Antibacterial properties of *Anthemis gayana* leaves essential oil

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**ABSTRACT:** Plants are a large origin of new bioactive molecules with remedial potentials. Only a small amount of living plants on Earth have been phytochemically investigated. The aim of this study was in vitro studying of antibacterial effects of the leaves essential oil of *Anthemis gayana*. In this study essential oil was obtained by hydro-distillation using Clevenger type apparatus for 4h. It was tested in vitro against 4 bateria. Several dilution of essential oil (1%, 2%, 5%, 10%, 15%, 20%, 40%, 80%) was prepared. Antibacterial activity was tested by using microdilution MIC determination and agar well diffusion assay. Data were analyzed by ANOVA test in the P-Value< 0.001. The results illustrated that essential oil in dilution 80% has more inhibitor activity and most activity were shown against *Escherchia coli* and *Acinetobacter baumannii*. The results of the present project indicate that the *Anthemis gayana* leaves inhibited bacteria growth but their effectiveness varied. These antibacterial activities are likely due to the presence of the terpenes in the essential oil. Therefore we will be able perform researches with extraction of this plant effective compound for the treatment of infectious disease.

**Key words:** *Anthemis gayana*, Antimicrobial, Essential oil, Leaves.

**INTRODUCTION**

Discovery healing powers in plants is an ancient idea (Cowan, 1999). Some medicinal plants used in traditional Iranian medicine are effective in treating various ailments caused by bacterial and oxidative stress (Chan et al., 2008). The use of phytochemicals as natural antibacterial agents commonly called “biocides” is gaining generality. There is growing concern in correlating phytochemical constituents of plant with its pharmacological activity. The antimicrobial properties of essential oils have been recognized for many years, and their rudiment have found applications as naturally occurring antimicrobial agents in the field of pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation, etc. The essential oil rudiment that possess antimicrobial activities have been the subject of many investigations resulting in the screening of a wide variety of plant species and have picked structurally unique biologically active compounds. However, less attention was given to the activities of their original components in the oils tested. The original advantage of natural factors is that they do not enhance the “antibiotic resistance”, a event generally encountered with the long-term use of synthetic antibiotics. There are reports of the active principles of essential oils from various plants with antifungal or antibacterial activity. The antimicrobial activity of essential oils is determined to a number of terpenoids, which also in pure form demonstrate high antibacterial activity. The essential oils and their components are known to be active against a large variety of microorganisms, including gram-negative and gram-positive bacteria. Gram-negative bacteria were shown to be commonly more resistant than gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane, but this was not always correct (Vukovic et al., 2007).

The genus Anthemis is the second largest genus in the Asteraceae family, tribe Anthemideae, distributed all over of Iran (Uzel et al., 2004). The species of the Anthemis genus are widely used in the pharmaceutics, cosmetics and food craft. The flowers of the genus have well-documented use as disinfectant and healing herbs, the main components being natural flavonoids and essential oils (Uzel et al., 2004). Some Anthemis spp. essential oils contain anti-aging activity (Savoglou et al., 2006). While *Anthemis cotula* essential oil has been proved to possess antioxidant confidants and essential oil from *Anthemis nobilis* flowers is generally used for pharmaceuticals, food additives, as well as an main source in aromatic and cosmetic industries (Savoglou et al., 2006).
Anthemis gayana Boiss. is an annual endemic plant of Asteraceae family (Sonboli et al., 2005). This plant grows in the Isfahan west region from Iran. Essential oil leaves were first investigated by Sonboli et al, 2005 and more than 34 compounds of the leaves oil representing 92.4% of total oil were identified, germacrene-D (30.2%), geranyl isovalevate (7.4%), bicyclogermacrene (6.7%) and β–caryophyllene (5.5%) as the major compounds (Sonboli et al., 2005).

Amjad et al. (2013) reported that methanolic extract of Anthemis gayana flowers and leaves were not active against Candida glabrata CBS 2175 and Candida albicans ATCC 62061, ATCC 1677. Flowers and leaves methanolic extract had more effect against Candida parapsilosis (Amjad et al., 2013), Thus, leaves methanol extract had more effect against Candida albicans ATCC 3153 (Amjad et al., 2012). The information about the in vitro antibacterial activity features of the Anthemis gayana has not been reported earlier. In this paper were showed the antibacterial activity of the leaves essential oil of Anthemis gayana for the first time.

MATERIAL AND METHODS

Plant material
Anthemis gayana Boiss. Was collected from around area of Isfahan province (Daran), Iran in the June of 2012. The voucher specimen was deposited at the herbarium of Research Institute of Isfahan Forests and Rangelands. They were air-dried in the shade at room temperature. The samples were homogenized in an electric grinder to fine powder.

Essential oil preparation
For essential oil's preparation, 50 gram of leaves powder of Anthemis gayana was obtained from hydro-distillation (500 ml distillate water) for 4h using a Clevenger-type apparatus (Oliveira et al., 2006). Oil sample was stored at 5°C in dark sealed glass vials until each experiment (Al-Janabi, 2011; Bajpai et al., 2008). The essential oils were dissolved in dimethylsulfoxide15% (DMSO15%) and tween80 10% (1:1, for easy diffusion) (Prabuseenivasan et al., 2006).

Strains and growth conditions
Microorganisms were isolated from patients and recognized as Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus aureus. Two strains of gram-negative bacteria Escherichia coli, Pseudomonas aeruginosa, and two strains of gram-positive bacteria Acinetobacter baumannii and Staphylococcus aureus were used. The cultures of bacteria were maintained in their appropriate agar slants at 4°C throughout the study and used as stock cultures.

