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Original Article

In Vitro Antibacterial Effect of *Pimpinella Anisum* Essential Oil on *Enterococcus Faecalis, Lactobacillus Casei, Actinomyces Naeslundii*, and *Aggregatibacter Actinomycetemcomitans*

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Abstract

Introduction: *Pimpinella anisum* is a medicinal plant with antimicrobial, antifungal, and anti-oxidative properties. Limited studies have assessed the antibacterial properties of *Pimpinella anisum* on oral and dental pathogens.

Aim: This *in vitro* study aimed to assess the antibacterial effect of *Pimpinella anisum* essential oil on *Enterococcus faecalis*, *Lactobacillus casei*, *Actinomyces naeslundii*, and *Aggregatibacter actinomycetemcomitans*.

Materials and methods: After obtaining the essential oil of *Pimpinella anisum*, its antimicrobial activity was evaluated using the agar disc diffusion test. The minimum inhibitory concentration and minimum bactericidal concentration of the essential oil were also determined; 0.2% chlorhexidine was used as the positive control.

Results: The mean diameter of growth inhibition zone was 39 mm for *Enterococcus faecalis*, 40 mm for *Lactobacillus casei*, 42 mm for *Actinomyces naeslundii*, and 18.5 mm for *Aggregatibacter actinomycetemcomitans*. The mean diameter of the growth inhibition zones for *Enterococcus faecalis*, *Lactobacillus casei*, and *Actinomyces naeslundii* was significantly greater than that of *Aggregatibacter actinomycetemcomitans* (p=0.001). Also, the mean diameter of the growth inhibition zone of *Actinomyces naeslundii* was significantly larger than that of *Enterococcus faecalis* (p=0.05). The minimum inhibitory concentration and minimum bactericidal concentration of the essential oil for *Enterococcus faecalis* were 4.88% and 4.88%, respectively. These values were 9.76% and 9.76% for *Lactobacillus casei*, 9.76% and 4.88% for *Actinomyces naeslundii*, and 9.76% and 9.76% for *Aggregatibacter actinomycetemcomitans*, respectively.

Conclusions: *Pimpinella anisum* essential oil was effective against all four microorganisms evaluated in this study. Since the lowest minimum inhibitory concentration and minimum bactericidal concentration were recorded for *Enterococcus faecalis*, this essential oil has maximum effects on *Enterococcus faecalis*. Future clinical studies are required to assess the antimicrobial efficacy of *Pimpinella anisum* essential oil in clinical samples.

Keywords

Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Enterococcus faecalis, essential oil, Lactobacillus casei, Pimpinella anisum

INTRODUCTION

Oral and dental conditions are a public health dilemma. Many patients suffer from dental caries, periodontal disease and pulpal and periapical diseases caused by streptococci, actinomyces, and lactobacilli.[1,2] *Streptococcus mutans* plays an important role in initiation of caries.[3,4] Lactobacilli are responsible for caries progression especially in tooth crown.[5] *Actinomyces* are responsible for root caries, and are involved in periodontitis and root canal infection.[6,7] *Enterococcus faecalis* is responsible for root canal treatment failure, and development of treatment-resistant refractory infections.[8] *Aggregatibacter actinomycetemcomitans* is a major periodontal pathogen.[9,10] Since bacteria are the main causative agents of oral and dental conditions, elimination of pathogenic microorganisms is the best strategy for prevention of oral diseases. At present, use of medicinal plants as an alternative to synthetic medications has been gaining increasing popularity.[11] Use of mouthwashes, along with mechanical plaque removal methods, is suggested for elimination of oral pathogens. Scientists are in search of a medicinal plant to prevent dental plaque formation. An ideal medicinal plant for this purpose should not adversely affect the normal flora of the mouth, tooth colour, or sense of taste. Also, it should taste good and exert antibacterial effects comparable to the antibacterial effects of chlorhexidine (CHX).[12-16] *Pimpinella anisum*, also known as anise, is a one-year plant 1 m high, with narrow leaves, and good scent, which is extensively used in the Iranian traditional medicine.[17] Its essential oil contains anethole with antibacterial effects against *Salmonella typhi*, and *Escherichia coli*. It is used mainly for gastrointestinal diseases, but is also useful in the treatment of upper respiratory tract infections, fever, and oral and pharyngeal mucosal inflammation.[18] Its extract has antimicrobial properties against different bacteria and fungi.[19,20] Another study conducted in India showed that coconut oil can inhibit the proliferation of *S. mutans* similar to CHX.[1] In a study conducted in Turkey, the antioxidant and antimicrobial activities of aqueous and ethanolic extracts of *P. anisum* seed were proven.[5]

