

Original Article

## In vitro Investigation of Hydro-alcoholic Extract of *Helichrysum leucocephalum* on the Inhibition of *Streptococcus Mutans* Growth

Motamedifar M<sup>a\*</sup>; Nozari A<sup>b</sup>; Azhdari Ghasrodashti E<sup>c</sup>

<sup>a</sup>Department of Bacteriology, Shiraz Medical School & Shiraz HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>b</sup>Department of Pediatric Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

<sup>c</sup>Student Research Committee, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran

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#### Corresponding Author:

Mohammad Motamedifar

Department of Bacteriology,

Shiraz Medical School,

Shiraz University of Medical

Sciences, Shiraz, Iran.

Email: [motamedm@sums.](mailto:motamedm@sums.ac.ir)

[ac.ir](http://sums.ac.ir)

Tel/fax: +98-71 3230 4365

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### Abstract

**Statement of Problem:** Indiscriminate use of antibacterial agents increases the antibiotic resistance, which consequently necessitates seeking alternatives such as herbal remedies.

**Objectives:** The aim of this study was to evaluate the antimicrobial effect of hydroalcoholic extract of *Helichrysum leucocephalum* (*H.leucocephalum*) on *Streptococcus mutans* (*S. mutans*) growth as a major cause of dental caries.

**Materials and Methods:** In this study, hydroalcoholic extract of *H. leucocephalum* was prepared. The antibacterial effects, minimal inhibitory concentration (MIC), and minimal bactericidal concentration (MBC) of hydroalcoholic extract of *H. leucocephalum* and penicillin were assessed. Agar well diffusion method and micro broth dilution assay were used on bacterial suspension adjusted to a 0.5 McFarland standard (equivalent to  $1.5 \times 10^8$  CFU/mL). Each test was repeated four times to minimize lab errors.

**Results:** In this study, the inhibitory zone of hydroalcoholic extract of *H.leucocephalum* in concentration of 100 mg/mL was  $34 \pm 0.1$  mm and for penicillin was 10 mm. The MIC and MBC of *H.leucocephalum* hydroalcoholic extract were  $5.6 \pm 6.25$ ,  $21.6 \pm 6.25$  mg/mL, respectively.

**Conclusions:** Hydroalcoholic extract of *H. leucocephalum* have antibacterial effect on *S.mutans* at a concentration of 12.5mg/mL, indicating possible application of this extract in prevention of dental caries; however, future researches are necessary.

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## Introduction

Dental caries or tooth decay is one of the most important infective diseases of people in the world with multifactorial etiology. A wide group mixture of microorganisms including *S. mutans* has a great role in dental caries [1].

*S. mutans* is recognized to be a highly cariogenic microorganism in humans [2]. It is gram-positive cocci and encapsulated bacterium that adheres to the enamel surface and produces different glucans and acidificate dental plaque. The presence of these glucans is critical in the development of dental caries [3]. One approach to decrease the incidence of dental caries is to develop therapeutic agents with antimicrobial and/or anti adherent properties to inhibit the bacterial proliferation on the tooth surface.

The rising use for herbal drugs have so far directed the consideration of pharmaceutical scientists towards medicinal plants as traditional sources of drugs for the treatment of different diseases [4]. Among various families of plants studied, the genus *Helichrysum* of *Asteraceae* family deserves some noticeable interests. It is one of the largest genera in the family *Asteraceae* includes around 600 species [5,6]. *Helichrysum* is known as the “immortal, everlasting flower”. The distribution of 19 species in Iran, growing on the mountainous area with clay soil, calcareous rocks, dry slopes and steppe areas is reported [7]. The *Helichrysum* oil inhibits microbial growth and protects the body against a long list of febrile microbial infections. *Helichrysum leucocephalum* (*H. leucocephalum*), one of the six endemic species of the country is the subject of this study. *Helichrysum* species have been so far demonstrated to have diverse therapeutic properties such as hepato-protective, anti-allergic, antichlorotic, anti-diarrheal and antidiabetic effects. These remarkable properties in *Helichrysum* species is attributed to the presence of flavonoids as their principal constituents [8]. This plant is also used in folk medicine for the treatment of kidney stone and gall bladder disorders [9]. Various studies clearly indicated antidiabetic, antioxidant, anti-lipid peroxidation and antimicrobial effect of other *Helichrysum* species; however, there is not any published report on anti-microbial effect of *H. leucocephalum* on

*S. mutans* [10,11]. The aim of this study was to evaluate the antimicrobial effect of hydroalcoholic extract of *H. leucocephalum* on *S. mutans* growth as a major pathogen of dental caries.

## Materials and Methods

This experimental study was done in Department of Bacteriology and Virology of Shiraz University of Medical Sciences, Shiraz, Iran. Standard strain of *S. mutans* (ATCC 35668) was purchased from Iranian Research Organization for Science and Technology, Tehran, Iran.

Different parts of *H. leucocephalum* were collected from areas around Shiraz, Fars province, Iran, 2017. Herbal number of the plant was proved and a voucher number (PM937) was received from Shiraz school of pharmacy. All parts of the plant were dried at room temperature for 2 weeks. Then were ground into a fine powder. Hydroalcoholic extract was processed by percolation (about 72h). The plant powder (200g) was extracted with hydroalcoholic solution (70% and 96%, 500mL) using a percolator. The extract was concentrated in rotary evaporator to separate alcoholic part and then, it was kept in refrigerator until used [12].

