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Research Article

The Study Effects Antimicrobial of *Foeniculum vulgare mill* and *Achilles mille folium* Plant on Bacterial Pathogens Causing Urinary Tract Infections and Nosocomial Infection

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ABSTRACT

Urinary tract infections due to global prevalence and their complications such as, kidney stones, miscarriage, urinary tract disorders, hypertension, uremia, are very important. Moreover, they increase antibiotics resistance and drug side effects; all these reasons fo explain the replacement herbal medicines and natural antimicrobial substances with chemical drugs. In this study, the standard strains (ATCC) was used. Next, *Foeniculum vulgare mill* and *Achilles mille folium* plants during the growing season were collected and dried in a darkened place. Then, aqueous and alcoholic extracts of the plants were prepared by the soaking mmethod distillation with water. The concentrations of 0.25, 0.50, 1, 2, µg/ml were prepared. Then, in order to determine antimicrobial effects, the disk and agar diffusion method and (minimum inhibitory concentration) MIC were used. The results of this study showed that the alcoholic and aqueous extraction plants had antibacterial effects on these bacteria: *Staphylococcus aureus*, *E. coli*, *Staphylococcus saprophyticus* and *Pseudomonas aeruginosa*. The antibacterial activity increased with increasing concentration. In this study, *Achilles mille folium* plant did not affect the inhibitory of *Pseudomonas aeruginosa*. The findings of this study and other studies in this field showed that, alcoholic and aqueous extracts *Achilles mille folium* and *Foeniculum vulgare mill* plants can have the inhibitory effect on pathogenic bacteria.

Keywords: Urinary tract infection, Minimum inhibitory concentration, Disk diffusion method

INTRODUCATION

Urinary tract infections (UTIs) are the most common bacterial infection in human that can be seen in the all ages. Lack of appropriate diagnosis and treatment can result in some complications such as, urinary tract disorder, scars in renal parenchyma, hypertension, uremia, and preterm labor in pregnant women and miscarriage. Nosocomial infections (NI), a major public health care problem, is more prevalent in developed countries because of the related mortality and socioeconomic costs[1-3]. Nearly 12% of men and 20-10% of women during their lives, have experienced an acute symptomatic UTI and asymptomatic bacteriuria in most of the developed nation[4,5]. This bacterial infection is a serious threat too public health[1,6,7]. however, the high prevalence of UTIs, combined with the costs associated with medical intervention, have significant financial ramifications: it is estimated that the overall costs associated with UTI are nearly $2 billion each year[1,7]. Over 50 years, antibiotics have been used for the prevention and treatment of infectious diseases passed. But inappropriate use and persistent of this antibiotics causes the phenomenon of resistance to antibiotics and emergence of resistant strains of bacteria. It's difficult to treat diseases in humans and animals[8]. Medicinal plants have been used in treatment of infection diseases in humans for long time. It is estimated that more than 10% of the thousands of species of plants have known medicinal uses. The World Health Organization (WHO) has estimated that approximately 80% of world populations have used medicinal plants for their health care aspects[9]. *Achillea* genus is the Persian name of *Asteraceae* family. This plants contains phenolic compound, terpene, which are justifies properties wound healing, hemostatic, antiseptic, astringent and diuretic.The steam plant is used in traditional medicine for the treatment of abdominal pain and gastrointestinal discomfort. There is a high amount of the phenolic compound in the plant approving antioxidant properties and this candidate the plant for microbiology studies[10]. Fennel is, with the scientific name (*Foeniculum vulgare Mill*), a perennial herb that has been both form wild and cultivated *Foeniculum vulgare Mill* in Iran. Foeniculum vulgare Mill have been used in antiseptic in toothpaste, mouth rinses used[11].

MATERIALS AND METHODS

Collection of plant materials

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Achillea mille folium

Foeniculum vulgare mill

Escherichia coli (ATCC 25922)
Agar dilution

*Staphylococcus aureus (*ATCC 25923) *method disk diffusion*

Figure 1: Determination of antibacterial activity by agar dilution and disk diffusion method

The *Achillea mille folium* and *Foeniculum vulgare Mill* medicine plant was collected from Nahavand Mountains.

Preparation of the plant for extraction using aqueous and ethanol

First, aqueous and alcoholic extracts of the plants and essential oil by water distillation were prepared by soaking the plant extracts using the percolation method. Then, the concentrations of 0.25, 0.50, 1, 2 µg /ml of the extract solution of dimethyl sulfoxide (DMSO) were prepared and 30 microliters of each concentration was inoculated to a blank disk. Next, disks prepared to the overnight inside dilutions were made. After wards, the disks were kept at 25 °C for 5 hours to be dried.

