Original Article

Investigation of Antibacterial Effect of *Cuminum cyminum* and *Carum carvi* against *Streptococcus mutans* and *Streptococcus pyogenes*

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Abstract

Background: *Streptococcus mutans* is major cause of tooth decay. In addition, there is some report about effects of *Streptococcus pyogenes* on odontogenic infections. Herbal drugs is now administrating for treatment of some disease. The aim of this study was to evaluate the antibacterial effect of *Cuminum cyminum* and *Carum carvi* against *Streptococcus mutans* and *Streptococcus pyogenes*.

Materials and Methods: In this study, standard strains of *Streptococcus mutans* and *Streptococcus pyogenes* were used, and ethanolic extract was prepared in the microbiological laboratory of Shahid Beheshti University of Medical Sciences. The experiment was repeated 6 times and chlorohexine 2% was used as a control.

Results: The best effect of *Carum carvi* was on *Streptococcus mutans*, whereas on *Streptococcus pyogenes* both extracts of *Cuminum cyminum* and *Carum carvi* showed the same effect.

Conclusion: This study showed, due to the fact that the *Cuminum cyminum* and *Carum carvi* extracts exhibited excellent antibacterial properties, they could be a suitable candidate in compounds such as mouthwash, toothpastes and Gums. Therefore, could be used in the prevention and treatment of future oral and dental diseases.

Keywords: *Cuminum cyminu, Cuminum cyminum, Streptococcus mutans and Streptococcus pyogenes*

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Introduction

More than 700 microorganisms colonized in the oral cavity like bacteria, some could cause oral disease like periodontitis and some species have been implicated in systematic diseases such as bacterial endocarditis, aspiration pneumonia, osteomyelitis in children, preterm low birth weight and cardiovascular disease[1].

Among bacteria which colonized in the oral cavity, *Streptococcus spp* is the most prevalent in which *Streptococcus mutans* considered a major causative agent of dental caries with the formation of dental biofilm[2]. *Streptococcus mutans* belongs to *Streptococcus spp*, which are facultative, anaerobic, gram-positive bacteria. *Streptococcus mutans* lives in the oral cavity, pharynx and intestine. The capacity to synthetize the polysaccharides in dental caries make

the S.*mutans* as a major cause of tooth decay. Therefore, it is essential to reduce the number of bacteria in the mouth[3]. Most oral infection occurs through the root apex of tooth and odontogenic infection rarely cause the oral infection except acquiring the bacteria like S.*pyrogenes* through dental implant which may cause a life- threatening systematic diseases[4].

Streptococcus pyogenes is a gram-positive bacterium, which is classified in the Lancefield group A antigen and is the causative agent of pharyngitis, localized skin infection (impetigo), erysipelas and cellulitis in humans[5]. The treatment of oral infection is administrating fluoride which inhibits the enolase activity of bacteria and chlorhexidine that interfere with adherence agent of bacteria or mechanical removal of plaque[6].

Infectious diseases have always been a concern for humankind. Today, Antibiotics are used to treat infectious diseases, but they have serious problems. For example, increased drug resistance and side effects have been identified. As a result, scientists are trying to find alternative ways to treat bacterial infections appropriately[5]. One of the alternatives that can be a good is herbal ingredients. These compounds can also be effective in the treatment of infectious diseases and reduce the effects of antibiotics[7]. Administrating of chemical antibacterial agent always have a side- effect beside its effectiveness. Therefore. global attention to herbal drugs has been increasing recently. According to world health organization (WHO), more than 4 billion people nowadays prefer using herbal medicine for healing of diseases. Herbal plants have a wide range of pharmacological activity such as antimicrobial, anticancer, anti-inflammatory which could be administered in the wide range of human disease[8].

Cuminum cyminum (cumin) and *Carum carvi* (caraway) belong to the family of Apaiaceae. Cumin and caraway seeds form *Cu. cyminum* and *Ca. carvi* respectively which were traditional Egyptian curative herbal drugs and has been cultivated in Middle East, India, China and Mediterranean countries[8].

