

In-vitro antibacterial effects of *Artemisia* extracts on clinical strains of *P. aeruginosa*, *S. pyogenes*, and oral bacteria

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Abstract

Background and objective: Some common problems in the health care system are Microbial resistance to antibiotics, the side effects of food additives, and preservatives. Considering the antibiotic resistance of microorganisms and the need to identify new compounds, the present study was conducted to determine the antimicrobial effects of *Artemisia* extracts.

Material And Method The study was performed in two stages including extraction and determination of antibacterial properties of aqueous, ethanolic, methanolic, acetone/ethanolic, and hydroethanolic extracts of *Artemisia* on standard *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and oral bacteria sample. The well diffusion method was performed to evaluate the concentration that had an inhibitory and bactericidal effect.

Result: Aqueous extract had an effect on the oral bacteria sample (8 mm), the methanolic extract had an effect on *S. pyogenes* and oral bacteria sample (15 mm) and finally acetone/ethanolic extracts had antimicrobial properties against *S. pyogenes* (16 mm). The concentration used for all five extracts was 50 mg/ml and showed an inhibition effect on the growth of *S. pyogenes* standard strain and oral bacteria sample. The less serial dilutions of extracts were tested but no antibacterial effects were seen. So, 50 mg/ml was the minimum concentration that had an inhibitory and bactericidal effect.

Conclusion: It can be inferred that aqueous, methanolic, and acetone/ethanolic extracts of *Artemisia* had the highest inhibitory effect on *S. pyogenes* and the oral bacteria sample. Consequently, by applying different extraction methods and by utilizing different solvents, it may be possible to more efficiently obtain biomaterials with antimicrobial properties from this plant.

Keywords: herbal antibacterial effects, *Artemisia* extracts, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, oral bacteria.

Introduction

In the new century, the tendency of consumers to use fewer chemicals and preservatives has led to extensive research on the benefits and harms of using natural compounds with antimicrobial properties. Further, the resistance of microorganisms to antibiotics poses problems that have become one of the major concerns of human society and the health care system (1). With the advent of antibiotic-resistant bacteria, the effectiveness of existing drugs has diminished, increasing the failure of antimicrobial treatments. In addition, the widespread use of industrial drugs, as well as the misuse of these drugs, can lead to many side effects (2,3). As a result, one way to prevent the development of pathogenic bacteria resistant to antibiotics is to use new compounds that are fundamentally different from the existing industrial source in terms of basic structure with antimicrobial drugs (4). Plants produce secondary metabolites including alkaloids, flavonoids, tannins, glycosides, terpenes, and phenolic compounds. Some of these compounds have complex molecular structures and antimicrobial properties, therefore, in recent years, there has been a strong desire to discover and introduce antimicrobial agents of plant origin (5). Knowledge of chemical compounds in plants is important not only for the discovery of medicinal drugs but also because the use of chemical products of plant origin that have antimicrobial properties help control resistant strains (6). In this regard, the World Health Organization has recommended the use of natural drugs as a reliable method for the treatment of microbial and non-microbial diseases (7). Numerous plants have been identified that have antimicrobial properties, but most of them have not yet been

adequately studied (8). Plants as a source of new drugs remain largely unknown, and screening studies to discover the effects of their biologically active compounds and molecules are necessary. The first step in achieving this goal is to investigate their antimicrobial activity in vitro (9).

Artemisia is one of the medicinal plants that is used in traditional medicine to treat some diseases and so far many studies have been performed on it to detect different degrees of inhibitory and antimicrobial effects. The plant is widely grown in a variety of climates, especially in arid regions where plant growth is somehow difficult due to climatic conditions; one of the reasons for this expansion could be the high ability of this plant to adapt to different climatic conditions. Chemically, *Artemisia* extracts often contain polypranoids and aromatic compounds that can have antimicrobial effects on a range of bacteria due to their phenolic groups in their structure (10).

