1. Introduction

Emergence of drug resistance among bacteria as a result of prolonged usage of broad-spectrum antibiotics has led to an essential need for novel antimicrobials to combat infectious diseases. Traditional knowledge has provided a valuable source to screen plants with beneficial effects to treat infectious diseases. Validated methods in combination with traditional knowledge prepare a primary source of discovery to identify potentially useful antimicrobial molecules.

In the Northern hemisphere, mostly Europe and Asia about 85 species of the genus, Achillea L. (Asteraceae) is represented (Könemann, 1999). Achillea is represented with 46 taxa, 25 of which are endemic in Turkey (Güner et al., 2000; Wagenitz, 1975). The plant name was originated of a legend regarding its wound healing effect using by the hero of Trojan, Achilles. The aerial part of Achillea millefolium L., a well-known species of Achillea genus, is traditionally used to remedy gastrointestinal disorders and hepatobiliary complaints, as well as for wound healing and skin inflammations in Europe (Benedek & Kopp, 2007). Other species of the Achillea are commonly applied for treatment of diarrhea and flatulence, wound healing, as diuretic, an emmenagog agents, and abdominal pain in Turkey (Sezik et al., 2001; Baytop, 1999; Sezik & Yesilada, 1999). However, the biological properties of A. tenuifolia have not been completely elucidated, therefore, in this study we examined the antibacterial effect of the plant extract growing in Iran.

2. Materials and Methods

2.1. Plant Material

All parts (leaves, stems, flowers and roots) of A. tenuifolia were collected from Qazvin province (1500 m) in June 2011, and identified by Dr. Yousef Ajani. A herbarium specimen (No. 1604) has been deposited at the Herbarium of Institute of Medicinal Plants, Jahade-Daneshgahi (ACECR), Karaj, Iran. The plant materials were cleaned and dried in shade at room temperature.
Antimicrobial properties of extracts and oils of different species of Achillea have been reported previously against various strains in vitro (Alsolahli & Al-fawwaz, 2014; Issabeagloo & Abri, 2012; Hasson, 2011; Stojanović et al., 2005). However, there are limited studies available regarding the chemical composition of the plant. The results of a study revealed that the main compounds of the seed oil of the plant were linoleic acid (69.4%) and oleic acid (14.5%) (Goli, Rahimmalek, & Tabatabaei, 2008). Another experiment revealed that essential oils of aerial parts of A. tenuifolia rich in limonene (23-25%) moderately inhibited the growth of

### Table 1. Antibacterial activity of A. tenuifolia against tested strains.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>E. fæcalis</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. thyphimurium*</th>
<th>E. coli*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (µg/mL)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IZD (mm)</td>
<td>12±0.0</td>
<td>11.6±0.5</td>
<td>10.3±0.5</td>
<td>14±0.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*: Isolated strains from diseased hen, IZD: Inhibition Zone Diameter, MIC: Minimum Inhibition Diameter.

3. Results and discussion

Antibacterial activity of the extract of A. tenuifolia is presented in Table 1. It was tested for their inhibitory effects against four standard bacteria E. coli, S. aureus, P. aeruginosa, and E. fæcalis, and two isolated strains from diseased hen including S. thyphimurium and E. coli using disk diffusion and microdilution methods to evaluate their IZDs and MICs, respectively. The extract of plant inhibited all the standard strains with IZD values ranging between 10 to 14 mm. Interestingly, P. aeruginosa, a gram negative bacteria was more susceptible to the extract with IZD of 14±0.0 mm, compared to other bacteria. The MIC values were evaluated the same for all the standard strains as 50 µg/mL. Growths of the both isolated bacteria were not inhibited by treatment of A. tenuifolia extract. All the tested bacteria were inhibited by thymol as positive control (100 mg/mL) with IZD values of >80 mm, while thymol with concentrations of 1 and 10 mg/mL were inactive against the strains.

The results of the present study showed that the extract of the plant was active against standard strains of E. coli, P. aeruginosa, S. aureus and E. fæcalis. While, isolated bacteria form diseased hen S. thyphimurium and E. coli were resistant to the extract of A. tenuifolia. Although, thymol, a well-known antibacterial agent, was totally suppressed the bacteria with concentration of 100 mg/mL, the plant extract inhibited the standard strains with much lower concentration (100 µg/mL).

2.2. Extraction

The powdered plant material was extracted (700 g) by maceration method using pure methanol, three times each last for 48 h at room temperature (3±48 h). The extracts were concentrated after removing the solvent by rotary evaporator and then lyophilized using a freeze dryer. The concentrated methanol extracts weighed 2.7 g (on the basis of dry weight). The extracts were then kept in opaque containers under cold and dry conditions until assay.

2.3. Antimicrobial activity test

Antimicrobial studies were carried out against four standard bacteria strains Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, and Enterococcus fæcalis ATCC 29212 alongside two isolated strains from diseased hen including Salmonella thyphimurium and E. coli. Microdilution method was employed to evaluate minimum inhibitory concentration (MIC) of the extract. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm. The suspension of the strains were prepared in normal saline and the turbidity adjusted to 0.08-0.1 at 600 nm.

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S. aureus, E. fæcalis, and E. coli, while they were not active against P. aeruginosa (Shafaghat, 2009). In contrast, P. aeruginosa was susceptible to the extract of A. tenuifolia in the present work. Antioxidant activity of the plant extract attributed to the presence of phenols and flavonoids contents in different extracts (Asgarirad et al., 2010) and cytotoxicity of A. tenuifolia extracts against the larvae of Artemia salina have been previously reported (Saeidnia & Gohari, 2006). Presence of secondary metabolites of different classes including sesquiterpene lactones, flavonoids, tannins, and sterols and also their probable synergistic interactions could be responsible for the observed effect of the extract (Manayi et al., 2012a).

4. Conclusion

In conclusion, the antibacterial activity of methanol extracts of A. tenuifolia was mild against tested bacteria. Interestingly, P. aeruginosa was more susceptible to the extract comparing to other strains. Isolation and identification of pure compounds of the plant extract might provide an active agent against pathogenic bacteria to treat infections.

5. Acknowledgment

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6. References


İonemann (1999). Botanica, the illustrated A–Z of over 10000 garden plants and how to cultivate them, Hong Kong: Gordon Cheers Publication.


