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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.

Keywords: *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 [1]. Plant, as sources of medicinal compounds have continued to play a dominant role in the mainlenance of human health since ancient times [2]. 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 antimalarial, antibacterial, antiviral, nematicidal and fungicidal for many years [3, 4]. *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 [5, 6]. The effect of plant extracts on microorganism have been studied by a very large number of researchers in different parts of the world [7,8,9,10,11,12.13,14].The aim of the present study was to evalute 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 [11]. 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* [12].

In study by Désirée *et al*. 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*) [7]. Appalasamy *et al.* 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 [12]. In Iran, Verdian-Rizi *et al*. and China, Li *et al*. assessed the antibacterial activity of *A. annua* on *S. aureus* and *E. coli* [13, 14]. In study by Juteau *et al*. *A. annua* essential oil remarkably inhibited the growth of tested Gram-positive bacteria *Enterococcus hirae* and both tested fungi (*Candida albicans* and *Saccharomyces cerevisiae*) [10].

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

Pathogens	Ethanol	Methanol	Ciprofloxacin	DMSO
S. aureus PTCC 1431	15±1.4	16.5±0.7	25	-
E. coli PTCC 1399	12±0.0	11.5±0.7	17	-
K. pneumoniae PTCC 1053	13±0.0	13±0.0	20	-
S. enterica PTCC 1231	13.5±0.7	15.5±0.7	22	-
Sh. dysenteriae PTCC *1188*	13±0.0	12.5±0.7	19	-

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

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.

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