Well-diffusion assay
100 ml of 1.5 × 10^8 CFU/ml bacteria suspension was spread monotonically onto the Mueller-Hinton Agar (MHA) plate using cotton swabs. Next, wells which arranged on the agar surface impregnated with 1%, 2%, 5%, 10%, 15%, 20%, 40%, 80% dilution of essential oil were placed onto agar plates. Inhibition diameters (in mm) were measured after incubation at 37°C for 24h (Uzel et al., 2004).

Determination of MIC by microdilution
Serial dilutions of the essential oil (40%, 20%, 10%, 2.5%, 1.25%, 0.625%, 0.313%, 0.156%, 0.078%) were load in a microdilution plate (96 wells). Then, the inoculums (150µl 1.5×10^6 CFU/ml) were added to each well. The microplates were incubated at 37°C for 24 h. The MIC was defined as a lowest concentration which resulted in inhibition of visual growth absorbance was read in an ELISA plate reader at 630 nm (ELISA, Stit fax 2100, USA). Minimal bactericidal concentrations (MBC) were determined by subculturing 10µl of the culture from each negative well and from the positive control, measured as explained (Chan et al., 2008).

Statistical analysis
The experiments were performed three times to minimize the error and the mean values are presented. Data were analyzed using ANOVA test in the P<0.001.

RESULTS AND DISCUSSION

The antibacterial activity of the essential oil of Anthemis gayana leaf evaluated against four of gram-positive and gram-negative bacteria with a MIC assay. The mean MICs and MBCs for the essential oil are given in Table 1. It should be noted that, it in dilution 80% had strong effect. As well as, it showed strong activity with MICs of 2.5%.
Essential oil was tested for its power of antibacterial activity using a well-diffusion assay which wells were plated onto MHA plates. Maximum inhibition zone (23.75±6.25 mm) of essential oil was obtained against *Acinetobacter baumannii*, in 80% dilution. In other, minimum inhibition zone (2.66±0.57 mm) of that was obtained against *Escherichia coli*, in 20% dilution. Results showed that, there is direct relationship between dilution and inhibition zone Fig.1. It is worth mentioning that there are significant changes between the essential oil and positive control sample, ciprofloxacin and cephalixin (P<0.001).

Table 1. Determination of MIC and MBC of *Anthemis gayana* leaves essential oil against bacteria.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (%)</th>
<th>MBC (%)</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2.5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. The antibacterial activity of the leaves essential oil of *Anthemis gayana* on different bacteria

<table>
<thead>
<tr>
<th>Concentration</th>
<th>20%</th>
<th>40%</th>
<th>80%</th>
<th>Control +</th>
<th>Control -</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>3.66±0.57</td>
<td>7.33±1.15</td>
<td>20±1.63</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>11.75±2.36</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>-</td>
<td>10</td>
<td>23.75±6.25</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>10.33±50.07</td>
<td>14.75±0.95</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

Inhibition zone: mm, Mean ±SD

Plants have an extremely ability to synthesize aromatic products. Most are secondary metabolites and in many cases, these products serve as plant defense mechanisms against predation by microorganisms and insects. Some, such as terpenoids (essential oils), give plants their smells (Cowan, 1999). The composition of essential oils from a particular species of plant can differ between harvesting seasons and between geographical sources (Burt, 2004). Researchers interpretation demonstrate that: first, the composition of essential oil is known to vary according to local climatic and environmental conditions (Chludil et al., 2008; Tim-Cushnie and Lamb, 2005). Secondly, the method used to evaluation antimicrobial activity, and the choice of test organisms. The results obtained by different methods may differ as many factors vary between assays. These include: volume of broth or agar, type of broth or agar, size of wells, size of paper disks, strains of a particular bacterial species used, incubation period and microbial growth (Singh et al., 2010; Tim-Cushnie and Lamb, 2005). Tertiary, the hydrophobic nature of most plant essential oils prevents the uniform diffusion of these substances through the agar medium (Tim-Cushnie and Lamb, 2005). Thus, The activity of the oils would be expected to relate to the respective composition of the plant volatile oils, the structural configuration of the constituent components of the volatile oils and their functional groups and possible synergistic interactions between components (Dorman and Deans, 2000).

In vitro studies in this work showed that *Anthemis gayana* leaves inhibited bacterial growth but their effectiveness varied. Totally, the gram–positive bacteria are more susceptible than gram–negative bacteria due to the differences in their cell wall structure. Gram–negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics (Parekh and Chanda, 2007; Afolayan and Ashafa, 2007; Vukovic et al., 2007). However, the results from Sonboli et al., 2005 study reveals that the leaves essential oil of *Anthemis gayana* are including: germacrene D (30.2%), geranyl isovalerate (7.4%), bicyclogermacrene (6.7%) and β- caryophyllene (5.5%). This compounds have been reported to display strong antibacterial effects. So that, germacrrenes are a class of volatile organic hydrocarbons, specifically, sesquiterpenes. Germacrrenes are typically produced in a number plant species for their antimicrobial and insecticidal properties (Burt, 2004).

The results of this study indicate the antimicrobial activity of leaves essential oil of *Anthemis gayana*. Studies aimed at the isolation and structure elucidation of antibacterial active constituents from this plant is in progress.

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REFERENCES


