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## Original Article

# Antibacterial Activity of Different *Artemisia dracunculus* Extracts Against Dental Caries-Related Pathogens

Bitā Arabestani<sup>1</sup> , Laleh Babaeekhou<sup>1\*</sup> , Maryam Ghane<sup>2</sup>

<sup>1</sup>Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

<sup>2</sup>Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

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## \*Corresponding author:

Laleh Babaeekhou,

Emails: babaeekhou@iaau.

ac.ir, lamid21@yahoo.com



## Abstract

**Background:** This study investigated the antibacterial properties of four types of tarragon (*Artemisia dracunculus*) extracts against two caries contributing bacteria, namely, *Streptococcus mutans* and *Streptococcus sobrinus*.

**Methods:** The extracts of *A. dracunculus* were prepared using n-hexane, ethyl acetate, methanol, and water solvents. In addition, the diameter of inhibition zones, minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) were determined, and finally, MTT (tetrazolium-based colorimetric assay) was used to analyze the cytotoxic effects of the extracts.

**Results:** The well-diffusion method showed the antibacterial property of four tested extracts against bacteria. Methanol and water extracts made the highest inhibition zone diameters ( $P < 0.001$ ). This was true for both tested bacteria. The MIC of the methanol, water, n-hexane, and ethyl acetate extracts were 0.78, 1.5, 3.1, and 1.5 mg/mL, as well as 0.78, 0.78, 1.5, and 1.5 mg/mL against *S. mutans* and *S. sobrinus*, respectively. The half-maximal inhibitory concentration (IC50) values for methanolic, aqueous, ethyl acetate, and n-hexane extracts were 0.78, 0.78, 1.56, and 3.12 mg/mL, respectively.

**Conclusions:** The results provided a rational reason for the traditional use of *A. dracunculus* extracts against anti-caries-related bacteria. The methanolic extract demonstrated better activity, thus methanol can probably extract a wider range of plant compounds with antibacterial effects.

**Keywords:** *S. mutans*, *S. sobrinus*, *A. dracunculus*, Extract, Methanol

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## Background

The bacterial plaque has a critical and primary role in the pathogenesis of dental caries. It is highly accepted that mutans streptococci (MS), including *Streptococcus mutans* and *Streptococcus sobrinus*, contribute to caries. These common oral pathogens have been isolated from human dental plaques, and their distribution has been investigated in epidemiological studies (1,2). According to the results of some studies, higher scores of decayed, missing, and filled teeth (DMFT) are reported in the co-presence of MS in mouth microflora (1,3-5). It is reported that 4-6 years old children with MS in plaque samples have higher scores of DMFT compared to those with *S. mutans* alone (2). This result is confirmed by studies on 6-30 years old individuals and pre-school/school children (1,3). Accordingly, the growth inhibition of both mentioned bacteria can be a rational strategy for caries control. It is shown that natural compounds derived from plants, including the plant extracts of *Zingiber officinalis* and *Polygonum cuspidatum*, can inhibit the growth and virulence of *S. mutans* and *S. sobrinus* (6-8). The genus

*Artemisia* from the Asteraceae family consists of more than 350 species, among which *Artemisia dracunculus* is native to Asia and has a wide application in food recipes or salads (9). The aerial parts of *A. dracunculus* have traditionally been used as antitumor, immunomodulator, antimutagen, and antioxidant, and have antibacterial and antifungal properties (10). Regarding the antimicrobial activity of *Artemisia*-derived compounds, Juteau et al reported that the essential oil (EO) of *A. annua* aerial parts can inhibit the growth of *Enterococcus hirae*, *Candida albicans*, and *Saccharomyces cerevisiae* (11). In a similar study, Behbahani et al (12) evaluated the antibacterial and antifungal effects of the *A. dracunculus* extract against *Candida albicans*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. It is accepted that extraction outcomes can change based on different factors, including solvent polarity. The extraction yield of phenolic and flavonoid contents and the antimicrobial capacity of the extracts greatly depend on solvent polarity (13), and more investigations should be conducted to identify the impact of solvents on the antimicrobial activity of plants. Hence,



to the best of our knowledge, this is the first study to investigate and compare the antibacterial activity of four types of *A. dracuncululus* extracts, including methanolic, aqueous, ethyl acetate, and n-hexane extracts against *S. mutans* and *S. sobrinus*. The half-maximal inhibitory concentration (IC<sub>50</sub>) value of each extract was determined in the second part of the study.

