

Evaluating antimicrobial effects of different orange blossom extract (*Citrus aurantium* L) on microbial species *in vitro*

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ABSTRACT

Citrus aurantium L is rich of flavonoids, many flavones, volatile compounds and monoterpenes hydrocarbons. Orange blossom extract has antioxidant and antimicrobial features. In Iranian traditional medicine, orange blossom is known as a comforting and sleep booster plant. According to disrupt and side effects of chemicals and its high costs, the use of herbs in the prevention and treatment of bacterial factors is very important, also different extracts and biological and pharmaceutical properties on the bacteria species were studied by sink and micro dilution methods. Extraction was done with aqueous methanol and acetone solvents using percolation method and then were dried. Different concentrations were prepared from concentrated extracts for disc diffusion and MIC. The results showed that the antimicrobial properties of acetone extract are better than methanol and water extracts by micro dilution method on *Klebseilla* and the related MIC is 100 mg/ml. The average

diameter of inhibition zone was 11.33 mm. Methanol extracts had better activity on *E.coli*. The related MBC and average diameter were 150 mg/ml and 12 mm, respectively. Orange blossom showed antibacterial effects for higher concentration of methanol and acetone.

Keywords: Antimicrobial effects, *Citrus aurantium L*, extract, microbial species

INTRODUCTION

Citrus aurantium L. (Rutaceae) is a tree with a height of 3-5 m with round crown flower that has seen in northern and southern area of Iran (Figure 1).



Fig. 1. *Citrus aurantium L.*

Flowers are single or multiple shapes distributed on branch axis. These flowers are consisted of thick and juicy aromatic petals with off-white color on which secretors cavities are easily seen and widely used in nutrition and pharmaceutical plants [1,2]. Orange is obtained from *Citrus* and also cultivation of *Citrus* is one of the most important commercial, industrial, and agriculture activities in the world. *Citrus* is a rich source of flavonoids, many flavones, volatile compounds

and monoterpenes hydrocarbons. Many flavones are in oil [3], and plant extracts that have been considered biologically active compounds [4,5] and also *Citrus* has also antimicrobial properties. *Citrus aurantium* has been used as food and medicine which has many medicinal properties and biological effects [7]. There are more than ten compounds in orange blossom extract which major parts are consisted of monoterpenes, β -pinene, limonen, linalool, nerylacetate, linalylacetate and α -terpinylacetate. It has antidepressant, disinfectant, antispasmodic, and sedative properties with any special side effect. Also, its usage is allowed during pregnancy, childbirth and after childbirth [8]. Orange blossom extract has antioxidant and antimicrobial properties. In Iranian traditional medicine, orange blossom is known as a comforting and sleep booster plant. The orange blossom for reducing anxiety is the same as diazepam so it can be replaced by diazepam [9]. The use of herbs in the prevention and treatment of bacterial problem is very important, especially the indiscriminate use of antiseptics and

antibiotics which leads to many problems such as drug resistance host and environmental problems [10]. However, high price of antibiotics and the high cost of treatment and chemical products which create tendency toward effective, cheap and low-risk natural ingredients. Many studies were done using plants to prevent the growth of pathogenic bacteria [11]. Plants and their natural products are wide group of compounds with therapeutic properties [12], not only as a medicine but also as an unique template which are used to make synthetic analogs [13], control of diseases, especially infectious diseases by natural remedies were increased in recent years. Researchers i have a great desire to use natural drugs to treat infections which have considerably lower effects than chemical drugs [14]. Many medicinal and antimicrobial properties of plants are active substances such as phenols and flavonoids [15,16] due to medical and health issue[17, 18], biological and pharmaceutical properties of orange blossom and its application in traditional medicine [19]. Today, natural products and herbs compared with a variety of industrial and chemical innovation. In this study, antibacterial activity of plant extract was evaluated in traditional medicine and modern medicine. The antibacterial effect of methanol, water and acetone extracts on *Escherichia coli* and *Klebseilla* were evaluated by sink and micro dilution methods.

