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Research Article

Antibacterial activity of *Achillea tenuifolia* Lam. extract against standard bacteria and isolated strains

Sahar Omidpanah[a], Abbas Hadjiakhondi[a,b], Azadeh Manayi[a*]

[a]*Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

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***Correspondence**
manayi@sina.tums.ac.ir
Tel/Fax: +982164121229

Abstract

Finding a new class of antibiotics against infectious diseases caused by different microorganisms is one of the most challenging issue for researchers in the recent decades. Drug resistance developed among bacteria due to the prolonged usage of the antibiotics; therefore, novel antimicrobial agents have been searched from different sources such as plants, which are used in traditional medicine. The aim of this study was to evaluate antibacterial effect of Achillea tenuifolia. The plant material was extracted by maceration method using methanol three times at room temperature. The extract was concentrated after removing the solvent by rotary evaporator and then lyophilized using freeze dryer. Inhibitory effect of the extract was examined against four standard bacteria and two isolated strains from diseased hen using disk diffusion method and microdilution method to evaluate their inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC), respectively. The results showed that the extract of the plant was active against standard strains including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis with IZDs of 10.3±0.5, 14±0.0, 12±0.0 and 11.6±0.5, respectively. However, growths of isolated strains were not inhibited in the presence of the extract. Although, the growths of isolated strains were not inhibited by the plant extract, the standard strains were moderately susceptible to the extract; among those P. aeruginosa was more sensible than other tested strains.

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 specious 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.

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

A. tenuifolia						
	S. aureus	***E. fecalis***	***E. coli***	***P. aeruginosa***	***S. thyphimurium****	***E. coli****
IZD (mm)	12±0.0	11.6±0.5	10.3±0.5	14±0.0	-	-
MIC (µg/mL)	50	50	50	50	-	-

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

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 faecalis* 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 0.5 McFarland with absorbance of 0.08-0.1 at 600 nm. The suspension of the bacteria diluted 1:100 (v/v) in Mueller-Hinton broth incubated at 37°C for 24 h. Briefly, bacterial suspensions (1×106 CFU/mL) were added to serial dilutions of the extract in a 96-well microtiter plate with concentrations ranged from 100-0.04 µL/well. The lowest concentration which was clear against a black background (no visible growth) were defined as MIC. Moreover, the disc diffusion method was applied for evaluation of inhibition zone diameters (IZD). Sterile cotton swab was used to spread microbial suspension on Muller-Hinton agar and all plates were incubated for 16 h at 37°C. Sterile paper discs (6 mm in diameter) were impregnated with 10 µL of methanol extracts (100 µg/mL) individually and concomitantly placed on the inoculated plates. All tests were performed in triplicate (Wayne, 2003). Thymol (100 mg/mL) was used as a positive control with concentration of 100 mg/mL.

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. faecalis*, 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. fecalis*. 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). Antimicrobial properties of extracts and oils of different specious of *Achillea* have been reported previously against various strains *in vitro* (Alsohaili & 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

S. aureus, *E. fecalis*, 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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