Although the determination of the active ingredients of the plants is pharmacologically important, it is necessary to first determine the effectiveness of the extracts obtained from them (11). In this regard, it has been noted that in the early stages of the discovery and production of new drugs, the selection of raw plant extracts may be more appropriate than the screening of compounds for this reason (12). In the present study, we conducted a preliminary screening to determine the antimicrobial effect of aqueous, ethanolic, methanolic, acetone/ethanolic and hydroethanolic extracts of *Artemisia* aqueous on standard *P. aeruginosa*, *S. pyogenes*, and oral bacteria sample from rotten teeth, in order to investigate the antibacterial effect of *Artemisia* for using as a natural mouth wash or antibacterial agent as preservative material in food, cosmetic, and the health industries.

Materials and Methods

Preparing the plant

Artemisia specimens were collected from the Isfahan Medicinal plants market. After collection, the sample was rinsed several times with water, then dried in the shade, and the sample was powdered with a grinder for easy extraction.

Extraction

The extract of the plant prepared in the separate stages with solvents including water, ethanol, hydroethanol, acetone/ethanol, and methanol. Extraction was done by the maceration method for all solvents, but hydroethanolic extract was performed using the Soxhlet method to determine whether the temperature in the extraction process affects the antibacterial properties of the extracts. To prepare the water extract, 24 grams of plant powder was added into an Erlenmeyer flask and increased the volume with distilled water to 100 ml. It was put in a shaker for 3 days, then the extract was first filtered with a sterile lace cloth and then with filter paper. After smoothing, the solution was poured into a petri dish and placed in an incubator at 37 C to evaporate water and dry completely (13). After drying, the extract was kept away from light. The above method was also used to prepare ethanolic, methanolic, and acetone/ethanolic extracts. For preparing hydroethanolic extracts, 24 g of dried *Artemisia* powder with 200 ml hydroethanolic solvent was placed in the Soxhlet extractor for 7 hours. The solvent was evaporated slowly and was concentrated at 37 C. Then, 5% of Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, United States) was utilized to prepare the extract. Finally, two types of solvents including water and DMSO were used to dissolve the various extracts. The aqueous extract of *Artemisia* in water and other

extracts in DMSO were completely dissolved and finally filtered with 0.22 µm MS® MCE Syringe Filter from Membrane Solutions Company (14,15).

Evaluation of the antibacterial activity of the extract

In the present study, *P. aeruginosa* and *S. pyogenes*, and the oral bacteria sample were used to evaluate the antibacterial activity of *Artemisia* extract. The oral bacterial sample was randomly chosen by a general sampling of a decayed tooth and no specific strains identification was done. *P. aeruginosa* and *S. pyogenes* were clinical strains and were provided from the Al-Zahra University Hospital of Isfahan, Iran. First, equal volumes of each bacterial suspension were prepared according to standard 0.5 McFarland (16) 7×10^8 . The bacteria were first cultured on Mueller Hinton Agar medium (QUELAB's, Canada). To investigate the antibacterial activity of each extract on studied bacteria, extracts were used at a concentration of 50 mg/ml. Dilution antimicrobial experiments were performed twice and in accordance with the recommendations of the Standards Committee of Clinical Laboratories (CLSI) (17). For this purpose, 15 ml of sterile culture medium with an appropriate temperature was transferred into 8 mm Petri dishes. The surface of the culture medium was covered with bacterial suspension, then 5 well with a diameter of 6 mm were made in each plate using a sterile Pasteur pipette. To assure the accuracy of the test and comparison conditions, description gentamicin and nalidixic acid antibiotic discs (Padtan Teb Company, Iran) were settled in two wells (positive control). DMSO was also utilized as a negative control sample in one of the wells. The plant extract suspension was loaded into two wells. Each extract was analyzed on *P. aeruginosa*, *S. pyogenes*, and the oral bacteria

sample. Finally, all the plates were incubated at 37 °C for 24 hours.

After finding the *Artemisia* extracts that had antibacterial effects, the different concentrations of those extracts were prepared and poured in the prepared wells on Mueller Hinton Agar medium. Seven vials were prepared. 900 microliters of distilled water was poured into each vial. About 100 µl was obtained from the main vial with a concentration of 50 mg/ml and added to 900 µl of distilled water in the first vial and well mixed. Next, 100 µl were removed from the first vial and added to the second vial, and this process continued until the seventh vial. Finally, 100 µl was removed from the last vial and poured out. In this way, a series of dilutions from the 50 mg/ml of extracts were prepared. An equal amount of the extract in each vial was transferred to wells in Mueller Hinton Agar medium containing the cultured bacteria to determine the antibacterial properties of each concentration. All plates incubated at 37 °C for 24 h. Then the lowest concentration that caused the non-growth zone was considered as the minimum inhibitory dosage. Units from the non-growth

area of each dilution were collected and to ACC at the position 1003. In fact, nucleotide transitional mutation of G to A has caused alanine 335 amino acid to be replaced by threonine.

