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Evaluation of antibacterial activity of methanolic extracts of *Francoeuria Undulata* in two harvest times on several aerobic bacteria causing vaginal infections

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ABSTRACT

Background & Aim: Vaginal disease has always been a great concern of women. Medicinal plants as natural resources with fewer side effects can effectively treat these infections given the diversity of these infections, resistance to antibiotics and side effects of existing drugs. The present study aimed to compare antimicrobial effects of methanol extract of *Francoeuria Undulata* on growth of several gram-positive and gram-negative bacteria causing vaginal infections.

Experimental: *Francoeuria Undulata* as an indigenous Iranian plant was collected in two harvest times (T_1 and T_2). Methanol extract of aerial parts of the plant was prepared. The effects of various concentrations (62.5, 125, 250 and 500 mg/ml)of methanol extracts were studied onKlebsiella and Enterobacter as gram-negative pathogens. Minimum Inhibitory Concentration (MIC) of bacterial growth and Minimum Bactericidal Concentration (MBC) were determined using macro-dilution method. Data analysis was performed using ANOVA.

Results: The results showed that methanol extracts of aerial parts of *Francoeuria Undulata* inhibit bacterial growth of *Klebsiella pneumoniae* and *Enterobacter aerogenes*. MICs varied from 125mg/ml to 250 mg/ml in the old and new harvest times. Therefore, the smallest diameter of inhibition zone belonged to *Klebsiella pneumoniae* at 62.5mg/ml concentration in the old harvest time and the largestdiameter of inhibitory zone belonged tothe second strains of *Klebsiellapneumoniae* at 500mg/ml concentration in the new harvest time.

Recommended applications/industries: The findings of the study indicated that methanol extracts of *Francoeuria Undulata* at two old and new harvest times had strong antibacterial and inhibitory effects on gram-negative bacteria causing vaginal infections.

1. Introduction

Medicinal plants are divine blessings with an important role in maintaining health and treating various diseases. These herbs are known as valuable resources for treating a variety of diseases for many years (Shanazis, 2007).

Nowadays, bacterial resistance and increased number of different antibiotic-resistant bacterial strains are great concerns of medical science. It is essential to find a suitable herbal alternative to combat pathogens. Valuable biodiversity and natural production of medicinal plants in Iran can lead to production of significant amounts of medicinal plants with proper planning (Rezaei *et al.*, 2008).

Francoeuria Undulata with local name of crisp-leaved fleabane has different species in various regions of Iran. Anticancer and antiviral properties of this plant have been reported in different studies (Algabr, 2012; Amin, 2002).

Barry *et al.* (1970) and Gerging *et al.* (1991) studied antibacterial activity of ethanol extract of this plant on several standard bacterial strains. They showed strong antibacterial activity of this extract on *Escherichia coli* (ATCC 25923), *Staphylococcus aureus* (ATCC25923), *Proteus vulgaris* (ATCC6380) and *Klebsiella pneumoniae* (ATCC 1312) (Ravandeh *et al.*, 2011; Barry *et al.*, 1970).

Female genital infections include a complex of gram-positive cocci (Streptococcus and Staphylococcus) and gram-positive bacilli as normal flora and gram-negative bacilli (Enterobacteriaceae) and gram-variable coccobacilli (*Gardnerellavaginalis*). Genital infections are the most common diseases among womenassociated with high social, emotional and physical consequences (Gerging *et al.*, 1991). Given increased drug resistance to antibacterial and antifungal drugs, it is essential to find herbal compounds with strong antimicrobial properties to treat vaginal infection with fewer side effects and lower costs (Steven, 2010).

Therefore, the study aimed to assess the effects of methanol extracts of aerial parts of *F.undulata* attwo harvest times on several gram-positive and gram-negative aerobic bacteria causing vaginal infections.

2. Materials and Methods

2.1. Extraction of methanolic extract of Francoeuria Undulata

In this study, *Francoeuria Undulata* was collected at two periods from Ardestantown in northeastern Isfahan: a) September 2014 andb) September 2015.

Aerial parts of the plant were dried out at room temperature in the shade. Dried-out samples were powdered using a grinder and kept at 4ºC (Nabipour, 2015). Then, powder was soaked in 80% methanol for 72 hours to prepare the extract. For this purpose, 50 grams of the powder was mixed with 500 ml of 80% methanol. The mixture was shaken to mix the powder with the solvent. Then, the flasks containing the mixture were covered with aluminum taps. The flasks were shaken at 150rpm for 72 hours at room temperature. These extracts were filtered with a filter paper and transferred to sterilized plates at ambient temperature until they were dried out. Then, the extracts were condensed with 10%dimethyl sulfoxide (DMSO) solvent. Then, the extracts were prepared at 62.5, 125, 250 and 500 mg/ml concentrations (Cowan, 1999; Skocibusic*et al.*, 2004).