MATERIALS AND METHODS

Collection of plants

In this experimental laboratory study, flowers of *Citrus aurantium* L were collected from orchards in Mazandaran province (central part of Sari, Esfivard-e Shurab rural district, village of Kordkhil_Galeh Kala Sofla) and they were identified by botanists in Payam Nor University (PNU).

Collected plants were dried in the shade, away from direct sunlight, and then were crushed into smaller pieces [20].

Preparation of plant extracts

To extract orange blossom extract, flowers of *Citrus aurantium* L were used and then dried herbs were divided into three equal parts and were added solvents such as acetone and methanol to each parts (Merck, Germany) for percolation extraction method. Each of the solvents was removed three times by vacuum rotary evaporator and were dried by freezing device at -50°C and 80°C [21,22].

Evaluating antimicrobial effects

Concentrated extracts of orange blossom (*Citrus aurantium* L) were prepared by DMSO 10 % to obtain concentrations of 150, 100, 75, 50, 37.5 and 25 mg/ml. Antibacterial properties were used by two sink and micro dilution methods on

Klebsiella (ATCC 7881) and *Escherichia coli* (ATCC: 25922) which were prepared from Tehran University [23]. Fresh and young culture of bacteria colonies were transferred to Mueller-Hinton broth (Fluka, USA) to prepare microbial suspension. Microbial suspension opacity was adjusted according to McFarland standard (equivalent opacity 1.5×10^8 bacteria per ml) [24]. In this the research, the antimicrobial effect of aqueous acetone, and methanol extracts were studied by agar well diffusion and micro dilution. Well diffusion method: microbial suspension was transferred and cultured against 1.5×10^8 CFU/ml on agar Mueller-Hinton and then concentrations of 150, 100, 75, 50, 37.5 and 25 mg/ml were added on the wells and used as a negative control using a solution of DMSO 5% and chloramphenicol antibiotic which was used as a positive control [25,26]. Plates were incubated for 24 h at 37°C and microbe cultures were measured [27]. Tube Dilution method: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanol extract were determined. Then all tubes were observed and related MBC was determined through culturing in plate [28]. Any of the above experiments were repeated 3 times to reduce the error. The analysis of variance was used to evaluate the significant differences between these groups [29].

RESULTS

To evaluate the antimicrobial property of acetone extract of *Citrus aurantium* L on *Klebsiella*, 100 mg/ml was chosen with higher concentrations of MBC, 150 mg/ml, and the maximum diameter of 12 ± 1 mm (Table 1). Antibacterial property of acetone extract of *Citrus aurantium* L and related MIC on *E. coli* was determined in a concentration of 100 mg/ml and related MBC was 150 mg/ml. It is also the same for 11.66 ± 2.3094 mm (Table 1).

To evaluate antibacterial properties of methanol extract of *Citrus aurantium* L, the concentration of methanol extract for MIC on *Klebsiella* was 100 mg/ml and for MBC was 150 mg/ml and related diameter was 11.33 ± 1.1547 mm (Table 2).

The effect of methanol extracts of *Citrus aurantium* L concentrations of *E. coli* in well and micro dilution methods:

The antimicrobial property of *Citrus aurantium* L methanol extract for MIC was better in the concentration of 100 mg/ml on *E. coli* on tube method and related MBC was higher concentration and the maximum diameter was 12.66 ± 1.5275 mm (Table 2).

The effect of *Citrus aurantium* L concentrations on *Klebsiella* by well and micro dilution methods:

Klebsiella does not show any sensitivity to different *Citrus aurantium* L water extract

concentrations and extract was ineffective on it (Table3).

The effect of water extracts of *Citrus aurantium* L concentrations of *E.coli* by well and micro dilution methods:

E.coli does not show any sensitivity to different *Citrus aurantium* L water extract concentrations and extract was ineffective on it (Table 3).

