Article

The Effects of *Artemisia spicigera* Essential Oil before and after Flowering against Bovine Mastitis Bacteria

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Abstract: The absence of side effects of herbal essential oils and their antimicrobial effects has led researchers to evaluate the antimicrobial effects of essential oils and plant extracts in vitro to control microorganisms with a natural source. Artemisia is one of the genera of the *Anthemideae* family, widely distributed in the world and containing more than 400 species. In this study, *Artemisia spicigera* before and after flowering was prepared from region Bostanabad of East Azerbaijan and essential oils were extracted using Clevenger apparatus. Constituents of essential oil was analyzed by GC/MS. Then, the antimicrobial effect of essential oil *Artemisia spicigera* against bacteria isolated from clinical cases of bovine mastitis (*Staphylococcus aureus* and *Escherichia coli*) was determined and with
some standard antibiotics as a positive control was compared. For the preparation of bacteria of 20 cows with clinical mastitis was sampled and after culturing milk samples, the bacteria were isolated. The results showed that the inhibitoriest effect of Artemisia essential oils, before and after flowering, was 15 and 30 mm on Staphylococcus aureus and 9 and 25 mm on Escherichia coli in 100% concentration, respectively. Also in antibiogram method most diameter of inhibition zone in culture medium against Staphylococcus aureus and Escherichia coli was related to ceftriaxone, 40 and 25 mm, respectively. Results also showed that the antimicrobial effect of essential oils of Artemisia spicigera after flowering was more than before flowering. Due to the significant antibacterial activity of Artemisia essential oil in compared to different antibiotics can be concluded that essential oil of Artemisia spicigera has impressive antimicrobial properties, so it can be used in combination with other preservatives as natural herbal medicine using in treatment of mastitis in cows.

**Keywords:** Artemisia spicigera, Essential oil, mastitis, antimicrobials, Staphylococcus aureus, Escherichia coli

### 1. Introduction

The absence of side effects of herbal essential oils and their antimicrobial effects has led researchers to evaluate the antimicrobial effects of essential oils and plant extracts in vitro to control microorganisms with a natural source (Ihsan-ul-Haq et al., 2012). The excessive use of antibiotics can lead to a serious health outbreak in humans. The main goal of the World Health Organization is to minimize the use of antibiotics in livestock breeding livestock. Therefore, safe and natural alternative methods should be developed to control infections (Zafalon et al., 2007).

Artemisia is one of the genera of the Anthemideae family, widely distributed in the world and containing more than 400 species. In Iran, about 34 species have been reported to be used as a traditional medicinal plant (Ghavamizadeh et al., 2013, Mozaffarian, 1996). For this plant, there are various healing properties, including antimalarial, antiparasitic, antibacterial, anti-diabetes, astringent, anti-fever, anticancer, anti-hepatitis, anti-inflammatory, anti-spasm, antiviral and anti-headache (Masoudi et al., 2012, El-Massry et al., 2002). Bovine mastitis is defined as inflammation of the mammary glands that can cause infectious and non-infectious causes, as a result of inflammation of the breast glands, changes in milk, including the decrease in the synthesis of casein-lactose and the quality of milk fat occurs. (Blowey and Edmondson, 2010).
The aim of this study was to evaluate the antimicrobial activity of essential oil of *Artemisia* before and after flowering stage against common bacteria isolated from milk samples of cows infected with clinical mastitis.

2. **Material and Methods**

2.1. **Prepare of Plant**

*Artemisia* plant from Bostanabad County, East Azarbaijan Province, was confirmed by the botanical group of Faculty of Pharmacy, Tabriz University of Medical Sciences with the number 14966 Herbarium.

2.2. **Prepare of Essential Oil**

For preparing of essential oil, the aerial parts of the plant were dried in a shade condition, completely powdered by an electric mill. Then, 200 g of that was transferred to a Clevenger and was extracted with water by distillation for 3 hours and kept in a refrigerator dark glass containers until it was used. (Ghajarbeygi et al., 2015).

