IN VITRO ANTIMICROBIAL ACTIVITY OF ARTEMISIA ANNUA LEAF EXTRACTS AGAINST PATHOGENIC BACTERIA

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ABSTRACT
In recent years, the usage of plant materials as food supplement and as alternative medicine has increased. Medicinal herbs are a rich source of antimicrobial agents. In this investigation, antimicrobial effects of leaf methanol and ethanol extracts of Artemisia annua against Staphylococcus aureus PTCC 1431, Salmonella enterica PTCC 1231, Klebsiella pneumonia PTCC 1053, Shigella dysenteriae PTCC 1188 and Escherichia coli PTCC 1399 were studied, using well diffusion method. Methanol and ethanol extracts obtained from leaves of A. annua exhibited antimicrobial activity against test microorganisms. The methanol extract of A. annua showed high inhibition of the growth of S. aureus PTCC 1431, S. enterica PTCC 1231 with inhibitory, 16.5 and 15.5 mm, with average 13.7 mm. The results indicate the fact that extracts of A. annua could be effectively used against diseases caused by selected human pathogens.

KEY WORDS: Artemisia annua, Antimicrobial activity, Plant extract

INTRODUCTION
In the last three decades, although pharmacological industries have produced number of new-antibiotics, but microbial resistance to these antibiotics has increased because of genetic ability of the bacteria to acquire and transmit the resistance against therapeutic agents. Herbal drugs have been used since ancient times as remedies for various diseases across the world (Darsanaki and Parsa, 2014). Plant, as sources of medicinal compounds have continued to play a dominant role in the mainlenance of human health since ancient times (Kirbag et al., 2009). The genus Artemisia belongs to the Asteraceae family and comprises more than 400 species. Essential oils of Artemisia spp. have been widely used for a variety of medicinal purposes such as antimarial, antibacterial, antiviral, nematicidal and fungicidal for many years (Ahameethunisa and Hopper, 2010; Ahameethunisa and Hopper, 2012). A. annua is available commercially in China and Vietnam as an antimalarial drug and is efficacious against drug-resistant strains of Plasmodium. However, the plant grows wild and become naturalized in many countries, including Europe and America. It is cropped on a large scale in China, Vietnam, Turkey, Iran, Afghanistan, Australia, Argentina, Bulgaria, France, Hungary, Romania, Italy, Spain, and the former Yugoslavia. Artemisia (Asteraceae, Compositae family) has 34 species in Iran (Mueller et al., 2004; Pirali-Kheirabadi and Teixeira da Silva, 2011). The effect of plant extracts on microorganism have been studied by a very large number of researchers in different parts of the world (Woerdenbag et al., 1993; Juteau et al., 2002; Maggi et al., 2005; Verdián-Rizi et al., 2008; Li et al., 2011; Désirée et al., 2013; Appalasamy et al., 2014). The aim of the present study was to evaluate the antimicrobial activity of A. annua leaf extracts against pathogenic bacteria.

MATERIALS AND METHODS

Plant material and extraction
Fresh leaves of A. annua were collected from Guilan Province, North of Iran. Plant leaves were cleaned with deionized water and dried at shade for a week. After drying, the leaves were powdered and sieved using gauze cloth. Powdered leaves (50 g) were extracted with methanol and ethanol by the aid of a Soxhelet apparatus. Finally, the obtained solution was passed through Whatman No.1 filter paper and stored at 4°C for further antimicrobial activity study.

Tested microorganisms
The standard strains used in this study were Staphylococcus aureus PTCC 1431, Salmonella enterica PTCC 1231, Klebsiella pneumonia PTCC 1053, Shigella dysenteriae PTCC 1188 and Escherichia coli PTCC 1399. The strains were obtained from collection center of fungi and bacteria, Tehran, Iran. The microorganisms were grown overnight at 37°C.
for 24 h in Nutrient Broth (Merck; Germany). Ciprofloxacin (0.3%w/v) was used as a standard antibiotic for in vitro antimicrobial activity.