Due to the increasing demand for use of herbal medicines as well as the optimal antimicrobial activity and low side effects of medicinal plants, researchers are attempting to synthesise herbal antimicrobial products against the pathogenic bacteria.

AIM

Considering the significance of this topic, this study aimed to assess the efficacy of the essential oil of *P. anisum* against *E. faecalis*, *Lactobacillus casei*, *Actinomyces naeslundii*, and *A. actinomycetemcomitans* in vitro.

MATERIALS AND METHODS

Bacterial species

This in vitro study evaluated standard strains *E. faecalis* (PTCC 1778), *L. casei* (PTCC 1608), *A. naeslundii* (PTCC 1201), and *A. actinomycetemcomitans* (JP2NOV99) obtained from the Pasteur Institute of Iran.

Preparation of *P. anisum* essential oil

The essential oil of *P. anisum* was prepared by an expert person in the School of Pharmacy of Shahid Beheshti University of Medical Sciences. For preparation of the essential oil, 100 g of *P. anisum* was placed in an Erlenmeyer flask and 500 mL of distilled water was added to it. The essential oil was obtained using the Clevenger device for 4 hours. Averagely, 4 mL of essential oil was obtained from each 100 g of *P. anisum*. The primary concentration of the essential oil for antibacterial tests was considered to be 100%. The essential oil was then sterilized by using a 0.22-µm microbiology filter.

Preparation of 0.5 McFarland standard concentrations

The bacteria were first cultured on brain heart infusion (BHI) agar and incubated at 37°C for 24 hours. Next, 0.5 McFarland [1.5×10^8 colony forming units (CFUs)/mL] bacterial suspensions were prepared for the agar disc diffusion test. For this purpose, 2 cc of sterile saline was added to each test tube and next, adequate amount of bacterial colonies from the 24-h culture was inoculated into the BHI agar. The contents of the tube were vortexed to obtain a homogenous suspension. The turbidity of the suspension was measured by a spectrophotometer at 600 nm wavelength; absorbance between 0.08-0.1 nm in this wavelength indicated 0.5 McFarland standard concentration.

Agar disc diffusion test

The agar disc diffusion test was used to assess the antimicrobial efficacy of the *P. anisum* essential oil. For this purpose, bacterial suspensions with 0.5 McFarland standard concentration were streak-cultured on BHI agar separately with sterile swabs. Each bacterial culture plate was divided into four regions. A blank disc dipped in 0.2% CHX was placed in one region as the positive control (0.2% CHX has proven antimicrobial effects and inhibits the bacterial growth; thus, a growth inhibition zone forms around the disc dipped in 0.2% CHX as the positive control). A blank disc was placed in another region as the negative control (the blank disc has no antimicrobial effect; thus, there would be no growth inhibition zone around it as the negative control), and discs containing *P. anisum* with primary concentration were placed on the remaining two regions.

Each test was repeated four times. Sterile gloves were worn during the entire experiment, and all procedures were performed next to the flame and under a hood. The plates containing *A. actinomycetemcomitans* and *L. casei* were placed in Gas-Pak anaerobic jars, respectively, for 48 hours. The plates containing *E. faecalis* and *A. naeslundii* were incubated in aerobic conditions at 37°C for 24 hours. After removing the plates from the incubator, a ruler was used to measure the diameter of the growth inhibition zone of the bacteria around the discs under a magnifier. The values were reported in millimetres.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC is determined to assess the inhibitory effect of a material on proliferation of microorganisms. A 96-well enzyme-linked immunosorbent assay (ELISA) plate was used for the microdilution test.

