

Ibactericidal and Bacteriostatic In Vitro Effects of *Teucrium Chamaedrys* Hydroalcoholic Extract on Two Bacterial Causative Agents of Tooth Decay

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ABSTRACT

Tooth decay is a prevalent dental disease and *Streptococcus mutans* and *Lactobacillus rhamnensis* are the most important bacterial causes of tooth decay. Because of the increased antibiotic resistance of bacteria and the side effects of antibiotics, this study was conducted to investigate the antibacterial effect of hydroalcoholic *Teucrium Chamaedrys* extraction *S.mutans* and *L.rhamnensis*. In this experimental-laboratory study, *T.chamaedrys* was extracted by maceration, and the standard strains of lyophilized *S.mutans* and *L.rhamnensis* were provided from Iranian Research Organization for Science and Technology for investigation of *T.chamaedrys* effect on their growth. The antibacterial effect of hydroalcoholic *T.chamaedrys* was investigated on *S.mutans* and *L.rhamnensis* and then minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The MIC of *T. chamaedrys* was obtained 128 and 32 µg/L against *S.mutans* and *L.rhamnensis*, respectively. The MBC of *T.chamaedrys* was obtained 256 and 64 µg/L against *S.mutans* and *L.rhamnensis*, respectively. *T.chamaedrys* bactericidal and bacteriostatic effects on *L.rhamnensis* and *S.mutans*, two bacterial causative agents of dental cavity show that it might be used for prevention of dental cavity due to these pathogens.

KEY WORDS: Hydroalcoholic extract, *Teucrium chamaedrys*, *Streptococcus mutans*, *Lactobacillus rhamnensis*

1. INTRODUCTION

Tooth decay is a prevalent infectious disease in children (Olak, 2007). Studies have reported that different factors are the risk factors for tooth decay, including oral micro flora and health habits (Gudkina and Brinkmane, 2010). Tooth decay which causes demineralization of oral tissues is a dental bacterial infection (Gudkina and Brinkmane, 2010). Nowadays, certain streptococcal species, such as *Streptococcus mutans*, and some lactobacillus species, including *Lactobacillus rhamnensis*, are considered important causative agents of dental plaques and caries (Thorild, 2002). *L.rhamnensis* and *S.mutans* are gram-positive and obligate anaerobic cocci (Clifford and Stuedevand, 1995). *S.mutans* is the most important and first microorganism known to cause tooth decay, and is present in plaques. To date, over 25 species of *S.mutans* have been demonstrated to cause loss of tooth enamel by producing lactic acid and fermenting sucrose. There has been no project to definitely exterminate tooth decay to date, and dental expenses were estimated approximately 70.3 billion dollars in the USA in 2003. There are over 2000 species of *Teucrium* with confirmed analgesic, anti-inflammatory, anticonvulsant, antipyretic and anti-burn properties, but some of them are toxic (Ansari, 2013, Baradaran, 2013). According to Iranian traditional medicine, *Teucrium Chamaedrys* is used as a disinfectant, bactericidal, and antipyretic agent. *T.chamaedrys* is a perennial, herbaceous plant with many branches, reaches to a height of 10 to 35 cm and is white as cotton. It usually occurs in barren areas, rocky shores and sandy regions across the world. Its leaves are narrow and long. The flowers are white, yellowish white, yellow and even purple. Flowering shoot is the usable part of this plant (Najafi F). *T.chamaedrys* is from Lamiaceae family. There are 12 species of *Teucrium* in Iran, of which three are exclusive to Iran. Because of the increased antibiotic resistance of bacteria and the side effects of antibiotics, the use of medicinal plants and is important. Medicinal plants are considered as a good source for preparation of new drugs (Delfan, 2014, Baharvand-Ahmadi, 2015, Saki, 2014, Nasri and Shirzad, 2013). As well, medicinal plants have several benefits such as low toxicity, low cost, availability, and agreeability to human nature (Sewell and Rafieian-Kopaei, 2014). According to ethnobotanical investigations in Lorestan, Iran, *T.chamaedrys* can be used to prevent tooth decay. Because *S.mutans* is a significant bacterial causative agent of tooth decay and the effect of *T.chamaedrys* has not yet been investigated on *S.mutans*, this study was conducted to investigate the antibacterial effect of hydroalcoholic *T.chamaedrys* extraction *S.mutans* and *L.rhamnensis*.