2.3. **Determination of Chemical Composition of Essential Oil of Artemisia**

The essential oil composition of the plant was measured by a mass spectrometer-attached gas chromatograph (GC / MS). (Mahmoudi and Nosratpour, 2013).

2.4. **Isolation of Bacteria from Bacterial Mastitis in Dairy Cows**

At first 20 specimens were collected in sterilized falcon tubes from clinical mastitis. After transfer to laboratory under appropriate conditions, 100 μl were cultured in Broth media. In the following, 100 μl of dilution of 1/1000 stokes was transferred to specific Parker Agar, Nutrient Agar, EMB and SDA cultural media and after 24 hours of incubation at 37 °C, colonies were examined for microscopy. Finally, *Staphylococcus aureus* and *Escherichia coli* isolates were isolated and were confirmed by biochemical tests including Catalase, Oxidase and Gram staining (Erdogrul, 2002).

2.5. **Determination of the Effect of Essential Oil of Artemisia against Staphylococcus aureus and Escherichia coli Isolated and Its Comparison with Different Antibiotics by Disk Diffusion Method**

At first, the number of *Staphylococcus aureus* and *Escherichia coli* bacteria was calculated in each ml of bacterial suspension in the Nutrient Agar after dilution. Then, 100 μl of dilution of $3 \times 10^8$ CFU / ml was cultured on the Muller Hinton Agar. Next, 6 mm sterile paper discs were placed on an empty plate and 20 μl of different concentrations of essential oil (3.125, 6.25, 12.5, 25, 50 and 100) were poured them. In the next step (after drying), the disks impregnated with essential oil were removed with sterile pins and placed on Muller Hinton Agar with a minimum of 2.4 cm spacing. At
the same time, the antibiotics of ceftriaxone, kanamycin, amikacin, neomycin, amoxiclav, erythromycin, chloramphenicol and tetracycline were also used to compare the effect of essential oil effects. For this, the bacteria were cultured in a uniform manner on the surface of the Muller Hinton Agar and then placed on the surface of the antibiotic disks. Finally, the plates containing the disk at 37 °C were incubated for 24 hours and the halo diameter lack of growth was measured by millimeter ruler (Ghajarbeygi et al, 2015, Raeisi et al., 2012).

3. Results and Discussion

3.1. Results

The results showed that the major components of essential oil of Artemisia, Before and after flowering stage, were Spacholenol H1 cycloprob (18.39%) and Biaseclu [0,1,3] Hexane-3-N, 4-Met (26.16% %). In this study, essential oil yield of Artemisia was determined to be 0.55% based on the dry weight of the plant. Investigating the effect of inhibitory effects of different concentrations of essential oil of Artemisia spicigera by disc diffusion method showed that the maximum inhibitory effect of essential oil before and after flowering stages on Staphylococcus aureus was 15 and 30 mm, respectively, on Escherichia coli was 9 and 25 mm at 100 concentrations (Table 1).

Table 1: Effect of inhibitory effects of different concentrations of essential oil of Artemisia spicigera by disc diffusion method in before and after flowering stages (mm)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Before flowering</th>
<th>After flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

In the antiogram method, the highest non-growth halo diameter in *Staphylococcus aureus* and *Escherichia coli* was 40 and 25 mm, respectively, in ceftriaxone (Table 2).