Five rows (each row had 12 wells) of the plate were used in this test. For testing of the essential oil with three repetitions (the test instructions were the same for all bacteria), three rows were used. Also, one row was used as the positive and one row as the negative control. At first, 100 µL of the BHI broth was added to each well in all five rows. Next, for all three rows (row numbers 1, 2, and 3) that were related to testing of the essential oil with three repetitions, 100 µL of the primary concentration of the essential oil was added to number 1 to 12 wells of each of the three rows. Next, 100 µL of the contents of the first well was removed and added to the second well. This process was repeated until the 12th well; 100 µL of the contents of the 12th well was discarded. In this way, the essential oil was diluted by half each time it was transferred from one well to the other. In the next step, 10 µL of the bacterial suspension (0.5 McFarland standard concentration) was added to each well (#1 to 12). Also, one row (row C+) was considered as the positive control (100 µL of the BHI broth plus 10 µL of the bacterial suspension were added to wells 1 to 12) and another row (row C-) was considered as the negative control [only 100 µL of the BHI broth plus 100 µL of the primary concentration of the essential oil were added to these wells (#1 to 12), without any bacterial suspension].

Next, the ELISA plates of *A. actinomycetemcomitans* and *L. casei* were placed in Gas-Pak anaerobic jars for 48 hours. The plates containing *E. faecalis* and *A. naeslundii* were incubated in aerobic conditions at 37°C for 24 hours.

After incubation, the turbidity of the wells was measured by colorimetry. For this purpose, 0.01% resazurin was added to all wells and the plates were then placed in an incubator at 37°C. After 2 hours, the colour of the dye changed from blue to pink due to the activity of bacteria.

The first chromatic well was considered, and the previous wells with no colour change (lowest dilution in which no discolouration was observed) indicated the MIC of the essential oil for the respective microorganism as a fraction (percentage) of the primary concentration (100%).

To determine the MBC of the essential oil for the bacteria, samples were taken from one well before and 3 wells after the MIC well, as well as the MIC well itself. Then, the samples were passaged on the BHI agar.

Bacterial growth was evaluated after 24-48 hours of incubation as explained earlier. The MIC showing no growth on BHI agar indicated the MBC of the essential oil for the respective microorganism. The value was reported as a fraction (percentage) of the primary concentration (100%).

Statistical analysis

Data were analysed using SPSS software version 21 (SPSS Inc., IL, USA). The mean, standard deviation, minimum and maximum values were reported for the quantitative data. Independent t-test was used to compare the diameter of the growth inhibition zones of the studied bacteria around the essential oil and CHX discs, and two-way analysis of variance (ANOVA) was used to analyse the effect of the type of antimicrobial agent, type of microorganism, and the interaction effect of these two factors. $P \leq 0.05$ was considered statistically significant.

RESULTS

In the agar disc diffusion test, the diameter of the growth inhibition zone around the essential oil disc was maximum for *A. naeslundii* (mean value of 42 mm) followed by *L. casei* (40 mm), *E. faecalis* (39 mm), and *A. actinomycetemcomitans* (18.5 mm). The mean diameter of the growth inhibition zones for *E. faecalis*, *L. casei*, and *A. naeslundii* was significantly greater than that of *A. actinomycetemcomitans* (p=0.001). Also, the mean diameter of the growth inhibition zone of *A. naeslundii* was significantly larger than that of *E. faecalis* (p=0.05).

Fig. 1 shows an agar disc diffusion test for *E. faecalis*. CHX disc was used as the positive control and a blank disc was used as the negative control.

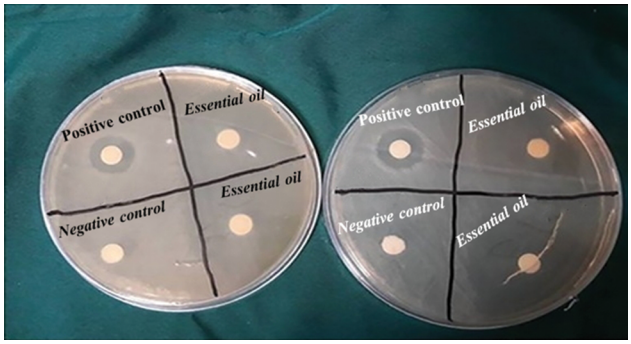

Figure 1. Measuring the diameter of the growth inhibition zones in *E. faecalis* culture; positive control: 0.2% CHX, negative control: blank disc, essential oil: essential oil of *P. anisum*.