2. MATERIALS AND METHODS

First, *T.chamaedrys* was provided from Larestan, southern Fars province, southern Iran. After identification and scientific confirmation of the plant, the samples were dried and pulverized. Extraction was done by maceration. For this, 100g of the plant was dissolved with one L of alcohol 80%. The solution was kept in darkness for 72 hours. After filtering with Whatman filter paper 0.5 in duplicate, the solution was distilled with rotary evaporator (vacuum distillation) and then incubated at 37°C. Finally, a honey-thick extract was obtained. A stock solution of this extract was prepared with dimethyl sulfoxide (DMSO).

In this work, the standard strains of lyophilized *S.mutans*, PTCV35668 and ATCC1683, and lyophilized *L.rhamnensis*, PTCC1637 and ATCC53103, were provided from the Iranian Research Organization for Science and Technology.

To prepare microbial suspension, 1.5×10^8 CFU/mL 24-h culture was conducted on blood agar, and then a suspension with 0.5 Mc Farland opacity was prepared in normal saline.

The antibacterial effect of hydroalcoholic *T.chamaedrys* extract was investigated on *S.mutans* and *L.rhamnensis* according to Broth Microdilution technique. Minimum inhibitory concentration (MIC) was tested in 96-well, sterile plates according to two-fold Broth Microdilution technique and the Clinical & Laboratory Standards Institute in triplicate.

First, a stock solution of the extract was prepared. In this work, the serial, two-fold dilutions, from 1 to 500 μ g/mL, were used. The determined dilutions were conducted in 96-well plates in triplicate for each sample. Then, 95 μ L of Mueller-Hinton agar was introduced into each well. Afterwards, 5 μ L of bacterial suspension, equal to 0.5 Mc Farland, and then 100 μ L of serial two-fold dilutions of hydroalcoholic *T.chamaedrys* extract was introduced into all the wells.

The final volume of each well was 200 μ L. After mixing the samples with a shaker at 300 rpm for 20 seconds, we incubated them at 37°C for 18-24 h. Then, optical densities of the samples were read at 450 nm wavelength with an ELISA reader (State Fax 2100, USA). No development of opacity was considered representative of minimum inhibitory concentration (MIC). Minimum bactericidal concentration (MBC) of the ethanolic *T.chamaedrys* extract was determined by Broth Micro dilution. To determine the MBC of each extract, nine sterile test tubes were used. Of these test tubes, eight were used to test different dilutions of each extract and one was considered control. After culture, all of the test tubes were incubated at 37°C for 24 h. After incubation, the tubes were investigated for the opacity due to inoculated microorganism's growth, specimens were taken from all the tubes in which no growth was observed, and cultured for determination of the MBC. The tubes with the lowest concentrations of the extract and no growth in the corresponding plates were considered MBC. The well containing 195 μ L of the extract-free culture medium and five μ L of bacterial suspension was considered negative control. Different concentrations of amikacin besides Mueller-Hinton Broth and five μ L of bacterial suspension were considered positive control.

3. RESULTS

Determination of the MIC and MBC of hydroalcoholic *T.chamaedrys* extract against *S.mutans* and *L.rhamnensis* by Broth Microdilution technique, indicated that two, four, eight, 32, and 64 μ g/L of *S.mutans* were resistant to *T.chamaedrys*, but 128 μ g/L and higher concentrations of *S.mutans* were susceptible to *T.chamaedrys*. Two, four, and eight μ g/L of *L.rhamnensis* were resistant to *T.chamaedrys*, but 32 μ g/L and higher concentrations of *L.rhamnensis* were susceptible to *T.chamaedrys* (Table 1).

Table.1. MIC of *T.chamaedrys* on *S.mutans* and *L.hamnensis*

Type of extract	Microorganism	<i>T.chamaedrys</i> concentration (mg/mL)							
		2	4	8	32	64	128	256	Control
Ethanolic	<i>S.mutans</i>	-	-	-	-	-	+	+	-
		-	-	-	+	+	+	+	-
Ethanolic	<i>L.rhamnensi</i>	-	-	-	+	+	+	+	-

Therefore, the most desirable MIC was obtained 128 and 32 μ g/L against *S.mutans* and *L.rhamnensis*, respectively. The most desirable MBC was obtained 128 and 64 μ g/L against *S.mutans* and *L.rhamnensis*, respectively (Table 2).