Result

The gained results have revealed that aqueous extract of *Artemisia* had no effects on *P. aeruginosa* and *S. pyogenes* but it has an inhibiting effect on oral bacteria sample. The ethanolic and hydroethanolic extract of *Artemisia* did not indicate any anti-bacterial effects on all three mentioned bacteria strains. The result of the methanolic extract has shown more impact on different bacteria.

As demonstrated in [Table 1](#), the methanolic extract had a significant antibacterial effect on *S. pyogenes* and the oral bacteria sample. Also, the acetone/ ethanolic extract had the most inhibiting effect on *S. pyogenes*.

50 mg/ml of *Artemisia* extracts was the minimum concentration, which had inhibitory and bactericidal effects. None of the fewer concentrations had an antibacterial effect. ([Table 2](#))

Table 1. Mean diameter of growth inhibitory zone of aqueous, ethanolic, methanolic, acetone/ ethanolic, and hydroethanolic extracts of *Artemisia* against selected bacteria in millimeters (mean ± standard deviation).

Concentration (mg/ml) / Bacteria Strain	Aqueous extract (Maceration) 50 mg/ml	Ethanolic extract (Maceration) 50 mg/ml	Methanolic extract (Maceration) 50 mg/ml	Acetone/ ethanolic extract (Maceration) 50 mg/ml	Hydro ethanolic extract (Soxhlet) 50 mg/ml	Nalidixic acid	Gentamicin
<i>P. aeruginosa</i>	0	0	0	0	0	0	25
<i>S. pyogenes</i>	0	0	15 ± 0.21	16 ± 0.09	0	15 ± 0.15	30 ± 0.14
oral bacteria sample	8 ± 0.1	0	15 ± 0.37	0	0	N/D	N/D

Table 2. The minimum concentration of aqueous, ethanolic, methanolic, acetone/ethanolic, and hydroethanolic extracts of *Artemisia* (mg/ml) with bacterial growth inhibitory and bactericidal effect.

Substance Bacteria Strain	Aqueous extract	Ethanolic extract	Methanolic extract	Acetone/ethanolic extract	Hydroethanolic extract
<i>P. aeruginosa</i>	-	-	-	-	-
<i>S. pyogenes</i>	-	-	50	50	-
oral bacteria sample	50	-	50	-	-

Discussion

Artemisia extract has been reported to contain many antimicrobial compounds such as phenols, cineol, ketones, camphor, and thymol, all of which interfere with the action of membrane enzymes by destroying cell walls and bacterial proteins; in fact, the presence of antibacterial activity of this plant can be attributed to the presence of these compounds (10,19,20). In *Artemis* species, there are high concentrations of beneficial substances along with antioxidants that are effective in maintaining the natural flora of the body. These antimicrobial effects are likely to be mediated by a variety of mechanisms, including disruption of the mitochondrial-related proton chain, disruption of membrane proteins and lipids, and inhibition of the sarcoplasmic reticulum calcium pump (21).

According to the findings of a survey, the quality of natural extracts and their antioxidant effects depend not only on storage time, geographical origin, and harvest time but also on the environment and technological factors used for the extraction process. Also, the type of solvent used in extraction is one of the other important factors that are important in causing antioxidant effects (22). The results obtained by Appalasamy *et al.* Also showed that the extract obtained from *Artemisia* at a concentration of 6 mg/ml led to inhibitory

effects on *Escherichia coli* and *Staphylococcus*. This result proves that the active ingredients in the crude extract have synergistic and intensifying effects, thus resulting in a higher overall antimicrobial activity (23). Another study by Jutea *et al.* revealed that *Artemis* extract significantly inhibited the growth of *Enterococcus hirae* (24). Findings of a study also stated that various extracts prepared from *Artemis* leaves have significant antioxidant properties compared to the standard curve of vitamin C and ferrous sulfate (25).