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The difference in the diameter of the growth inhibition zone around the essential oil and CHX discs was significant for all bacteria (p<0.001), indicating that the essential oil was more potent than CHX against *L. casei*, *A. naeslundii*, and *E. faecalis*; while the antimicrobial efficacy of CHX against *A. actinomycetemcomitans* was higher than that of the essential oil **(Table 1)**. No growth inhibition zone was noted around the blank disc.

In addition, the results of two-way ANOVA showed that the effect of the type of antimicrobial agent, type of microorganism, and the interaction effect of these two factors were significant on the diameter of the growth inhibition zone (p<0.001).

The MIC test showed that the essential oil was effective against all four microorganisms. The essential oil had maximum inhibitory effect on *E. faecalis* and *A. naeslundii* with MIC=4.88%. The MIC of the essential oil against *A. actinomycetemcomitans* and *L. casei* was 9.76%.

Fig. 2 shows the results of MIC testing for *A. actinomycetemcomitans* after reaction with resazurin.

Regarding the MBC, the essential oil had maximum bactericidal effect on *E. faecalis* with MBC=4.88%. The MBC of the essential oil was 9.76% for *L. casei*, *A. actinomycetemcomitans*, and *A. naeslundii* .

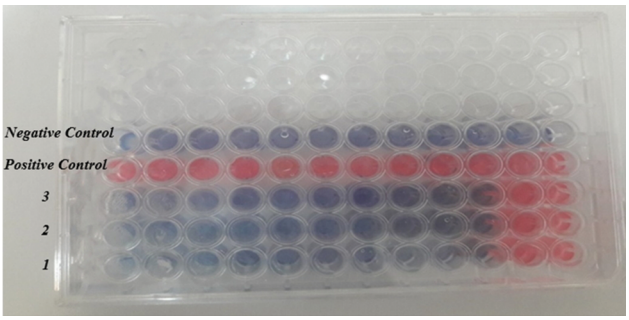

Figure 2. MIC test results of *A. actinomycetemcomitans* after reaction with resazurin; rows 1, 2, and 3: for testing of the essential oil with three repetitions, BHI broth plus essential oil of *P. anisum* with bacterial suspension, negative control: BHI broth plus essential oil of *P. anisum* without any bacterial suspension, positive control: BHI broth plus bacterial suspension, blue colour: presence of bacteria without activity (the colour of resazurin is blue), red colour: presence of bacteria and their activity (the colour of resazurin changed from blue to red).

Fig. 3 shows the MBC test result for different concentrations of the essential oil against *A. actinomycetemcomitans*. As shown, no colony grew in concentrations <1.1024.

According to both the MIC and MBC values, the essential oil had a maximum effect on *E. faecalis*. The MIC of the essential oil was 4.88% of the primary concentration (100%) for *E. faecalis* and *A. naeslundii*. The MIC of the essential oil for *A. actinomycetemcomitans* and *L. casei* was the same (9.76% of the primary concentration) **(Fig. 4)**. Complete bacterial growth was noted in the positive control while no bacterial growth was noted in the negative control.

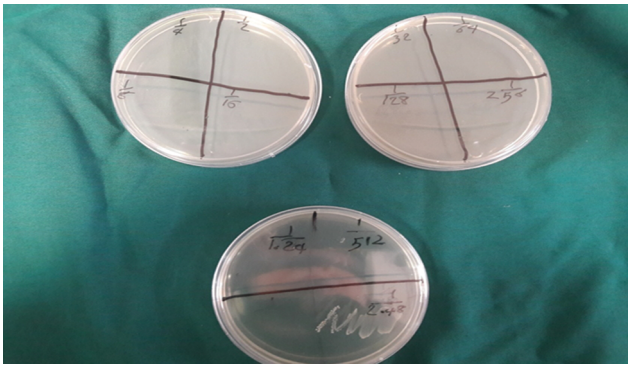

Figure 3. MBC test results of *A. actinomycetemcomitans* after incubation. Different concentrations of the essential oil were tested. As seen, no colony grew in concentrations <1.1024.