**Antimicrobial Activity Assay**

Agar well diffusion method used to detect antimicrobial was activities of leaf Extracts. Muller Hinton agar media (Merck, Germany) was used for conducting antimicrobial tests. The pathogenic strains (S. aureus PTCC 1431, S. enterica PTCC 1231, K. pneumonia PTCC 1053, Sh. dysenteriae PTCC 1188 and E. coli PTCC 1399) were adjusted to a density of 109 CFU/ml by adding sterile water and spread on the surface of MHA. 100 μl of leaf extract, placed into each well. The culture plates were incubated at 37°C for 24 h and antimicrobial activity was evaluated by measuring the diameter of the inhibition zone and presented in millimeter. The experiments were performed in duplicate and the mean values were observed. Statistical analyses were performed using SPSS software.

**RESULTS AND DISCUSSION**

Natural products are considered an important source of new antibacterial agents. Medicinal plants continue to be used world-wide for the treatment of various diseases and have a great potential for providing novel drug leads with novel mechanism of action (Vatľák et al., 2014). Table 1 showed the antimicrobial activity of leaf methanol and ethanol extracts of Artemisia annua by the agar diffusion method against selected pathogenic bacteria. Methanol and ethanol extracts obtained from leaves of A. annua exhibited antimicrobial activity against test microorganisms. The methanol extract of A. annua showed high inhibition of the growth of S. aureus PTCC 1431, S. enterica PTCC 1231 with inhibitory, 16.5 and 15.5 mm with average 13.7 mm and ethanol extracts showed antimicrobial activity against S. aureus PTCC 1431 and S. enterica PTCC 1231 with inhibitory, 15 and 13.5 mm with average 13.3 mm. Artemisia spp. have been widely used for a variety of medicinal purposes for many years. Many studies confirm positive role of A. annua extract in inhibitory pathogenic bacteria. Artemisinin, one of the bioactive compounds, with antimalarial activity has been successfully isolated from A. annua. Other than antimalarial activity, artemisinin was found to be a good antibacterial, antifungal, antileishmanial, and antitumor agent. The antibacterial properties of artemisinin had been tested on a wide range of bacteria, such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Mycobacterium intracellulare (Appalasamy et al., 2014).

Table 1. Antimicrobial activity of Artemisia annua leaf extracts by agar well diffusion method

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Ciprofloxacin</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus PTCC 1431</td>
<td>15±1.4</td>
<td>16.5±0.7</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>E. coli PTCC 1399</td>
<td>12±0.0</td>
<td>11.5±0.7</td>
<td>17</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumonia PTCC 1053</td>
<td>13±0.0</td>
<td>13±0.0</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>S. enterica PTCC 1231</td>
<td>13.5±0.7</td>
<td>15.5±0.7</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>Sh. dysenteriae PTCC 1188</td>
<td>13±0.0</td>
<td>12.5±0.7</td>
<td>19</td>
<td>-</td>
</tr>
</tbody>
</table>

Values, including the diameter of the well (6 mm), are means of duplicates ± standard deviation. Ciprofloxacin (positive control), DMSO (negative control).

In study by Désirée et al. (2013) antimicrobial activity of the essential oil extracted from A. annua grown in West Cameroon revealed that it was active against most isolates tested. Inhibition zone diameters varied from 6 (Pseudomonas aeruginosa and Shigella flexneri) to 45 mm (Vibrio cholerae). Appalasamy et al. (2014) reported that in vitro cultured plantlets of A. annua can be used as the alternative method for production of artemisinin and its precursor with antimicrobial activities. In Iran, Verdian-Rizi et al. (2008) and China, Li et al. (2011) assessed the antibacterial activity of A. annua on S. aureus and E. coli. In study by Juteau et al. (2002) A. annua essential oil remarkably
inhibited the growth of tested Gram-positive bacteria *Enterococcus hirae* and both tested fungi (*Candida albicans* and *Saccharomyces cerevisiae*).

**CONCLUSION**

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from microorganisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. In conclusion, the results clearly indicated that using *A. annua* extract had the beneficial effect in controlling the microbial infections.

**REFERENCES**