Table 2. Non-growth halo diameter in antiogram method (mm)

<table>
<thead>
<tr>
<th>Antibiotic Bacteria</th>
<th>Amikacin</th>
<th>Kanamycin</th>
<th>Neomycin</th>
<th>Amoxiclav</th>
<th>Erythromycin</th>
<th>chloramphenicol</th>
<th>ceftriaxone</th>
<th>tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
<td>20</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>24</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>20</td>
<td>25</td>
<td>15</td>
</tr>
</tbody>
</table>

3.2. Discussion
In this study, the major components of essential oil of Artemisia before and after flowering stages were Spacholenol H1 cycloprob (18.39%) and Biaseclu [0,1,3] Hexane-3-N, 4-Met (26.16% %). In study of Ghajarbeygi et al, the major components of essential oil of Artemisia before flowering stage were Spacholenol H1 cycloprob (18.39%), Epizonarene (9.62%), Biaseclu [0,1,3] Hexane-3-N, 4-Met (6.76%), and Jumacarine D (6.33%) and after flowering stage were Biaseclu [0,1,3] Hexane-3-N, 4-Met (26.16%), 1.8 Cineole 2 Oxabicyclo (26.15%) and Conifer Bicyclo [2.2.1] Heptane (17.46%) (Ghajarbeygi et al., 2015).

Antimicrobial effect of Artemisia essential before and after flowering stage, which was done by disc diffusion method, showed that the antibacterial effect of essential oil on Gram-positive bacteria of Staphylococcus aureus is higher than the Gram-negative bacteria of Escherichia coli and essential oil of Artemisia after flowering stage has the most antibacterial effects. In various studies, it has been argued that Gram-positive bacteria are more susceptible to essential oils of Artemisia than Gram-negative bacteria than (Ihsan-ul-Haq et al., 2012). The walls of the Gram-positive bacteria have mucopeptide compounds, while Gram-negative bacteria have a thin layer of mucopeptide compounds and have more lipoprotein and lipopolysaccharide like compounds, and this difference probably causes herbal essences and essential oils to be more effective on Gram-positive bacteria (Ramezani et al., 2005).

The results also showed that the diameter of the inhibition zone for both bacteria increased with increasing essential oil concentration. By comparing the diameter of the non-growth region of antibiogram discs with the diameter of halo region of the essential oil fractions (100), it was found that all antibiotics except ceftriaxone had less inhibitory effect than the essential oil on the bacteria.

In studies in Iran on various species of Artemisia, it has been shown that in Artemisia kermanensis, the inhibitory effect is on the bacteria of Klebsiella and Pseudomonas, and Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Aspergillus niger fungus have shown a fairly good sensitivity (Kazemi et al., 2011).

Asghari et al., in a study showed that Artemisia essential oil had inhibitory effects on Staphylococcus aureus in four successive consecutive dilutions of 100, 50, 25 and 12.5, but had an inhibitory effect on Escherichia coli bacteria only at concentrations of 100 and 50, growth halo in Staphylococcus aureus was more than Escherichia coli (Asghari et al., 2012).

Sefidkon et al. showed antimicrobial activity of essential oil of methanolic, chloroform, butanol and water extracts against 8 Gram positive and gram negative bacteria, including Escherichia coli and Staphylococcus aureus, that except Gram negative bacterium Pseudomonas aeruginosa, the non-growth halo diameter of other bacteria was more than 22 mm in comparison to the essential oil, that it was equal to or greater than the effect of tetracycline and erythromycin (Sefidkon et al., 2013).
Study of antibacterial activity of essential oil of *Artemisia annua* L. extract by MIC and MBC method by Massiha et al. showed that the essential oil of this plant at 32 μg / ml concentration had the most antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* (Massiha et al., 2013).

A study in Pakistan also showed that methanolic extract of *Artemisia dubia* have effects against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* (Ihsan-ul-Haq et al., 2012).

### 4. Conclusion

Considering the significant antibacterial effects of essential oil of *Artemisia spicigera*, in comparison with various antibiotic discs, it can be concluded that the essential oil of this plant has an antimicrobial activity better than some antibiotics and can be used combined with other preservatives as a natural herbal drug in the treatment of mastitis. Therefore, it is suggested that further research is needed to evaluate the effects of the essential oil of this plant as a natural source on animal models for controlling diseases and also in the food industry as antimicrobial inhibitors and flavors.

### References