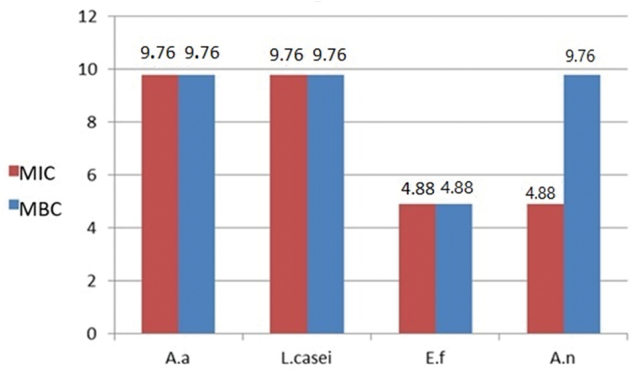

Figure 4. MIC and MBC of the essential oil against *E. faecalis*, *L. casei*, *A. naeslundii*, and *A. actinomycetemcomitans*.

Table 1. Comparison of the diameter (mm) of the growth inhibition zone of the studied bacteria around the *P. anisum* essential oil and chlorhexidine discs

Bacteria	Essential oil Mean ± SD*	Chlorhexidine Mean ± SD	Difference in means **	*P*-value
E. faecalis	39±1.15	16±1.15	23	<0.001
L. casei	40±1.63	20±0.00	20	<0.001
A. naeslundii	42±1.63	15±0.00	27	<0.001
A. actinomycetemcomitans	18.50±1.29	35.50±0.57	-17	<0.001

* Standard deviation; ** Absolute difference between the mean values in two different groups

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DISCUSSION

P. anisum has long been used in the Iranian traditional medicine as a disinfectant, antimicrobial, antifungal, antiviral, and antioxidant agent, muscle relaxant, and analgesic, anti-coagulative, and anti-inflammatory agent.[20] Considering the significance of treatment of oral infections and the side effects of chemical mouthwashes, this study aimed to assess the effect of *P. anisum* on oral pathogens. The results confirmed the inhibitory effect of *P. anisum* on four types of microorganisms. This effect was greater on *E. faecalis*, and *A. naeslundii*. According to the diameter of the growth inhibition zone, the essential oil of *P. anisum* had a smaller effect on *A. actinomycetemcomitans* compared with 0.2% CHX as the positive control. The reason for this finding may be the fact that *A. actinomycetemcomitans* is Gram-negative while the other tested bacteria are Gram-positive. Unlike Gram-positive bacteria, a lipopolysaccharide layer is present in the cell wall of Gram-negative bacteria. Thus, materials cannot easily pass through their cell wall, and only hydrophilic compounds with special structure can pass through it and affect the microorganism. Moreover, *A. actinomycetemcomitans* is a slow-growing microorganism and may show resistance to this essential oil, or might have protective mechanisms against it. On the other hand, *L. casei* was cultured in microaerophilic conditions and due to its slow growth, the essential oil had a smaller effect on it compared with *E. faecalis* and *A. naeslundii*. Evrendilek et al. evaluated the antimicrobial effects of the essential oils of 14 plant species including *P. anisum* on 10 microorganisms including four Gram-positive microorganisms such as *Listeria innocua*, coagulase negative *staphylococcus*, *Bacillus subtilis*, and *S. aureus* and six Gram-negative bacteria namely *Proteus mirabilis*, *E. coli*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Yersinia enterocolitica*, and *Klebsiella oxytoca* by the agar disc diffusion method. They concluded that *P. anisum* had maximum antibacterial effects on Gram-positive bacteria such as *S. aureus* and *Listeria*. It had minimum antibacterial effects on *Salmonella enteritidis* and *Proteus*. Similarly to our study, they used the agar disc diffusion test, but did not assess the MIC and MBC. They evaluated a higher number of medicinal plants and also assessed the antimicrobial and anti-oxidative activity of the components by gas chromatography. They showed that *P. anisum* had significant antibacterial effects.[21] Kermanshah et al. in Iran evaluated the antibacterial effect of the hydroalcoholic extract of *P. anisum*, *Satureja hortensis* (savory) and *Salvia officinalis* (sage) on *S. mutans*, *Lactobacillus rhamnosus*, and *Actinomyces viscosus* using the agar disc diffusion test, and determined the MIC and MBC values as well. According to the obtained MIC value and the diameter of the growth inhibition zone, they concluded that all tested plant species had antibacterial activity of different levels. They also reported that the hydroalcoholic extract of anise had antibacterial effect on all three microorganisms. It had a maximum effect on *S. mutans* followed by *L. rhamnosus*. It had lower antibacterial activity against *A. viscosus*. In line with our findings, they confirmed the antibacterial effects of anise on *S. mutans* and *L. rhamnosus*.[19]