In traditional medicine cumin is used for the treatment of colic pain, bronchitis and fatulence. cumin seeds have been shown to have antibacterial, antifungal, anti-carcinogenic, anti-diabetic, anti-thrombotic, and antioxidant properties. Administrating the cumin and caraway as a herbal medicine has been widespread from Northern Europe to Mediterranean regions[9]. In Iranian tradition, it has been administered to gastrointestinal, gynecological and respiratory disorders as well as treatment of toothache, epilepsy, and diarrhea[10].

The composition of cumin and caraway are alkaloid, coumarin, anthraquinone, flavonoid, glycoside, protein, resin, saponin, tannin and steroid[11]. Recently, by exploring the chemical content of these herbal plant, researcher aimed to find the exact mechanism of action in different disease. Todays, we faced with increasing of antibiotic resistance of bacteria to chemical agent, herbal drugs could be good alternative for treatment of bacterial disease. The aim of this study was to evaluate the antibacterial effect of *Cuminum cyminum* and *Carum carvi* against *Streptococcus mutans* and *Streptococcus pyogenes*.

Methods

Seeds of Cumin and Caraway were obtained from Iran center of herbal drugs and were extracted according to following protocol:

The seeds of Cumin and Caraway (60 gram of each seed) were socked with 75% ethanol (300ml*2) as a solvent, and then shacked for 3 hours at room temperature in order to extract the material of seeds. After that, extracts were kept in dark room for 72 hours to avoid some unrelated chemical reactions. After that, the extracts were filtered with watman paper and dried at 50˚C in to the incubator to evaporate the solvent and become a powder. The obtained powder was kept for further analysis[12].

The standard bacteria of *Streptococcus mutans* (lot number PTCC 1683) and *Streptococcus pyrogenes* (lot number PTCC 1762) strains were obtained from Iranian research organization for science and technology and subculture in blood agar in microbiology department of Shahid Beheshti University of Medical Sciences. The screening test has been performed to reassure about strains.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) determinartion with miro-diluton method: MIC value

of extracts means the minimal concentration of extract, which inhibit the growth of bacteria after 48 hours of incubation at 37˚C. According to CLSI recommendation, 20mg of each extracts were dissolved in 2% DMSO and sterilized through micropore filter (45mM)[13]. The MIC of seeds extracts were determined in sterile 96-well microplates according to the microdilution method of clinical and laboratory standard institute, M07-A8 each test was repeated for 6 times. A total 100µl of prepared extract were added to each column and then diluted serially with adding brain-heart infusion (BHI) broth as diluent except in column 4 and 8 that were as positive control.

The inoculum was prepared by adding one colony in two normal saline suspensions and incubated at 37^0 C until the turbidity is equivalent to 0.5 McFarland (using a bacterial suspension with density of 0.5 mg/ml, which is account for 1.5×108 CFU/ml). After standardization, 100µl bacterial inoculum was added into each rows containing seed extracts and mixed gently.

The MIC was determined one day after incubation of 96-well plate. The lowest concentration without any sign of bacterial growth or any visible turbidity was determined as MIC. Wells without any turbidity were sub-cultured in fresh nutrient agar media at 37^0 C for 18-48 hours and the highest dilution without any bacterial growth on nutrient agar was taken as MBC.

Results

This was an *in vitro* experimental study conducted at microbiology department of Shahid Beheshti University of Medical Sciences. Two seeds species (*Cuminum cyminum* and *Carum carvi*) were investigated to evaluate their antibacterial activity against oral pathogen (*streptococcus mutans*) and most prevalent bacteria in the skin (*streptococcus pyrogenes*) using dilution methods. Results demonstrated that both *Cuminum cyminum* and *Carum carvi* have an antibiotic activity against these two pathogens. Although, *S. pyrogenes* was most susceptible to cumin. Hnce, the minimal inhibitory concentration (MIC) activity and minimal bactericidal concentration (MBC) of *Cuminum cyminum* and *Carum carvi* against *S. pyrogenes* and *S. mutans* has been determined.