Preparation test on test organisms

In order to evaluate the antimicrobial properties, four microorganisms were used. Two grams of positive bacteria *Staphylococcus saprophyticus* (ATCC 1440) and *Staphylococcus aureus* (ATCC 25923) and two grams of negative bacteria *Escherichia coli* (ATCC 25922), *pseudomonas aeruginosa* (ATCC 27853) microorganism strains were employed for determination of antimicrobial activity.

Determination of antibacterial activity

The disk diffusion method was performed using the standard procedure (NCCLS). The two solvent extracts for obtained 4 (dilutions: 0.5, 0.25, 1, 2 µg/ml) were investigated for the antibacterial activity by the disk diffusion method against clinical isolate of *Staphylococcus saprophyticus, Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa*. The bacterial suspension with a turbidity equivalent to 0.5 McFarland (1.5×10^8) CFU/ml in the BHI broth (Brain Heart infusion broth) was prepared. Then, the suspension on Muller Hinton Agar was cultured. The prepared disks of different concentrations antibiogram conditions were placed on the medium with the disk diffusion method. Then, the disk containing DMSO as a negative control and the antibiotic disk antibiotic Gentamicin (10mg) and Vancomycin (30mg) (Mast England) was used as a positive control. The plates were then incubated at 37 ℃ for 24 hours. Finally, the antibacterial activities of the plant extracts were evaluated (fig 1).

Determination of minimum inhibitory concentration (MIC)

Extracts that showed potent antibacterial activity was further tested to determine the minimum inhibitory concentration (MIC) for the bacterial samples. The MICs of these extracts were determined by the broth microdilution method.

Statistical analysis

Statistical package for the social sciences (SPSS) version 20 was used to analyze the data obtained.

RESULTS

In this study, the antibacterial activity of ethanol and water extract (*Achillea mille folium* and *Foeniculum vulgare Mill*) were survey on four bacterial strains of pathogenic bacteria *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Staphylococcus saprophyticus* (ATCC1440) performans. The results have been shown in (tables 1 – 8). Based on the data from (Table 1), the ethanol extracts of fennel (Foeniculum vulgare mill) in a dilution of 50 and 100 and 200 mg/ml respectively, of 6 and 10 and 11mm zone of inhibition against *Staphylococcus aureus* at concentrations of 100 and 200 mg /ml respectively, 6 and 10 mm zone of inhibition against *Staphylococcus saprophyticus* showed. The antibacterial activities of this plant ethanol extract against the gram-positive. The antibacterial effects of this plants ethanol extract against gram-negative bacteria was the way that the concentration of 200 mg /ml on *Pseudomonas aeruginosa* zone growth at a rate of 12 mm zone of inhibition was created. The plant ethanol extracts in the concentrations of 50, 100 and 200 mg /ml inhibition was observed in *E. coli*. The MIC of the aqueous extracts of *Achilles mille folium* showed that, the aqueous extracts of the plant inhibited the growth of *Staphylococcus saprophyticus* at the concentration of 5000 ppm, while at concentration of 10000 ppm, the aqueous extracts inhibited the growth of *Staphylococcus aureus;* also, the aqueous extracts of *Achilles mille folium* inhibited the growth of *Pseudomonas aeruginosa and Escherichia coli* at the concentrations of 20000 ppm and 2500 ppm, respectively. The MIC of the ethanol extracts of *Achilles mille folium* showed that, at the concentration of 10000 ppm, the ethanol extracts of the plant inhibited the growth of *Staphylococcus saprophyticus*, while, at the concentration of 5000 ppm, the ethanol extracts inhibited the growth of *Staphylococcus aureus;* further, the ethanol extract of *Foeniculum vulgare mill* inhibited the growth of *Pseudomonas aeruginosa and Escherichia coli* at the concentrations of 20000 ppm and 2500 ppm. The MIC of the aqueous extracts of *Foeniculum vulgare mill* showed that, at the concentration of 2500 ppm, the aqueous extracts of the plant inhibited the growth of *Staphylococcus saprophyticus* and *Escherichia coli; while*, at the concentration of 5000 ppm, the aqueous extracts inhibited the growth of *Staphylococcus aureus;* moreover, the aqueous extracts of *Foeniculum vulgare mill* inhibited the growth of *Pseudomonas aeruginosa* at the concentration of 20000 ppm. The MIC of the ethanol extracts of *Foeniculum vulgare mill* showed that, at the concentrations of 2500 and 5000 ppm, the ethanol extracts of the plant inhibited the growth of *Staphylococcus saprophyticus and Staphylococcus aureus*, respectively. While, the ethanol extracts inhibited the growth of *Pseudomonas aeruginosa* at concentration of 10000ppm*: and*, the ethanol extract of *Foeniculum vulgare mill* inhibited the growth of *Escherichia coli* at a very high concentration of 10000 ppm.