Abu-Darvish et al. evaluated the antibacterial effects of the essential oil and hydroalcoholic extract of several medicinal plants including anise, thyme, and *S. officinalis* (sage) against *S. aureus*, *Pseudomonas aeruginosa*, and *E. coli* using the agar disc diffusion test. They reported that *P. aeruginosa* was resistant to the essential oil and hydroalcoholic extract of anise. However, the essential oil and hydroalcoholic extract of anise had mild antimicrobial effect on *E. coli* and moderate antimicrobial effect on *S. aureus*. Similarly to our study, they used the agar disc diffusion test; however, they used the essential oil and hydroalcoholic extract, and did not determine the MIC. Also, they used ciprofloxacin as the positive control.[22] Kermanshah et al. compared the antibacterial effect of the hydroalcoholic extract of *S. officinalis* (sage) and anise on *S. mutans*, *L. rhamnosus*, and *A. viscosus* in vitro by determining the MIC and MBC values. They reported that both herbs effectively inhibited the bacteria while *S. officinalis* (sage) had lower MIC and MBC compared with anise. They reported significant inhibitory effect of hydroalcoholic extract of anise on the bacteria.[18] Akhtar et al. in India evaluated the antimicrobial effects of the aqueous and alcoholic (acetone, methanolic, and ether) extracts of anise in vitro on four pathogens namely *S. aureus*, *Streptococcus pyogenes*, *E. coli*, and *Klebsiella pneumonia*. They concluded that the aqueous extract of anise was more effective than other extracts against the studied bacteria. The effect of methanolic extract of anise was lower than its aqueous extract. Acetone and ether extracts had no antibacterial effect. Ciprofloxacin served as the positive control and was more effective than all extracts. Their study had some differences with the present study. They used the aqueous form of the extract while we used its essential oil. They used ciprofloxacin as the positive control while we used CHX for this purpose. Moreover, they only performed the agar disc diffusion test while we performed MBC and MIC tests in addition to the agar disc diffusion test. They did not determine the MIC and MBC. They concluded that the aqueous extract of anise can serve as a cheap alternative to antibiotics.[23] Al-Bayati et al. in Iraq evaluated the antibacterial effect of alcoholic extract and essential oil of *Thymus vulgaris* and anise alone and in combination on *E. coli*, *Proteus vulgaris*, *Proteus mirabilis*, *S. typhi*, *Klebsiella*, *P. aeruginosa*, *S. aureus*, and *Bacillus cereus* by determining the MIC value, and concluded that the essential oil and alcoholic extract of these two plants had antibacterial effects against these pathogens. *Thymus vulgaris* was more effective than anise against all pathogens except for *P. aeruginosa*. However, the combination of both extracts was more effective than each of them alone against the pathogens particularly *P. aeruginosa*. Also, Gram-positive bacteria were more sensitive to the plant extracts compared with Gram-negative bacteria, except *Proteus vulgaris*. *E. coli* and *Klebsiella pneumonia* were resistant to *P. anisum*. Also it had minimum and maximum effects on *S.*