Minimum inhibitory concentrations (MIC's) *Cminum cyminum* and *Crum carvi* seeds extracts: The MIC of these two seeds extracts has been evaluated by dilution method to determine their bacteriostatic properties. MICs of effective seeds extracts were reported in table 1. The inhibitory effect of caraway was 0.625mg/ml against *S. mutans* and *S. pyrogenes* while the inhibitory effect of Cumin were 0.625 mg/ml against *S. mutans* and 2.5mg/ml against *S. pyrogenes*. The results indicated that *S. pyrogene* was most resistant to caraway whilst both cumin and caraway had a same inhibitory effect against *S. mutans* (figuer1).

Minimum bactericidal concentrations (MBC's) of the effective seeds extracts: The MBC was confirmed by the absence of bacteria growth of plates without any turbidity on nutrient broth. The MBC of effective seeds extracts (cumin and caraway) has been listed in table 1. The bactericidal effect of cumin against both *S. mutans* and *S. pyrogenes* was 1.25mg/ml whereas *S. pyrogenes* was more resistant to caraway (5mg/ml).

The results of MBC and MIC of cumin and caraway suggest that although both had a bactericidal activity against these two strains but cumin had a better effect and can be used for control of *S. pyrogenes* and *S. mutans*.

Table 1: The MIC and MBC of cumin and Caraway against S.pyrogenes and S.mutans.

Seeds Pathogens	*Cumin*		*Caraway*	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
S.pyrogenes	0.625	1.25	2.5	5
S.mutans	0.625	1.25	0.625	1.25

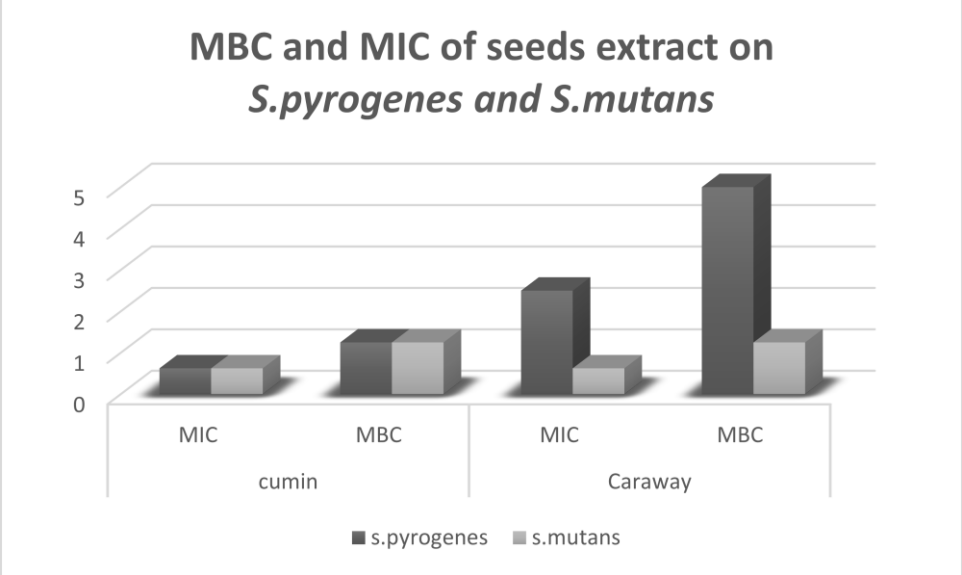

Figure 1. MBC and MIC of seeds extract on *S.pyrogenes and S.mutans*.