## Methods

### MS Strains

*Streptococcus mutans* PTCC 1601 and *S. sobrinus* PTCC 1683 were obtained from the Iranian Research Organization for Science and Technology (IROST). The MS bacteria were cultured anaerobically on TYCSB agar (Tryptone Yeast Cysteine Sucrose agar with 0.2 U/mL Bacitracin, Merck, Germany) at 37°C for 24 hours.

### Plant Extraction

*Artemisia dracuncululus* was prepared from the Medicinal Plants and Drugs Research Institute (MPDR) of Shahid Beheshti University of Iran. The fresh leaf of the plant was dried, and the plant extract was obtained by the maceration method (14). The plant's names were checked in <http://www.theplantlist.org>, and genus and species were confirmed by macroscopic and molecular analyses. In this study, four solvents, namely, water, methanol, n-hexane, and ethyl acetate (Merck, Germany) were used for the extraction procedure. Further, 200 g of powdered *A. dracuncululus* leaves were separately dissolved in 150 mL of the solvents. Next, the samples were placed in a shaker incubator for 24 hours (at 30°C). The suspensions were then filtrated using the filter paper (Whatman No. 1) 4 times, and the insoluble parts of suspension were taken accordingly. A vacuum rotary machine (Heidolph, Germany) was applied to remove the solvents from the extracts, and the concentrations of 400 mg/mL were prepared with 1% dimethyl sulfoxide (DMSO, Merck, Germany). Finally, the extracts were sterilized using bacteriological filters and stored in the refrigerator (-30°C) for microbial tests (14).

### Antibacterial Activity

#### Well Diffusion Methods

All extracts were tested for antimicrobial activity by the well diffusion method (15). For the test, the overnight culture of MS bacteria in the TYCSB broth was employed as bacterial inocula and adjusted to contain 1.5×10<sup>8</sup> CFU/mL bacterial cells (0.5 McFarland standard) with sterile phosphate-buffered saline (pH: 7.2). Subsequently, 10 μL of the bacterial suspension was spread entirely over Mueller-Hinton II agar (Merck, Germany) plates, and the wholes with 6-8 mm diameter were punched with a sterile Cork borer. Next, 100 μL of 25, 50, and 100 mg/mL of each extract solution was introduced into the wells, and the inhibition zones were measured after incubation at 37°C

for 24 hours. In this study, 1% dimethyl sulfoxide (DMSO, Merck, Germany) and 25 μg/mL Ampicillin (AppliChem, Germany) were used as negative and positive controls, respectively.

### Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations of the Extracts

The microdilution method was employed for MIC and MBC evaluation (16). Two-fold serial dilutions of the plant extracts (50-0.18 mg/mL) were prepared in the TYCSB broth. Next, 1% DMSO was added to the medium to increase the solubility of the extracts. Then, 100 μL of the solutions and 2 μL of the bacterial inocula (~1.5×10<sup>8</sup> CFU/mL) were added to each well (96-well microtiter plates; SPL, South Korea). DMSO-medium and bacteria without extracts were applied as a negative control. After anaerobically incubation (37°C for 24 hours), the lowest concentration of the extract with no growth turbidity was considered as the MIC. For MBC determination, 5 μL of each well with no turbidity was transferred on the TYCSB agar and incubated anaerobically (37°C for 24 hours). The concentration was considered MBC if the growth was decreased by ≥ 99.9%.