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typhi and *S. aureus*, respectively. They determined the MIC values similarly to our study but did not determine the MBC and did not use agar disc diffusion test.[24] Their results regarding optimal antibacterial efficacy of anise were in line with our findings. Tepe et al. in Turkey evaluated the anti-oxidative and antimicrobial activity of *P. anisum* and *Pimpinella flabellifolia* against 5 microorganisms namely *Streptococcus pneumonia*, *B. cereus*, *Acinetobacter luufi*, *E. coli*, and *K. pneumonia* and two fungi namely *Candida albicans* and *Candida krusei* using the agar disc diffusion test and MIC. *P. anisum* caused the maximum diameter of the growth inhibition zone in *Acinetobacter luufi* culture (18 mm) while *Pimpinella flabellifolia* caused the minimum diameter of the growth inhibition zone in *E. coli* culture (8 mm). The MIC of *P. anisum* and *P. flabellifolia* ranged from 9 to 72 mg/mL. The results showed that *P. anisum* and *P. flabellifolia* had moderate antibacterial effects on the tested microorganisms, and the antibacterial effect of *P. anisum* was stronger than that of *P. flabellifolia*. They assessed the antibacterial activity and anti-oxidative activity of two medicinal plants against several microorganisms. However, their results regarding the optimal antibacterial effect of *P. anisum* on the microorganisms were in agreement with our findings.[25]

CONCLUSION

Considering its optimal antibacterial activity, and fewer complications compared with chemical agents, it may be suitable for addition to toothpastes and root canal irrigating solutions. However, further studies on other properties, toxicity, and range of action of this essential oil against microorganisms are required prior to its use as a mouthwash.

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Disclosure statement

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Антибактериальное действие эфирного масла *Pimpinella Anisum in vitro* на *Enterococcus Faecalis, Lactobacillus Casei, Actinomyces Naeslundii* и *Aggregatibacter Actinomycetemcomitans*

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Резюме

Введение: *Pimpinella anisum* – лекарственное растение с противомикробными, противогрибковыми и антиоксидантными свойствами. Ограниченные исследования оценивали антибактериальные свойства *Pimpinella anisum* в отношении оральных и стоматологических патогенов.

Цель: Это исследование *in vitro* было направлено на оценку антибактериального действия эфирного масла *Pimpinella anisum* на *Enterococcus faecalis, Lactobacillus casei, Actinomyces naeslundii* и *Aggregatibacter actinomycetemcomitans*.

Материалы и методы: После получения эфирного масла *Pimpinella anisum* его антимикробную активность оценивали с помощью диффузионного теста с агаровым диском. Также определяли минимальную ингибирующую концентрацию и минимальную бактерицидную концентрацию эфирного масла; в качестве положительного контроля использовали 0.2% раствор хлоргексидина.

Результаты: Средний диаметр зоны задержки роста составил 39 мм для *Enterococcus faecalis*, 40 мм для *Lactobacillus casei*, 42 мм для *Actinomyces naeslundii* и 18.5 мм для *Aggregatibacter actinomycetemcomitans*. Средний диаметр зон ингибирования роста для *Enterococcus faecalis, Lactobacillus casei* и *Actinomyces naeslundii* был значительно больше, чем у *Aggregatibacter actinomycetemcomitans* (p=0.001). Кроме того, средний диаметр зоны подавления роста у *Actinomyces naeslundii* был значительно больше, чем у *Enterococcus faecalis* (p=0.05). Минимальная ингибирующая концентрация и минимальная бактерицидная концентрация эфирного масла в отношении *Enterococcus faecalis* составляли 4.88% и 4.88% соответственно. Эти значения составили 9.76% и 9.76% для *Lactobacillus casei*, 9.76% и 4.88% для *Actinomyces naeslundii* и 9.76% и 9.76% для *Aggregatibacter actinomycetemcomitans* соответственно.

Заключение: Эфирное масло *Pimpinella anisum* было эффективным против всех четырёх микроорганизмов, оцениваемых в этом исследовании. Поскольку самая низкая минимальная ингибирующая концентрация и минимальная бактерицидная концентрация были зарегистрированы для *Enterococcus faecalis*, это эфирное масло оказывает максимальное воздействие на *Enterococcus faecalis*. Необходимы будущие клинические исследования для оценки антимикробной эффективности эфирного масла *Pimpinella anisum* в клинических образцах.

Ключевые слова

Actinomyces naeslundii, Aggregatibacter actinomycetemcomitans, Enterococcus faecalis, эфирное масло, Lactobacillus casei, Pimpinella anisum