dogs, large and medium breeds are most commonly affected by dental caries with the last premolar and first molar teeth at the highest risk (Allmuca et al. 2016). Dental plaques (biofilm) are also very important in oral disease processes such as periodontal disease, dental caries and systemic illnesses in both humans and animals (Zambori et al. 2012).

Recent studies indicate a higher occurrence of oral disorders in dogs compared to older studies. These findings may be a result of an increasing prevalence of these disorders or an increase in owner requests for dental treatment in dogs (Kyllar and Witter 2005). A diet based on soft homemade foods, is often followed by the accumulation of plaques and dental calculus. Mutans streptococci are the main pathogens implicated in the aetiology of dental caries (Amoroso et al. 2003). Zambori et al. (2012) studied the role of biofilm in oral disease of dogs and cats and indicated that bacteria that form biofilm (dental plaque) release acids that promote dental surface demineralisation and dental caries. Amongst these bacteria, Gram-positive, facultative anaerobic streptococci have a particularly important role in plaque formation. In this study, we were able to determine the presence of mutans streptococci in 10 (50%) of 20 dental plaque samples according to metabolic activity and PCR analysis.

Cariogenic bacteria, especially mutans streptococci, are resistant to many antibiotics generally used to treat oral infections (Golestannejad et al. 2015). Fayaz et al. (2014) studied the prevalence and antibiotic susceptibility patterns of human dental biofilm-forming bacteria. Their results indicate that dental biofilm-forming bacteria vary in their resistance patterns. Some bacteria were found to be resistant to commonly known drugs (Fayaz et al. 2014). The antibiotic resistance patterns of *Streptococcus mutans* isolated from dental caries patients were identified by Karikalan and Mohankumar in 2016. Out of a total of 14 antibiotics tested, a 50% rate of resistance was observed for penicillin, ceftriaxone, cefepime, cefotaxime, ampicillin and cefaclor. Moderate resistance was seen against vancomycin (43%), erythromycin (14%), gentamycin (29%) and amoxicillin (29%). The least resistance (7%) was found against tetracycline, chloramphenicol and kanamycin. Only clindamycin showed 100% effectivity against the tested isolates. Here, the identification of antibiotic resistance patterns revealed a resistance rate of 100% among *Streptococcus sobrinus* isolates when using penicillin, ceftriaxone, cefotaxime, vancomycin, erythromycin, tetracycline and chloramphenicol. Also, 100% of *Streptococcus mutans* isolates were resistant to penicillin, 87% were resistant to cefotaxime, vancomycin, erythromycin and chloramphenicol, 75% to ceftriaxone and 50% to tetracycline, which indicates that the rate of resistance was considerable amongst our isolates, especially in *Streptococcus sobrinus* isolates.

The prevalence of oral diseases, antibiotic resistance in microorganisms and the side effects of chemical antibiotics such as vomiting, diarrhoea, tooth staining and alteration of the oral microbiota has led to the use of plant products like essential oils as an effective alternative.

It has been demonstrated that natural products exert antimicrobial activity against cariogenic bacteria. For example, benzoin derived from *Styrax tonkinensi*

doi: 10.17221/180/2016-VETMED

is an oral disinfectant or neem (*Azadirachta indica*) is used as a dental gel (Kabra et al. 2012).

*Carum copticum* essential oil has been proven to have various therapeutic activities such as antimicrobial, antifungal and cytotoxic activities. The main components of *Carum copticum* are carvacrol, γ-terpinene and p-cymene. The chemical composition of *Carum copticum* from the Kam firuz and Eghlid areas of Iran has been assessed and the results indicate thymol (35% to 60%) to be the major constituent. The nonphenolic fraction contained γ-terpinene, para-cymene, α- and β-pinenes, α-terpinene and carvacrol (Jeet et al. 2012).

Our GC-MS analysis of *Carum copticum* essential oil indicated that phenolic compounds were the major constituent of the oil, whereas the nonphenolic fraction was mainly composed of benzene methyl, γ-terpinene, and β-pinenes. Rabiey et al. (2014) used *Carum copticum* essential oil to control *Listeria monocytogenes* growth in a fish model system. The main components of the oil were thymol (57%), p-cymene (22%) and γ-terpinene (13%). They reported that this essential oil could markedly decrease the number of bacteria in their system. Compared to other studies, we found lower amounts of phenolic compounds but higher amounts of thymene constituents.