Comparing bacteria on different extracts of water, acetone and methanol *Citrus aurantium* L: According to comparison and analysis of different flowers mean diameter on *Citrus aurantium* L and *E.coli* (Figure 2) and *Klebsiella* (Figure 3) showed that there is a significant difference ($p \leq 0.05$) between different concentrations of each extract on *Klebsiella* and

E.coli. In ANOVA test with calculating standard deviations, on the *E. coli* has better effectiveness and they can be used in antimicrobial activity in cases of diseases, and it is better to be used in this way. Therefore, due to the favorable antibacterial effect of *Klebsiella* and *E.coli* extracts of water, acetone and methanol of flower *Citrus aurantium* L; in this study, it appears that secondary compounds in leaf organs can have an effective treatment of pneumonia and urinary tract infection. The antimicrobial effect of *Citrus aurantium* L methanol extract compared with aqueous and acetone extracts on *E.coli* (Figure 2), acetone extract and methanol extracts on *Klebsiella* (Figure 3) was more effective.

Table. 1. Mean diameter of microbial standard strains (mm) with different concentrations of acetone on *Citrus aurantium* L

Concentration of each extract of the aerial parts (mg/ml)								
Microbial standard strain/Density	150	100	75	50	37.5	Positive control	Negative control	P-value
<i>Klebsiella</i>	12±1	8±1.7321	4.33±1.5275	R* ¹	R	20.5	-	0.001
<i>Escherichia coli</i>	11.66±2.3094	7.33±0.5774	3.66±1.5275	R	R	22	-	0.018

Table. 2. Mean diameter in microbial standard strains (mm) compared with different concentrations of methanol extract of *Citrus aurantium* L

Diameter and concentration of each extract (mg/ml)	
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Microbial standard strain/Density	150	100	75	50	37.5	Positive control	Negative control	P-value
<i>Klebsiella</i>	11.33±1.1547	7±2.0817	4±1.7321	R	R	20.5	-	0.001
<i>Escherichia coli</i>	12.66±1.5275	7.66±1.1547	4.66±2.3094	R	R	22	-	0.018

Table 3. Mean diameter of non-growth halo in microbial standard strains (mm) compared with different concentrations of water extract of *Citrus aurantium* L

Non-growth halo diameter in concentration of each extract of the aerial parts (milligrams per milliliter)							
Microbial standard strain/Density	150	100	75	50	37.5	Positive control	Negative control
<i>Klebsiella</i>	R	R	R	R	R	20.5	-
<i>Escherichia coli</i>	R	R	R	R	R	22	-

*R= It is ineffective

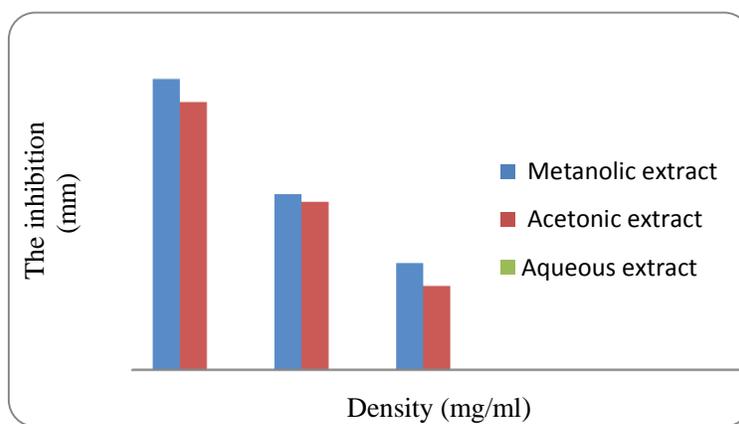


Fig. 2. *Escherichia coli* different extracts of

coli bacteria on *Citrus aurantium* L.