DISCUSSION

Nowadays, due to the increasing microbial resistance to commonly used to antibacterial drugs, finding new drugs for control and prevention of infections, particularly nosocomial infections, caused by resistant bacteria has been of interest to investigators and research organizations[12]. In the present study, the plant extracts of *Achillea mille folium* and *Foeniculum vulgare mill* with different dilutions for the antibacterial properties of these plants on pathogenic bacteria caused by urinary tract infection and nosocomial infection were used. The effect of the plant extracts on pathogenic bacteria in study, which significant results antibacterial effect showed in the disk diffusion method. This is significantly reason of development using natural medicine and herbal products in traditional medicine and complementary medicine. From between this medicinal plant, *Achillea mille folium* and *Foeniculum vulgare mill* wide range of therapeutic properties due to special attention from researchers in the world. *Achillea mille folium* is a medicinal plant known for thousands of years in the treatment of different types of disorders and diseases, especially infectious diseases[13,14]. In the current study, the results obtained from the disk diffusion method and MIC showed that the extracts (alcohol and aqueous) is effective on pathogenic bacteria and with comparing the results *Achillea mille folium* inhibitory effect of high on *Staphylococcus saprophyticus* and *Staphylococcus aureus* and the lowest prevention was *Pseudomonas aeruginosa*. *Foeniculum vulgare mill* plant showed the most effect on *Escherichia coli;*according to the results obtained, *Foeniculum vulgare mill* more effective on gram-negative bacteria. Notably, in this study of *Pseudomonas aeruginosa* resistant to these compounds

Table 1: Diameters of inhibition zone of the ethanol extracts, *Foeniculum vulgare mill* tested against microorganisms.

Ethanol extracts	Bacteria	Inhibition zone diameter and dilutions of extract ethanol (mg / ml) by the method of and dick diffusion (mm)				Control negative	Gentamicin (10mg	Vancomycin (30mg)
		25	50	100	200			
(Foeniculum vulgare mill)	*Staphylococcus saprophyticus*	0	0	6	10	0	0	18mm
	Staphylococcus aureus	0	5	9	11	0	0	18mm
	Pseudomonas aeruginosa	0	0	0	12	0	25mm	0
	Escherichia coli	0	8	11	14	0	25mm	0

Table 2: Diameters of inhibition zone of aqueous extracts, *Foeniculum vulgare mill* tested against microorganisms.

Aqueous extracts	Bacteria	Inhibition zone diameter and dilutions of extract ethanol (mg / ml) by the method of and dick diffusion (mm)				Control negative	Gentamicin (10mg)	Vancomycin (30mg)
		25		100	200			
(Foeniculum vulgare mill)	*Staphylococcus saprophyticus*	0	0	4	7	0	0	18mm
	Staphylococcus aureus	0	0	7	9	0	0	18mm
	Pseudomonas aeruginosa	0	0	0	9	0	25mm	0
	Escherichia coli	0	0	8	10	0	25mm	0

Table 3: Diameters of inhibition zone of ethanol extracts, *Achillea mille folium* tested against microorganisms.

Athanol extracts	Bacteria	Inhibition zone diameter and dilutions of extract ethanol (mg / ml) by the method of and dick diffusion (mm)				Control negative	Gentamicin (10mg)	Vancomycin (30mg)
		25	50	100	200			
(Achilles mille folium)	*Staphylococcus saprophyticus*	0	7	9	10	0	0	18mm
	Staphylococcus aureus	0	4	7	8	0	0	18mm
	Pseudomonas aeruginosa	0	0	0	2	0	25mm	0
	Escherichia coli	0	0	6	8	0	25mm	0

plants. In this research, *Achillea mille folium* and *Foeniculum vulgare mill* of the alcoholic extract of *Escherichia coli* and *Staphylococcus saprophyticus* was more inhibitory effect compared to the aqueous extract, while *Achillea mille folium* and *Foeniculum vulgare mill* aqueous extract and the alcoholic extract has very little effect on *Pseudomonas aeruginosa* ($P < 0.001$), while *Achillea mille folium* plant had no inhibitory effect on *Pseudomonas aeruginosa*. These results suggest that the antimicrobial activity depends on the type of microorganisms and compounds found in plants; these results are consistent with many studies. In a similar study, *Tajik et al.* evaluated the antibacterial effect of aqueous and alcoholic extracts of *Achillea mille folium* on pathogenic bacteria in laboratory conditions. The results of this study showed that *Achillea mille folium* had the most antibacterial effect on *Staphylococcus aureus* which results of in study with our study were similar that can *Achillea mille folium* approve antibacterial effect on *Staphylococcus aureus* in vitro[15]. Another study also showed the antibacterial effect of *Achillea mille folium*. *Aljancic et al.* reported that *Foeniculum vulgare mill* in the in vitro had a significant inhibitory effect on *Candida Albicans* and *Bacillus subtilis*. According to opinion these researcher's flavonoids in existence in of *Foeniculum vulgare mill* in addition to having an inhibitory effect on organisms that has an inhibitory effect on pathogenic fungi[16]. *Dehkordi et al.* studied the effect of antiparasitic *Achillea mille folium* on *Trichomonas vaginalis*[17]. Like this research, the study by *Amjad et al.*, showed the effect significant antibacterial of *Achillea mille folium* on *Staphylococcus aureus*[18]. Our study was similar in determination of dilution with the study by Amjad. In any of the concentrations, *Achillea mille folium* had no antibacterial effect on *Pseudomonas aeruginosa* but, in present study, the antibacterial effect of extracts *Achillea mille folium* in dilution of 200 mg / ml on *Pseudomonas aeruginosa*. The results demonstration that the *Achillea*