Agar well diffusion technique was used to assess the antimicrobial effect of hydroalcoholic extract of *H. leucocephalum* on *S. mutans* growth. First, *S. mutans* was cultured in blood agar for 24 hours. Then, a bacterial suspension with 0.5 McFarland turbidity ( $1.5 \times 10^8$  cfu/mL) was adjusted in brain heart infusion (BHI) broth (Merck, Germany). By using a sterile cotton swab, the bacterial suspension was applied on Muller Hinton agar (MHA, Merck, Germany) with 5% blood sheep. The holes were prepared by using Cork borer in MHA (6mm in diameter and 4mm in height with 25mm distance). Each well was filled by 100 $\mu$ L of different concentrations of hydroalcoholic extract of *H. leucocephalum* (3.125-50 mg/mL). One hole was filled with 10U/ml of penicillin as positive control. The plates were incubated at 37°C in 5% incubator for 24 hours. Finally, inhibition zones were measured in millimeter [13]. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of hydroalcoholic extract of *H. leucocephalum*, micro broth dilution method was used. First, 100 $\mu$ L of

BHI were added to each well of 96-well micro plates. Then, 100 $\mu$ L of highest concentration of extract (100 mg/mL) was added to the first well. Other wells were filled with two fold lower concentrations until the seventh well, respectively (3.125-50 mg/mL). One well contained just BHI medium as negative control and one of the wells contained BHI and bacterial suspension as the control with bacterium. The plates were incubated at 37°C in 5% CO<sub>2</sub> incubator for 24 hours. The first well in those series that showed no sign of visible growth of bacteria was considered as MIC. The MBC was determined by culturing 10 $\mu$ L of contents of wells that did not show any sign of bacterial growth on blood agar. The plates were incubated for 24 hours at 37°C in 5% CO<sub>2</sub> incubator. The least concentration that inhibited colony forming of *S. mutans* on agar was considered as MBC. All data were expressed as descriptive data by SPSS (mean and standard deviation) [14].

## Results

The anti-bacterial effects of (3.125-50 mg/mL) concentrations of hydroalcoholic extract of *H.leucocephalum* and penicillin as a control group were tested by using well diffusion agar technique.

The results of this study showed that 100 mg/mL concentration of extract, which created zones with the mean diameter of 34 $\pm$ 0.1 mm, had the highest antibacterial effect on *S. mutans* compared to other concentrations. The mean inhibition zones for 50 mg/mL were 21 $\pm$ 0.10 mm and the lower concentrations could not inhibit the growth of *S.mutans*. The mean diameter of zones created by penicillin was 10 mm (Table 1).

MIC of the extract on *S. mutans* was 15.6 $\pm$ 6.25

mg/mL and MBC was 21.6 $\pm$ 6.25 mg/mL. MIC and MBC for penicillin were detected both 5 U/mL.

## Discussion

There is a considerable interest in many kinds of plant essential and extracts as the antibiotic alternatives. The results of this study showed that *H.leucocephalum* extract could inhibit the growth of *S.mutans* as the most important microorganism involved in dental caries. Various medicinal and antimicrobial properties of this plant were studied in many investigations. Preliminary research evidenced bactericidal activity of *H. leucocephalum* extracts, as also reported that antimicrobial screening revealed higher degree of inhibition for essential oil of the aerial parts of *H. leucocephalum* against *Staphylococcus aureus* and *Escherichia coli* both with MIC value of 16 $\mu$ g/mL [15]. In the *Helichrysum compactum* species, the antimicrobial activity has been attributed to flavonoids and chemically related compound [16]. The data obtained in the present study on the specific activity of *H.leucocephalum* extracts against *S.mutans* are in agreement with the results previously reported for *Helichrysum italicum* by Nostro *et al.* [17]. Another in vivo study has shown that a regular daily rinsing with mouthwash containing *Helichrysum litoreum* ethanol extract could reduce on 50% of subjects the salivary levels of *S.mutans*, which are the most virulent cariogenic pathogens in the oral cavity [18]. Antioxidant activities have been previously detected in another *H. pseudoplicatum* Nab [19].

The antibacterial activity of *Helichrysum* phenolics including coumarates, benzofurans, pyrones, and heterodimeric phloroglucinols was evaluated in a

**Table 1: The effect of hydroalcoholic extract of *H.leucocephalum* on *S. mutans* by agarwell diffusion method**

Extract Concentration (mg/mL)	Inhibition zone Mean $\pm$ Standard deviation (mm)
100	34 $\pm$ 0.1
50	21 $\pm$ 0.10
25	0
Penicillin (10 U/mL)	10

study showed potent antibacterial effect against multidrug-resistant *Staphylococcus aureus* isolates [20]. The essential oil of *H. arenarium* had a significant anti-yeast activity ( $P < 0.05$ ); therefore, it can be used as an antifungal agent in the food and pharmaceutical industries [21]. Using the same method (micro broth dilution) as the current study, the essential oil of *Helichrysum aureonitens* in South Africa indicated antimicrobial activity against gram positive and gram negative bacteria with MICs in the range of 2.500–0.039 mg /mL [22]. The results of another study indicated that the extract of *H. plicatum* had significant antimicrobial activity [23]. Inhibition potential of *H. buddleioides* extract was indicated on gram positive bacteria such as *Staphylococcus aureus* [24].

Concentrations of 100 and 50 mg/mL of the extract made inhibition zones of bacterial growth around the wells while other concentrations could not create inhibition zone. As evidenced, the hydroalcoholic extract of *H. leucocephalum* exhibits significant antimicrobial activity against *S. mutans* and therefore could be considered as a source of natural antimicrobial compounds, but studies are necessary.

## Conclusions

The results of antibacterial assays in the current study might be helpful in developing useful products for inhibiting the progress of dental caries and pharmaceutical purposes. However, future researches are necessary.

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**Conflict of Interest:** None declared.

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