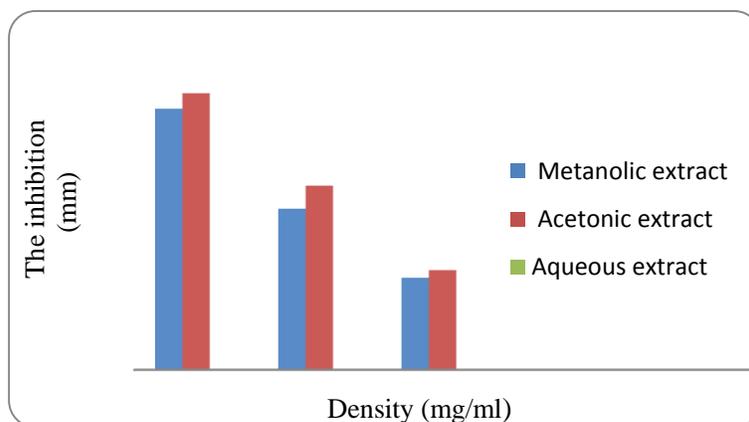


Fig. 3. *Klebsiella* bacteria on different extracts of *Citrus aurantium* L.

DISCUSSION

Medicinal plants have been used for many years. Nowadays, many researches have done identification and using active substances of medicinal plant [30]. Some microbes and fungi resistance to the drug have been increased that lead to difficulties in treatment process. Therefore, researchers and scientists are developing the use of new natural antimicrobial substances [31]. Some plant compounds could act like an antibiotics [32]. In a study, the effect of orange blossom smell was reviewed on the quality of heart patients in special care sections and was shown that aromatherapy with orange blossom can be used as an auxiliary treatment for sleep disorders in heart patients [33]. In another study, the antioxidant and antibacterial effects of extracts of leaf was assessed [34]. In the present study, the inhibition diameter related to methanol extract and acetone extract were 12.66 ± 1.5275 mm and 12 ± 1 mm, respectively. In another study, it was found that aromatherapy of orange

blossom and lavender can be used in relieving pain of burning [35]. According to many studies, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* bacteria are sensitive [36]. In this study, aqueous extract have the most sensitive to *Escherichia Coli* and *Klebsiella*. In a study, antibacterial activity of *Pistacia lentiscus* methanolic extract on *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sanguis*, *Pseudomonasaeruginosa* showed 16 mm and 12 mm, respectively [37]. It was found that orange blossom has biological, antioxidant and antibacterial properties [2]. In another study, n-hexane, ethyl acetate, chloroform, acetone, methanol and aqueous extracts were prepared by maceration. Antibacterial activity was carried out using agar well diffusion method. The aqueous extract showed maximum inhibition of *E. coli*, *S. typhimurium*, *E. amnigenus*, *S. pyogenes*, and *S. aureus*, (18.0 ± 1.0 mm, 12.5 ± 0.7 mm, 17.0 ± 0.0 mm, 11.0 ± 0.0 mm and 15.3 ± 2.0 mm), respectively. Similarly, n-hexane extract showed

maximum inhibition of *E.coli*, *E. amnigenus*, *S. aureus* (11.6±1.5mm;11.3±1.5mm;13.3±0.5 mm), [38]. In this study, methanol or acetone extracts have antibacterial effect and aqueous extract was ineffective. In another study, the results showed that orange blossom plant and its extracts have antiemetic effect on chickens [39]. In a study, it was tried to determine the efficacy of aromatherapy with *Citrus aurantium* oil in reducing anxiety during the first stage of labor. The levels of anxiety in the aromatherapy group were significantly lower in comparison with the control group and hence is confirmed aromatherapy with *C. aurantium* blossom oil as a simple, inexpensive and effective intervention to reduce anxiety during labor [40].

CONCLUSION

Many medicinal properties and antimicrobial due to the presence of alkaloids such as phenol and flavonoid and that the plant had already been reported and there has scientific and research activities in traditional medicine. Results showed that the methanol extract and acetone extract have good activity against tested bacteria. Many medicinal properties and antimicrobial plants due to the presence of alkaloids, phenol and flavonoid.

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