### Cytotoxicity Assay

Cytotoxicity assessment was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (17). RPMI-1640, in addition to 10% fetal bovine serum -streptomycin-penicillin (Gibco, UK), was used as a medium to culture the Vero cells, which were incubated for 24 hours at 37°C in humidified air containing CO<sub>2</sub> (5%). After the distribution of the cells in 96-well plates and at the cell density of 2×10<sup>4</sup> to 2×10<sup>5</sup> per well, they were incubated for the same above-mentioned time and condition. Thereafter, the extracts at different concentrations (0.39-12.5 mg/mL) were added and the plates were incubated for an additional 24 hours under the same conditions. Afterward, the cells were treated with a 10 μL MTT dye solution (5 mg/mL) for 4 hours (at 37°C). The absorbance was measured (490 nm) after the removal of the MTT dye solution and the addition of 100 μL of DMSO (Merck, Germany) using a microplate reader (ELx808, BioTek, USA). The concentration of extracts with a 50% cell growth inhibition (IC<sub>50</sub>) was considered cytotoxic. All tests were performed in triplicate, and the mean values were recorded accordingly. Cells treated with 0.1% DMSO were employed as the control group (17).

### Statistical Analysis

The assays were performed in triplicate. The mean and standard deviation were calculated, and data were processed for analysis using SPSS software (version 20.0/PC; Chicago, IL, USA). A comparison of values and means of the zones of inhibition was performed by the Tukey

HSD test, and a  $P < 0.05$  was considered to be statistically significant.

## Results

### Antibacterial Activity

#### Well Diffusion Methods

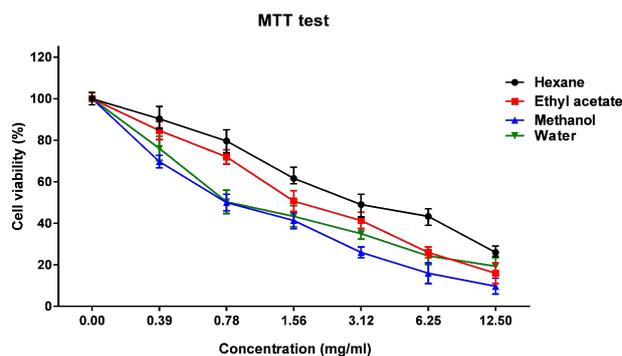
According to the results of the well diffusion, all the studied extracts in the tested concentrations (25, 50, and 100 mg/mL) produced growth inhibition zones against *S. mutans* and *S. sobrinus*. For both tested bacteria, methanolic extracts produced a significantly higher mean of the zone of inhibition ( $P < 0.001$ ) compared to ethyl acetate in all tested concentrations (Table 1). There was no significant difference between aqueous and methanolic extracts in the zone of inhibition ( $P = 0.22$ ). The aqueous extract significantly ( $P < 0.001$ ) produced a larger zone in comparison with ethyl acetate and n-hexane in 50 and 100 mg/mL concentrations, and no significant difference was observed between n-hexane and ethyl acetate ( $P = 0.34$ ). The correspondence inhibition zone of these extracts is presented in Table 1.

#### MIC and MBC Values

Table 2 provides the results of the MIC and MBC for all the extracts against *S. mutans* and *S. sobrinus*. The results showed that the most antibacterial activity against the two studied bacteria was related to water and methanolic extracts ( $P < 0.001$ ), while the least effect belonged to the n-hexane extract ( $P < 0.001$ ). These results confirmed the finding of the well diffusion test.

#### Cytotoxic Concentrations

MTT was applied to evaluate the effect of plant extracts on cell viability. The obtained results revealed a dose-dependent reduction of cell viability (from 0.39 to 12.5 mg/mL) by the extracts. IC50 values for methanolic, aqueous, ethyl acetate and n-hexane were 0.78, 0.78, 1.56, and 3.12 mg/mL, respectively. As shown in Figure 1, the most cytotoxic effect against Vero cells is related to the methanolic and aqueous extracts and the least to the n-hexane.



**Figure 1.** Viability of the Vero Cells in Different Extract Concentrations of *Artemisia dracunculus*. Note. MTT: Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide.