The bacteria were isolated from 100 inpatients and outpatients aged from 20 to 50 who visited the Shahid Beheshti Gynecologic Clinic in Isfahan.

2.2. Antibacterial assay

Blood Agar and EMB (Daruash-Tehran) media were used for primary culture of the isolates. Biochemical and confirmatory tests were used to detect the bacteria (Sokmen *et al.*, 2003; Mardane, 2012).

Bacterial genus and species were detected and confirmed using the above tests (Naghsh, 2013). Antibiogram patterns were determined using antibiotic discs (5µg trimethoprim, 30µg ceftazidime, 30µg cefotaxime, 5µg ciprofloxacin, 10µg gentamicin, 30µg cefalotin, 5µgcefixime, 30µg tetracycline, 10µg streptomycin, 10µg penicillin). Ciprofloxacin was identified as a positive control given antibiotic sensitivity and resistance of each bacterium to various antibiotics and common sensitivity tociprofloxacin.

Isolated bacteria were glycerolizedin a freezer at 20°C. All isolates were revived by standard methods. Fresh cultures of multi-colony bacteria were transferred to Mueller-Hinton broth to prepare bacterial suspension. Turbidity of bacterial suspension was set in accordance with McFarland Standard as1.5×10^6cfu/ml. Antimicrobial effects of methanol extracts of aerial parts of *F. undulata*at two harvest times on several gram-positive and gram-negative aerobic bacteria causing vaginal infections were studied using two methods of serial dilutions and well-plate. Various concentrations of the extract were prepared for both old and new harvest times.

In well-plate method, 100 ml of microbial suspension with concentration of 1.5×10^6cfu/ml was evenly spread in Mueller-Hinton agar medium. Then, three wells with 6mm diameter at 2.5cm distance were created in each medium. Then, 100ml of each concentration was transferred to each well. The wells were sealed (bottom of the wells were closed). Negative control was determined as 10% DMSO and 10% Tween80. Positive control was determined as 5µg ciprofloxacin. All media were incubated for 24h at 37°C. Then, bacterial cultures were evaluated in terms of presence or absence of inhibition zone around the wells (Rajabpour *et al.*, 2013). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of methanol extracts were determined using tube dilution method (macro-dilution) (Talebi, 2014).

To determine the MICs, 62.5, 125, 250 and 500 mg/ml dilutionsof methanol extracts were prepared. In addition, one ml bacterial suspension with a concentration of 1.5×10^6cfu/ml was added to every dilution. Tubes of positive control (with bacterial medium) negative control (without bacterial medium) were prepared. The tubes were incubated for 24 h at 37°C. After incubation, the tubes were studied in terms of turbidity and growth of inoculated bacteria.

2.3. Statistical analysis

Each test was repeated 3 times. Then, mean and standard deviation were calculated. Data analysis was performed using SPSS20, ANOVA and LSD. The difference between groups was determined at $P<0.001$ significance level.

3. Results and discussion

The results of various concentrations of methanol extracts of the plant in both old and new harvest times using well-plate method are shown in Table 1.

The discs impregnated with 10% DMSO and 10%Tween80were determined as negative controls and 5µg ciprofloxacin disc was determined as positive control. The results showed that methanol extract of either old or new harvest inhibited growth of a large number of *Klebsiella pneumoniae* and *Enterobacter aerogenes*. The inhibitory effect was increased with increasing concentration of methanol extract of the bacteria. Increased inhibition was manifested as increased diameter of inhibitory zone.

The results showed that diameter of the inhibition zone was increased byincreasing concentration of the methanol extract.Increase in both isolates of *Klebsiella pneumoniae* and *Enterobacter aerogenes* represent the presence of Enterobacteriaceae Family. Thisincrease was also observedin both old and new harvest. However, diameter of the inhibitory zone in new extract was smaller than the old extract.

The findings suggested thatthe smallest diameter of inhibition zone belonged to several isolates of *Klebsiella pneumoniae* with 62.5mg/ml concentration of the old extract. There was no significant difference between the old extract and the negative control in terms of inhibition zone. The largest diameter of inhibition zone belonged to several isolates of *K. pneumoniae* with 500mg/ml concentration of the new harvest. There was a significant difference between the new extract and the negative control in terms of inhibition zone. The difference may be attributed tosub-strains, which can be evaluated using molecular identification.

Contents of Table 2 show MICs and MBCs ofthe methanol extracts regarding two types of bacteria. The results showed that125mg/ml concentration of methanol extract of the plant has lethal effects on both bacteria in the new harvest. In addition, 250mg/ml concentration of methanol extract of the plant has lethal effect onboth bacteria in the old harvest.