Discussion

The oral cavity is a habitat for most bacteria which mostly cause oral diseases or even some systematic diseases like endocarditis, pneumonia and cardiovascular diseases[1]. *Streptococcus* species are most prevalent in the oral cavity in which *S. mutans* is the major causative agent for dental caries (tooth decay)[6]. Also, there is some evidence that *S. pyrogenes* due to biofilm formation could be a dental bacteria as well[4]. Nowadays, an occurrence of multidrug- resistant species is a major health problem. Therefore, there is an urgent need for the eradication of pathogen via using some antibacterial agents rather than chemical antibiotics.

More than 4 billion people around the world are using herbal drugs as an alternative for treatment of diseases as herbal drugs have a wide range of pharmacological effect[5, 8]. The current study focused on the effect of *Cuminum cyminum* (cumin) and *carum carvi* (caraway) on *streptococcus mutans* and *Streptococcus pyrogenes*.

Our results demonstrated that the caraway had a better effect on *S. mutans* (MIC=0.625mg/ml) while it had a minor effect of *S. pyrogenes* (MIC=2.5mg/ml). In addition, the cumin had the same effect on both *S. mutans* and *S. pyrogenes* (MIC=0.625mg/ml).

We also evaluate the bactericidal effect of caraway and cumin on *S. pyrogene* and *S. mutans* and the results revealed that the caraway could eradicate the *S. mutans* at 1.25mg/ml concentration while for eradication of *S. pyrogenes* the higher concentration was needed (MBC=5mg/ml). In addition, the cumin had a similar bactericidal effect on *S. mutans* and *S. pyrogenes* (MBC=1.25mg/ml).

Soleimai et al, evaluate the antimicrobial effect of the *cumin* seeds on gram-positive and gram-negative bacteria (*E. coli*, *S. aeruginosa*, *S. aureus*, *S. flexneri*, *B. cereus*, *E. faecalis* and *S. typhimurium*), using disk diffusion method. Their results demonstrated that the cumin had a better inhibitory effect on *E.coli* also; cumin could increase the antibacterial effect of gentamicin. The cumin could be a good alternative for treatment of multidrug resistance pathogens[14].

The minimal inhibitory concentration of three families of *Apaiaceae*, *Fedtsch Bunium perscicum*, *Carum copticum* and *Cuminum cyminum L* on *E.coli*, *S. aureus*, *B. cereus*, *S. enteritidis* and *L. monocytogenes* was evaluated by micro-dilution and Eliza methods. The results showed that Cumin and Bunimum had lower effect in comparison with *Cuminum cyminum* and administrating of both Persian cumin and cumin had a synergism in inhibition of gram-positive bacteria[15].

In addition, one study conducted by Safari et al, the antimicrobial effect of cumin and caraway has been

identified which the MIC of these two seeds was notable inhibition of *Streptococcus iniae*[16].

Mahmoodi et al, evaluated phytochemical composition, antioxidant and antibacterial property of oil extract of *Cumin*um *cyminum* L. the antimicrobial property of extracted oil has been performed using standard serial dilution method and the MIC and MBC of extracted oil were 37.5-9600µg/ml and 150-9600µg/ml respectively. In addition, they demonstrated that the cumin has a better inhibitory effect on gram-positive bacteria. Our result also revealed that the cumin has an inhibitory effect on *Streptococcus pyrogenes* and *Streptococcus mutans*, although the effect of cumin on *S. mutans* was noticeable[17].

Our study had some limitation; we evaluated the antibacterial properties of cumin and caraway oil extract against standard strains of bacteria that it could not show the real mechanism of these extracted oils on patients. In addition, just one concentration (20%) of extraction were administered and it is better to evaluate the different concentration of extracts on bacterial growth. In addition, we recommended that the antibacterial effect of oil extracts compare with relative antibiotics and determine if these oil extracts have a positive effect on the mechanism of antibiotics and in the following research the in vivo studies could be performed to get a better understanding about the effect of oil extracts of *cumin* and *caraway* on patients.

Conclusion

It can be concluded that these extracted oils could be a good alternative in the prevention of oral infection by administrating them into mouth rinse, toothpaste and chewing gums instead of chlrohexidine.

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