## Discussion

According to reports, different *Artemisia* species have a wide range of biological properties in traditional medicine and have been used as anti-malarial, anthelmintic, antidiabetic agents, and in treating wounds, bronchitis, ulcers, and tuberculosis (18). Higher scores of DMFT are reported in the co-existence of MS in mouth microflora (1, 3-5). Thus, the present study focused on evaluating the antibacterial activity of different extracts of *A. dracunculus* against dental pathogens (*S. mutans* and *S. sobrinus*). In this study, the extraction procedure was performed using water, methanol, n-hexane, and ethyl acetate solvents, and antimicrobial assay results confirmed the antibacterial activity for all extract types against *S. mutans* and *S. sobrinus*. The water and methanolic extracts with a MIC of 0.78 mg/mL indicated more anti-MS activity, and the lowest antibacterial activity was observed for the n-hexane extract (3.1 mg/mL,  $P < 0.001$ ). The difference in the antibacterial activity of the extracts can be related to different polarities of the solvents which can affect the variety of the extracted compounds (19). Considering that the biological activities of plant extracts are completely associated with their components, the extraction solvent type can directly affect the extract's activities (20). Oliveira et al focused on *Corylus avellana* and evaluated the effects of solvents with different polarities on the outcome of the

**Table 1.** Zone of Inhibition Means Yielded From *Artemisia dracunculus* Extract Effects on *Streptococcus mutans* and *Streptococcus sobrinus*

Extracts (mg/mL)	Mean ± Standard Deviation (mm)							
	<i>Streptococcus mutans</i>				<i>Streptococcus sobrinus</i>			
	25	50	100	P Value	25	50	100	P Value
N-hexane	21.5±0.5 <sup>abA</sup>	21.9±0.66 <sup>ba</sup>	23.03±0.68 <sup>ba</sup>	0.173	21.5±0.4 <sup>ba</sup>	21.9±0.66 <sup>ba</sup>	23.03±0.68 <sup>ba</sup>	0.221
Ethyl acetate	21.07±0.7 <sup>bb</sup>	21.97±0.85 <sup>baB</sup>	23.57±0.67 <sup>ba</sup>	0.016	21.4±0.6 <sup>bb</sup>	22±0.89 <sup>baB</sup>	23.6±0.56 <sup>ba</sup>	0.017
Methanol	23.43±0.61 <sup>ab</sup>	24.97±0.16 <sup>aAB</sup>	26.13±0.65 <sup>aA</sup>	0.009	23.4±0.6 <sup>ab</sup>	25±0.26 <sup>ab</sup>	27.03±0.55 <sup>aA</sup>	<0.001
Water	22.37±0.85 <sup>abb</sup>	24.93±0.8 <sup>aA</sup>	26.47±0.5 <sup>aA</sup>	0.001	22.5±0.5 <sup>abb</sup>	24.03±0.45 <sup>ab</sup>	26.5±0.36 <sup>aA</sup>	0.002
P value	0.021	<0.001	<0.001		0.024	<0.001	<0.001	

Note. The comparison of the means of the zones of inhibition by Tukey HSD test. Means followed by the same lower-case letters in a column and capital letters on the lines do not significantly differ by the Tukey test ( $P < 0.05$ ). The mean of the zones of inhibition by 100 µL ampicillin (25 µg/mL) as a positive control was 22±0.45. No zone of inhibition was observed in the well containing 100 µL of 1% DMSO as a negative control.

**Table 2.** MIC and MBC (mg/mL) of Different Extracts of *Artemisia dracunculus* Against *Streptococcus mutans* and *Streptococcus sobrinus*

Bacterium	Extract	MIC	MBC
<i>Artemisia dracunculus</i>	N-hexane	3.1 <sup>c</sup>	6.2 <sup>d</sup>
	Ethyl acetate	1.5 <sup>b</sup>	3.1 <sup>c</sup>
	Methanol	0.78 <sup>a</sup>	1.5 <sup>b</sup>
	Water	1.5 <sup>b</sup>	3.1 <sup>c</sup>
	<i>P</i> value	<0.001	<0.001
<i>Streptococcus sobrinus</i>	N-hexane	1.5 <sup>b</sup>	3.1 <sup>c</sup>
	Ethyl acetate	1.5 <sup>b</sup>	3.1 <sup>c</sup>
	Methanol	0.78 <sup>a</sup>	1.5 <sup>b</sup>
	Water	0.78 <sup>a</sup>	1.5 <sup>b</sup>
	<i>P</i> value	<0.001	<0.001

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration. Values followed by the same lower-case letters in a column do not differ significantly (Tukey HSD test,  $P < 0.05$ ).

extraction. They revealed that they could increase the number of extracted phenolic compounds and improve the antioxidant and antibacterial activities of the extracts by changing the type of the solvent (21). Principally, the influencing factors on the biological activity of the plant extract are the methods of drying and extraction and the type of the solvent (20). Our study results on *A. dracunculus* (tarragon) demonstrated that among the tested solvents, water and methanol were better options for the extraction of compounds with antimicrobial properties.