The results showed significant difference between antibacterial properties of methanol extracts of the plant ($P<0.001$). In other words, antimicrobial effect of the plant decreased in the old harvest compared to the new harvest.

The findings of the study showed that methanol extract of aerial parts of *F. undulata*in the new harvest in 125mg/ml concentration inhibited growth of several aerobic gram-negative bacteria causing vaginal infection. However, methanol extract of aerial parts of the plant in the old harvest at 250mg/ml concentration inhibited growth of aerobic gram-negative bacterial pathogens.

Ethanol extract of the *F.undulata*has the greatest antibacterial effect on gram-negative bacteria (Ravandeh *et al.*, 2011; Barry *et al.*, 1970).

Table 1. Mean ± SD of diameter of inhibition zone of two different genera of aerobic bacteria causing vaginal infections compared to methanol extracts of the plant.

Bacteria		Concentration of the extract (mg/ml)								Control		
		62.5		125		250		500		Positive		Negative
		A	B	A	B	A	B	A	B	A	B	A, B
K. pneumoniae	K1	6	4±0.2	12	10	19.8±0.2	17.7±0.2	26.4±0.3	24±0.3	26	24	6
	K2	9.8±0.2	8	18.3±0.4	15.7	22±0.2	24.2±0.2	30	28±0.2	22	23	6
E. aerogenes	En1	12	10	17.8±0.2	15.5±0.2	21.8±0.2	20	26	26	23	22	6
	En2	12	10±2	18±0.2	14±0.5	22	20±0.2	24.4±0.4	24±0.2	23	22	6

The old and new harvest at 62.5, 125, 250 and 500 mg/ml concentrations
Positive control: Ciprofloxacin
Negative control: 10% DMSOand 10% Tween80.
Diameter of the wells is 6mm diameter.
K1, K2: the first and second strain of *K. pneumoniae*
En1, En2: the first and second strains of *E. aerogenes*
A: new harvest time
B: old harvest time

Table 2. MICs and MBCs of methanol extracts of the plant in the new and old harvest times regarding studied bacteria based on mg/ml

Bacterial species	MIC (mg/ml)		MBC (mg/ml)	
	A	B	A	B
K. pneumoniae (K1, K2)	125	250	62.5	125
E. aerogenes (E1, E2)	125	250	62.5	125

• A: the new harvest
• B: the old harvest

Barry *et al.* (1970) showedstrong antibacterial effect of ethanol extract of aerial parts of Francoeuria on *E. coli*, *Pseudomonas*, *Staphylococcus aureus* and *Proteus* (Ravandeh *et al.*, 2011).

Gerging *et al.* (1991) studied antibacterial effect of *Francoeuria Undulata* and *Francoeuri acripsa* on *Staphylococcus aureus*, *Bacillus subtilis* and *Klebsiella pneumoniae*. They showed that MIC was larger in *Francoeuria Undulate* compared to *Francoeuriacripsa* (Barry *et al.*, 1970).

Mohammadi *et al.* (2010) studied the effect of methanol extracts of *Wilhelm aquila*. They studied gram-positive and gram-negative bacteria due to a specific structure between walls of these two types of bacteria (Khanafari and Hosseini, 2000).

Amjad *et al.* (2010)showed thatmethanol extract of leaves and flowers of *Achillea wilhelmsii* has significant effects on gram-positive bacteria including Staphylococcus. This effect was weaker on gram-negative bacteria. This may be due to presence of lipopolysaccharide cellular wallsof the gram-negative bacteria (Amjad *et al.*, 2010).

Methanolic extract of aerial parts of *F. undulate* inhibits growth of gram-negative bacteria due to specific structure of cellular walls of these of bacteria.

These bacteria have an outer membrane, which acts as a solid wall and prevents passage of large and hydrophobic molecules. Since majority of compounds in the extracts are hydrophobic, it can be concluded that methanol extract of the plant inhibits access to gram-negative bacteria as gram-positive bacteria. The extract significantly inhibits growth of gram-negative bacteria, especially the bacteria causing aerobic vaginal and urinary tract infections. The extract can be considered as a natural herbal product (Juven *et al.*, 2009; Mckeegan *et al.*, 2002).

4. Conclusion

The results of the present study indicated that the plants assayed possess antibacterial properties. This explains the use of plants in folk medicine for the treatment of various diseases whose symptoms might involve bacterial infections and underline the importance of the ethnobotanical approach for the selection of plant in the discovery of new bioactive compounds. Further phytochemical research is needed to identifythe active principles responsible for the antibacterial effects of this medicinal plant.

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