In a study conducted on *Artemisia* from Kerman province of Iran, it was found that the essential oil of this plant has antibacterial and fungal effects. Minimum inhibitory concentrations were obtained for *Staphylococcus aureus* (4 µg/ml), *Salmonella typhi* (32 µg/ml), *E. coli* (32 µg/ml), *Candida albicans* (8 µg/ml), and *Aspergillus niger* (4 µg/ml) (26). Another study on *Artemisia* from the city of Babol in Iran also confirmed that all aqueous, ethanolic, and methanolic extracts of *Artemisia* were able to have significant antibacterial properties in comparison with the blank and antibiotic sample. Aqueous extract with a 30% antibacterial effect of ciprofloxacin on *E. coli* and methanolic extract with the highest antimicrobial activity with 71% of vancomycin showed an inhibitory effect on the growth of *Enterococcus faecalis*.

Although the aqueous extract had the least inhibitory effect on *E. coli* and *Enterococcus faecalis*, the inhibitory effects of this extract were significant compared to the blank sample (27). Based on an in vitro study on aqueous, ethanolic, and methanolic of *Artemisia* from Markazi province of Iran, it was identified that their MIC were 6250, 3125, and 6250 µg/ml, respectively, so it was concluded that different extracts affect *Candida albicans* growth and prevent its growth (28). The results of an investigation on *Artemisia* From the western regions of Isfahan province, Iran, determined that flower essential oil had an inhibitory effect on the growth of pathogenic bacteria, especially gram-positive types (*Staphylococcus aureus*, *Enterococcus faecalis*, *E. coli*) because the diameter of the non-growth zone for was in the range of 8.3-45 mm and the least MIC was 2.5%. However, this essential oil did not affect *P. aeruginosa* (29).

In the present study, the bacterial effects of different concentrations of aqueous, ethanolic, methanolic, acetone/ethanolic, and hydroethanolic extracts of *Artemisia* under standard laboratory conditions on *P. aeruginosa*, *S. pyogenes*, and oral bacteria sample were investigated.

In most Iranian previous studies, one or two types of solvents have been used for extraction, while in the present study, attempts were made to use different types of *Artemisia* extracts for investigation. Also, to select the bacterial strain, the species that had not been used in Iranian studies on *Artemisia* were selected.

Taking into account these two factors (the simultaneous study of several types of extracts and selection of untested strains), it was tried to obtain new and complete information about the antibacterial effect of

this plant and to use it for pharmaceutical studies.

The findings showed that aqueous, methanolic, and acetone/ethanolic extracts of *Artemisia* had significant antibacterial effects on *S. pyogenes*. All effective extracts at a concentration of 50 mg had antimicrobial effects. Therefore, by using different extraction methods and various solvents, it may be possible to more effectively obtain biomaterials with antimicrobial properties from this plant. Considering that both in this study and in one of the previous studies on the essential oil of the Iranian *Artemisia* plant, it was found that they have less antimicrobial effects on *P. aeruginosa*, it seems that in future studies, higher concentrations of these extracts are better to be used for assessment. But overall, it can be concluded with certainty that this plant has a significant effect on gram-positive bacteria and is, therefore, a good choice for use in the food and pharmaceutical industries.

The oral bacterial sample was also randomly selected by a general sampling of a decayed tooth and no specific identification of the strains was performed to evaluate *Artemisia* antimicrobial overall effect on tooth decaying bacteria. And because of the positive results obtained from inhibitory tests, *Artemisia* extracts may be used in general for producing all types of mouthwashes and toothpaste. It is a better option compared to the chemical compounds used in these products.

Conclusion

It can be deduced that aqueous, methanolic, and acetone/ethanolic extracts of *Artemisia* have antibacterial effects on both *S. pyogenes* bacteria and the oral bacteria sample. This study also revealed that none of the extracts of *Artemisia* had an antibacterial effect on *P.*

aeruginosa. According to the findings of this study, Artemisia plant extracts can be used in food, cosmetics, and pharmaceutical industries due to its antibacterial properties.

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