Erdogrul et al (22) examined the antibacterial activities of methanol, ethyl acetate, acetone, and chloroform extracts from four plant species against 12 bacterial species and concluded that the most antimicrobial property in *Artemisia* is related to the ethyl acetate extract. The results of the mentioned study are not in agreement with those of our study, and this may be related to the extraction procedure, the tested bacteria, and the type and number of various components of the plant extracts. Previous studies declared that *A. dracunculus* is mainly composed of phenolic (e.g., carvacrol,  $\gamma$ - and  $\alpha$ -terpinene) and flavonoid contents, and it is well accepted that extracts and EOs rich in terpinene and phenolic components can represent noticeable antimicrobial activities (20). Likewise, Raesi et al (23) investigated the antibacterial effects of the *A. dracunculus* EO on *Escherichia coli* and *S. aureus*. In this study, the observed MIC for *E. coli* and *S. aureus* were 2.5 and 1.25 mg/mL, respectively. They further reported that all the EO concentrations for each tested bacterium reduced bacterial count in cheese samples compared to the control (23). Similarly, Mohammadi et al (24) confirmed the anti-quorum sensing and anti-biofilm activities of the *A. dracunculus* EO on *S. aureus* and *Salmonella enterica* serovar Typhimurium.

The safety of plant extracts toward eukaryotic cells should be evaluated since the results of studies on these extracts can be used for complementary development to be consumed by humans. The  $IC_{50}$  of various concentrations

of *A. dracunculus* extracts on the eukaryotic Vero cells was investigated in this study.  $IC_{50}$  values for the tested extract were 0.78 mg/mL for methanolic and aqueous, as well as 1.56 and 3.12 mg/mL for ethyl acetate and n-hexane extracts, respectively. To the best of our knowledge, there is no such report that could highlight the comparative cytotoxic effect of various *A. dracunculus* extracts. In their study, Motamedifar et al (25) compared the anti-*S. mutans* activity of *Peganum Harmala L.* with 0.2% chlorhexidine, and then investigated the cytotoxicity of the ethanolic extract of the plant on Vero cells by MTT. The concentrations of more than 0.5 mg/mL caused over 50% of cell destruction. However, the anti-*S. mutans* activity of the studied plant was lower compared to *A. dracunculus*.

## Conclusions

The results of this study confirmed the strong anti-*S. mutans/S. sobrinus* property of all types of *A. dracunculus* extracts. However, considering higher inhibition zone diameters and lower MIC/MBC values of the methanolic and aqueous extracts, these solvents can extract more effective components from the plant. MTT results showed the viability of the Vero cells after exposure to all extract types in bacteriostatic concentrations. Compounds with synergistic activities can also be used to decrease the MIC of the extracts. Thus, *A. dracunculus* extracts are potential candidates for further analysis and use in the development of herbal medicinal compounds against dental caries.

## Author's Contribution

**Conceptualization:** Laleh Babaeekhou.

**Data curation:** Laleh Babaeekhou, Maryam Ghane.

**Formal analysis:** Laleh Babaeekhou, Maryam Ghane.

**Funding acquisition:** Laleh Babaeekhou, Maryam Ghane.

**Investigation:** Laleh Babaeekhou, Maryam Ghane.

**Methodology:** Laleh Babaeekhou, Maryam Ghane.

**Project administration:** Bitra Arabestani, Laleh Babaeekhou, Maryam Ghane.

**Resources:** Laleh Babaeekhou, Maryam Ghane.

**Software:** Laleh Babaeekhou, Maryam Ghane.

**Supervision:** Laleh Babaeekhou.

**Validation:** Laleh Babaeekhou.

**Visualization:** Laleh Babaeekhou, Maryam Ghane.

**Writing—original draft:** Laleh Babaeekhou.

**Writing—review & editing:** Laleh Babaeekhou.

## Competing Interests

None.

## Ethical Approval

Not applicable.

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None.

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