Upadhyat et al. (2010) screened the antibacterial activity of *Carum copticum* essential oil against pathogenic bacterial strains and indicated that this essential oil was highly lethal to *Streptococcus pneumoniae, Staphylococcus aureus and Lactobacillus acidophilus*. In our study, we also determined antibacterial activities for this essence.

Jain et al. (2015) evaluated the antibacterial efficacy of *Aloe vera*, amla (*Phyllanthus emblica*), garlic (*Allium sativum*), ginger (*Zingiber officinalis*), neem (*Azadirachta indica*) and tulsi (*Ocimum sanctum*) extracts against *Streptococcus mutans* using MIC and MBC and also the disc diffusion method. The results of antibacterial susceptibility testing showed that inhibition zones varied from 6 to 25 mm in diameter. Here, antibacterial susceptibility testing of *Carum copticum* essential oil against *Streptococcus mutans* resulted in smaller inhibition zones of 2–5 mm. This may be explained by the significant difference between the volume of the essence we used (10 µg) and the volume used in the study of Jain and co-workers (100 µg). Jain et al. reported MIC values ranging from 6.25 to 100 mg/ml and MBC values of between 12.5 mg and 200 mg. However, in our study, both MIC and MBC values were considerably lower than these previous findings. The antibacterial activities of *E. caesia* Benth, *C. cyminum* L. and *S. hortensis* L. were evaluated by Golestannejad et al. (2015). The results of a disc diffusion assay indicated that the inhibition zone was larger at higher concentrations, which is in accordance with our findings. The MIC values of these essential oils were 34.8, 19.5 and 13.2, respectively, against *S. mutans*. Therefore, the MIC value of *E. caesia* Benth essential oil is higher than that of *Carum copticum* essential oil. This value is similar in *C. cyminum* L. and *Carum copticum* essential oil. Finally, the MIC value of *S. hortensis* L. is lower than that of *Carum copticum* essential oil.

Therefore, we conclude that *Carum copticum* essential oil has considerable antibacterial activities against mutans streptococci isolated from dog dental plaques and may be used instead of common antibiotics in the treatment of oral bacterial pathogens that are resistant to antibacterial agents. However, it is necessary to verify how animals will respond to the application of *Carum copticum* active substances. If these tests will be successful, there will also be a need to determine the suitable form in which to administer the essential oil. It may also be possible to use the above-mentioned active substances in the treatment of, for example, dermatitis, otitis, digestive problems or other diseases frequently observed in dogs, cats or other animals such as birds, reptiles, fishes, bees etc.

## Acknowledgements

The authors acknowledge Tabriz University for supporting this work.

## REFERENCES

Al-Mudallal NHA, Al-Jumaily EFA, Muhimen NAA, Al-Shaibany AW (2008): Isolation and identification of mutans Streptococci bacteria from human dental plaque samples. Journal of Al-Nahrain University 11, 98–105.

Allmuca H, Zalla P, Andoni E, Mazari B (2016): Prevalence of oral diseases in dogs in Tirana urban area. Indian Journal of Animal Research 50, 740–746.

Amoroso P, De Avila FA, Gagliardi CMO (2003): Prevalence of different Streptococci species in the oral cavity of children and adolescents. Brazilian Journal of Oral Sciences 2, 164–168.

doi: 10.17221/180/2016-VETMED

Beighton D, Russell RRB, Hayday H (1981): The isolation and characterization of Streptococcus mutans serotype h from dental plaque of monkeys (Macaca fascicularis). Journal of General Microbiology 124, 271–279.

Fayaz M, Sivakumaar PK, Melvin Joe M (2014): Prevalence and antibiotic susceptibility pattern of dental biofilm forming bacteria. International Journal of Current Microbiology and Applied Sciences 3, 46–50.

Franco e Franco TCC, Amoroso P, Marin JM, de Avila FA (2007): Detection of Streptococcus mutans and Streptococcus sobrinus in dental plaque samples from Brazilian preschool children by polymerase chain reaction. Brazilian Dental Journal 18, 329–333.