Table.2. MBC of *T.chamaedrys* on *S.mutans* and *L.rhamnensis*

Type of extract	Microorganism	<i>T.chamaedrys</i> concentration (mg/mL)							
		2	4	8	32	64	128	256	Control
Ethanolic	<i>S.mutans</i>	-	-	-	-	-	-	+	-
		-	-	-	-	+	+	+	-
Ethanolic	<i>L.rhamnensi</i>	-	-	-	-	+	+	+	-

DISCUSSION

As previously stated, *S.mutans* and *L.rhamnensis* are the most important causative agents of tooth decay. Tooth decay is indeed an infectious-microbial disease that causes demineralization of calcareous oral tissues. Tooth decay occurs in a specific site of tooth when the population of *S.mutans* bacteria reaches to 50% of all bacterial population in that site. Therefore, changes in oral microbial ecology are considered important to prevent tooth decay by decreasing *S. mutans* and *L.rhamnensis* populations.

Acidogenic bacteria, such as *S.mutans*, increase tooth decay dramatically (Fabricant and Farnsworth, 2001). In the present study, hydroalcoholic *T.chamaedrys* extract exerted limited in vitro antibacterial effects on *S.mutans*.

In contrast, in a study on methanol and oil extracts of *Ziziphora clinopodioides*, the methanol *Z. clinopodioides* extract exerted inhibitory effects on the growth of a number of gram-positive and negative bacteria (Chitsaz, 2006). A study on in vitro effects of polyphenols in some plants, including tea, demonstrated that these effects were dose-dependent on some bacterial species (Taguri, 2004). However, in the present work, the MIC of *T.chamaedrys* was determined higher than 128 and 32 µg/mL against *S.mutans* and *L.rhamnosis*, respectively.

Investigations on antibacterial and antifungal properties of hydroalcoholic extract of *Salvia officinalis* leaves, *Mentha pulegium*, and *Pimpinella anisum* demonstrated that these plants exerted stronger antibacterial effects than *T.chamaedrys* on bacterial causative agents of tooth decay, as the MICs of *S. officinalis*, *M. pulegium*, and *P. anisum* were derived 6.25, 12.5, and 12.5 µg/mL against *S.mutans* and 1.56, 3.12, and 12.56 µg/mL against *L.rhamnosis*, respectively (Kermanshah H). Moreover, a study on green and black tea extracts demonstrated that both types of teas had bactericidal property, but bactericidal property of black tea was greater on oral Streptococci than green tea (Hamdi, 2008). In contrast, the present work demonstrated that hydroalcoholic *T.chamaedrys* extract exerted a smaller inhibitory effect on the bacterial causative agents of tooth decay compared to the plants investigated in that study (Hamdi, 2008). Regarding the findings of the present study and other works, we can argue that *T.chamaedrys* hydroalcoholic extracts has antibacterial effects on *S.mutans* and *L.rhamnosis* and might be beneficial for prevention of tooth decay. The antimicrobial mechanism of these plants is not clear. Phenolic compounds which are abundant in this plant have antimicrobial activity (Sharafati-Chaleshtori and Rafieian-Kopaei, 2014, Bahmani, 2014, Chaleshtori, 2011, Karamati, 2014, Rahimian, 2013) and these compounds might be responsible for the plant effect. There are numerous plants which possess phenolic compounds (Shanafelt, 2002; Sarrafchi, 2016; Bahmani, 2016; Shayganni, 2015; Rahimi-Madiseh, 2016; Taherikalani, 2016). These plants also might have antimicrobial activities which should be examined.

4. CONCLUSION

Antibacterial effects of hydroalcoholic *T.chamaedrys* extract on *S.mutans* and *L.rhamnosis* causative agents of tooth decay is limited. Hence, regarding the limited antibacterial effect of hydroalcoholic *T.chamaedrys* extract on *S.mutans* and *L.rhamnosis*, and consequently tooth decay, we recommend that other bactericidal plants be investigated in future works to offer an economical treatment, with minimal side effects, for tooth decay. Moreover, future studies are recommended to investigate effects of simultaneous use of this plant and different concentrations of other bactericidal plants, and compare these plants' effects and antibiotics'.

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