Table 4: Diameters of inhibition zone of aqueous extracts, *Achillea mille folium* tested against microorganisms.

Aqueous extracts	Bacteria	Inhibition zone diameter and dilutions of extract ethanol (mg / ml) by the method of and dick diffusion (mm)				Control negative	Gentamicin (10mg)	Vancomycin (30mg)
		25	50	100	200			
(Achilles mille folium)	*Staphylococcus saprophyticus*	0	0	11	12	0	0	18mm
	Staphylococcus aureus	0	0	8	10	0	0	18mm
	Pseudomonas aeruginosa	0	0	0	2	0	25mm	0
	Escherichia coli	0	0	0	9	0	25mm	0

Table 5: Minimum inhibitory concentration (MIC) of the aqueous extracts of *Achilles mille folium*

Aqueous extracts	Bacteria	Concentration in (PPM)			
		2500	5000	10000	20000
(Achilles mille folium)	*Staphylococcus saprophyticus*	-	*	-	-
	Staphylococcus aureus	-	-	*	-
	Pseudomonas aeruginosa	-	-	-	*
	Escherichia coli	*	-	-	-

* = indicate MIC

Table 6: Minimum inhibitory concentration (MIC) of the ethanol extracts of *Achilles mille folium*

Ethanol extracts	Bacteria	Concentration in (PPM)			
		2500	5000	10000	20000
(Achilles mille folium)	*Staphylococcus saprophyticus*	-	-	*	-
	Staphylococcus aureus	-	*	-	-
	Pseudomonas aeruginosa	-	-	-	*
	Escherichia coli	*	-	-	-

* = indicate MIC

Table 7: Minimum inhibitory concentration (MIC) of aqueous extracts of *Foeniculum vulgare mill*

Aqueous extracts	Bacteria	Concentration in (PPM)			
		2500	5000	10000	20000
(Foeniculum vulgare mill)	*Staphylococcus saprophyticus*	*	-	-	-
	Staphylococcus aureus	-	*	-	-
	Pseudomonas aeruginosa	-	-	-	*
	Escherichia coli	*	-	-	-

* = indicate MIC

Table 8: Minimum inhibitory concentration (MIC) of the ethanol extracts of *Foeniculum vulgare mill*

Ethanol extracts	Bacteria	Concentration in (PPM)			
		2500	5000	10000	20000
Foeniculum vulgare mill	*Staphylococcus saprophyticus*	*	-	-	-
	Staphylococcus aureus	-	*	-	-
	Pseudomonas aeruginosa	-	-	*	-
	Escherichia coli	-	-	*	-

* = indicate MIC

mille folium had antimicrobial effect at different dilutions[18]. Foeniculum vulgare mill in two forms wild and cultivated seen in Iran, so that all the parts of the plant Properties treatment, but most of the seeds of this plant used contain this volatile essential oil and the chemical composition. The anethol of this plants that most of the properties antibacterial related of this compound. On the other hand, the presence of anethole and estragole causes sweet taste in Foeniculum vulgare mill and is a volatile essential oil and due to the composition of this plant as a disinfectant and bactericide in toothpaste, mouth rinses

used[11]. There is a few studies on the antibacterial effect Foeniculum vulgare mill on urinary pathogens.These are reasons for the selection of this plant used in this study. The study by *Ranjbaran et al.* investigated the antibacterial effect foeniculum vulgare mill on *helicobacter pylori*. 64.34% of *helicobacter pylori* strains were isolated from patients sensitive to these plant extracts[19]. In onother study,it was shown the antibacterial effect of fennel plants on *Salmonella typhimurium*[20].

CONCLUSION

According to the antibacterial effect of the plant extracts on Gram-positive and Gram-negative and due to their chemical composition, it can be concluded that the use of some of these plants in the pharmaceutical industry can reduce the cost of healthcare. Furthermore, increasing restrictions on the use of antimicrobial chemicals and complications and drug resistance, it is required to replace these plants with natural materials. This issue could be in the context of studies for the replacement of plant material in controlling food and human diseases.

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