Galvao LCDC, Furletti VF, Bersan SMF, Cunha MGD, Ruiz ALTG, Carvalho JED, Sartoratto A, Rehder VLG, Figueira GM, Duarte MCT, Ikegaki M, Alencar SM, Rosalen PL (2012): Antibacterial activity of essential oils against Streptococcus mutans and their antiproliferative effects. Evidence-Based Complementary and Alternative Medicine, doi: 10.1155/2012/751435.

Golestannejad Z, Mohammadi E, Motamedi A, Gavanji SH, Fallah N, Bagherie SH, Farzane G, Safaripour M, Vally A, Doost Mohammadi M, Larki B, Bakhtari A (2015): Chemical composition and antibacterial activity of some herbal essential oils against Streptococcus mutans. Advanced Herbal Medicine 1, 1–8.

Goudarzi GHR, Saharkhiz MJ, Sattari M, Zomorodian K (2011): Antibacterial activity and chemical composition of Ajowan (Carum copticum Benth and Hook) essential oil. Journal of Agricultural Science and Technology 13, 203–208.

Jain I, Jain P, Bisht D, Sharma A, Srivastava B, Gupta N (2015): Comparative evaluation of antibacterial efficacy of six Indian plant extracts against Streptococcus mutans. Journal of Clinical and Diagnostic Research 9, 50–53.

Jeet K, Devi N, Narender T, Sunil T, Shalta L, Raneev T (2012): Trachyspermum ammi (adjwan): a comprehensive review. International Research Journal of Pharmacy 3, 133–138.

Kabra P, Loomba K, Kabra SH, Majumdar DSP, Kumar N (2012): Medicinal plants in the treatment of dental caries. Asian Journal of Oral Health and Allied Sciences 2, 12–16.

Karikalan S, Mohankumar A (2016): Antibiogram of Streptococcus mutans isolated from dental caries patients. International Journal of Medical and Health Research 2, 79–83.

Kyllar M, Witter K (2005): Prevalence of dental disorders in pet dogs. Veterinarni Medicina 50, 496–505.

Mahmoudi R, Kosari M, Barati SH (2013a): Phytochemical and biological properties of Ferula sharifi essential oil. Journal of Biologically Active Products from Nature 3, 331–338.

Mahmoudi R, Ehsani A, Tajik H, Pajohi Alamoti M (2013b): Evaluation of phytochemical and antibacterial properties of some medicinal plants from Iran. Journal of Biologically Active Products from Nature 3, 310–322.

Maripandi A, Kumar A, Al Salamah AA (2011): Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different tooth pastes against Streptococcus spp. African Journal of Microbiology Research 5, 1778–1783.

Momtaz H, Rahimi E, Moshkelani (2012): Molecular detection of antimicrobial resistance genes in E. coli isolated from slaughtered commercial chickens in Iran. Veterinarni medicina 57, 193–197.

Percival SL, Knap JS, Williams DW, Heritage J, Brunton LA (eds) (2011): Biofilms and Veterinary Medicine. Springer-Verlag Berlin Heidelberg. 129–142.

Rabiey S, Hosseini H, Rezaei M (2014): Use Carum copticum essential oil for controlling the Listeria monocytogenes growth in fish model system. Brazilian Journal of Microbiology 1, 89–96.

Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K (2014): Antimicrobial drug resistance in strains of Escherichia coli isolated from food sources. Revista do Instituto de Medicina Tropical de Sao Paulo 56, 341–346.

Rawashdeh RY, Malkawi HI, Al-Hiyasat AS, Hammad MM (2008): A fast and sensitive molecular detection of Streptococcus mutans and Actinomyces viscosus from dental plaques. Jordan Journal of Biological Sciences 1, 135–139.

Upadhyat RK, Dwivedi P, Ahmad SH (2010): Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian Journal of Medical Sciences 2, 152–158.

Zambori C, Tirziu E, Nichita I, Cumpanasoiu C, Gros RV, Seres M, Mladin B, Mot D (2012): Biofilm implication in oral diseases of dogs and cats. Animal Science and Biotechnologies 45, 208–2012.

Zomorodian K, Ghadiri P, Saharkhiz MJ, Moein MR, Mehriar P, Bahrani F, Golzar T, Pakshir K, Fani MM (2015): Antimicrobial activity of seven essential oils from Iranian aromatic plants against common causes of oral infections. Jundishapur Journal of Microbiology 8, doi: 10.5812/jjm.17766.

Received: December 13, 2016
Accepted after corrections: October 18, 2017