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Full Length Research Paper

Evaluation of antibacterial activity of three Iranian medicinal plants

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Medicinal plants are the oldest known source for treatment of disease. Using pharmaceutical plants and plant extracts have been at great attention. In this research, the antibacterial activity of the methanolic and aqueous extracts of three Iranian medicinal plant species namely *Capparis spinosa* L.*, Adiantum capillus-veneris* L. and *Sambucus ebulus* L. were tested against six bacteria strains including (*Staphylococcus aureus*, *Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa*). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for each plant were determined by microdilution method. The maximum antimicrobial activity of aqueous extracts was exhibited by *S. ebulus*. The methanolic extract of *S. ebulus* had the best MIC values against *S. epidermidis, S. aureus*, *B. subtilis* and *K. pneumonia* (25, 50, 100 and 100 mg/ml, respectively) and the methanolic extract of *A. capillus-veneris* had the best MIC value against *S. aureus* and S. *epidermidis* (12.5 and 50 mg/ml) and the methanolic extract of *C. spinosa* had the best MIC value against *E. coli* (100 mg/ml).

Key words: *Sambucus ebulus*, *Adiantum capillus-veneris, Capparis spinosa,* Antibacterial effects, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in Iran, India and China.

In recent years, multiple drug resistance in human has been developed due to indiscriminate use of commercial antibacterial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search new antibacterial agents in various sources like medicinal plants (Kumar et al., 2006). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Jeyachardran et al., 2010). *Adiantum capillus-veneris* L and *Sambucus ebulus* L. grow in the northern regions of Iran especially in the Mazandaran Province. The leaves and rhizomes of *S. ebulus* (Caprifoliaceae) are used in Iranian traditional medicine for treating some inflammatory cases such as, bee and nettle, arthritis, and sore-throat, anti-hemorrhoid, anti bacterial toward *Helicobacter pylori*, treating burns and infectious wounds, edema, eczema, urticaria, the cold, inflammation and rheumatism (Ebrahimzadeh et al., 2009). *A. capillus-veneris* is (Polypodiaceae) used as expectorant, diuretic, febrifuge, as hair tonic, in chest diseases, in catarrhal infections, to treat hard tumours in spleen and it is anticancerous (Piyali et al., 2005). *A.capillus-veneris* is traditionally used to treat infectious diseases (Singh et al., 2008). *Capparis spinosa* (Capparidaceae) can be found in different parts of Iran e.g. Alborz province. The flowers, roots and fruits are used as antirheamatic, antiinfertility, stimulant, treat ears pain, antiseptic, anti-inflammatory, diuretic and for flu treatment (Ali-Shtayeh et al., 1998). Fermented capers are an important seasoning in the Mediterranean kitchen

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and are greatly appreciated for their flavor, appetite and digestive properties (Pulido et al., 2005).

In the present study, the methanolic and aqueous extracts of these three plants which have been used in Iranian folk medicine were screened for their antibacterial activity against 6 standard bacterial strains including *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 10031) and *Pseudomonas aeroginos*a (ATCC 27853) by cup plate method. MIC and MBC values using microdilution method determined also.

MATERIALS AND METHODS

Plant material

The fresh leaves *S. ebulus* and the aerial parts of *A. capillus-veneris* were collected from Mazandaran province, north of Iran in July 2010. The fresh flowers of *C. spinosa* were collected from Alborz Province, Iran in August 2010. All plants were identified by Mr. M.Kamalinejad in the Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Voucher specimens No. 1286, 1511, 1186 for *C. spinosa, A. capillus-veneris* and *S. ebulus* have been deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Bacterial strains

Gram-positive bacteria including *S. aureus (*ATCC 25923*)*, *S. epidermidis (*ATCC 14990*)* and *B. subtilis (*ATCC 6051*),* and Gram-negative bacteria including *E. coli (*ATCC 25922*)*, *K. pneumonia (*ATCC 10031*)* and *P. aeruginosa* (ATCC 27853) were obtained from Iranian Research Organization for Science and Technology, Persian Type Culture Collection, (PTCC), Tehran, Iran.

Plant extraction

50 g of dried ground materials were separately extracted by 500 ml water and methanol using maceration method. The methanolic extracts were concentrated by Rotary Evaporator apparatus and the solvent removed to produce dark brown gummy solids. The aqueous extracts were kept in room temperature to dry. The resulting extracts were kept separately in clean vials in a dark and cool place for further tests.

Antibacterial assay

Preliminary antibacterial activity study of the methanolic and aqueous extracts of *S. ebulus, A. capillus-veneris and C. spinosa* were investigated against six bacterial strains by the Cup plate method (Fazly-Bazzaz et al., 2005). The Muller-Hinton agar medium was purchased from Merck Company, Germany. The cups each of 6mm diameter were made by scooping out medium with a sterilized cork borer in a Petri dish which was streaked with the microorganism's saline suspension from overnight bacterial agar culture with a turbidity equivalent to a 0.5 Mc Farland standard. 50, 100, 200 and 400 mg of each extract were dissolved in 1 ml DMSO/ Water 10%(V/V) and then 80 µl of each dilution of extracts solution were added in cups and petri dishes were subsequently incubated at 35 to 37°C for 24 h. Dimethyl sulphoxide 10% was also added to one cup as a control which did not reveal any inhibition. Zone of inhibition produced by each dilution of extracts was measured in mm after 24 h. The experiments carried out 3 times and the results were presented as mean.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

After confirmation the antibacterial activity in methanolic and aqueous extracts of *C. spinosa*, *A. capillus-veneris* and *S. ebulus,* MIC and MBC of the extracts were determine by broth microdilution method using Muller-Hinton Broth medium(Merck, Germany) against the test organisms as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2006). This test was performed in sterile 96-well microplate.

The methanolic and aqueous extracts were properly prepared in DMSO 10% and transferred to each microplate well in order to obtain a twofold serial dilution of the original extract (from 1:2 to 1:128 starting from the concentration of 400 mg/ml. Each well contains approximately 5×10^5 CFU/ml of microorganism after inoculation. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added) and the DMSO 10% inhibitory effect. Plates were aerobically incubated at 35 to 37°C for 16 to 20 h in an ambient air incubator. The MIC was determined as the lowest concentration of the extract that completely inhibits visible growth of the microorganisms.

To confirm MICs and to establish MBC, 10 µl of each well with no visible growth was removed and inoculated in MHA plates. After 48 h of aerobic incubation at 35 to 37°C, the numbers of surviving microorganisms were determined. MBC was defined as the lowest extract concentration at which no growth of bacteria was seen. Each experiment was repeated at least three times.

RESULTS

The antimicrobial activities of methanolic and aqueous extracts of *C. spinosa*, *A. capillus-veneris* and *S. ebulus* in different concentrations were assayed against six Gram positive and negative bacteria by cup plate method and the results of inhibition zones have shown in Tables 1 and 2. The aqueous extracts of leaf of *S. ebulus* indicated significant antibacterial activity with inhibition zone diameters ranging from 8.6 to 27.6 mm against Gram-positive bacteria including *S. aureus, S. epidermidis and B. subtilis,* and Gram-negative bacteria including *E. coli, K. pneumonia* and *P. aeruginosa*.

The minimum inhibitory concentration (MIC) and minimum Bactericidal concentration (MBC) of the extracts were determined by micro dilution method and the results were shown in Tables 3 and 4.

The aqueous extract of *S. ebulus* leaves had the maximum antimicrobial effects *against* all tested Gram positive and Gram negative bacterial strains with the largest diameter of inhibition zones 27.6 mm against *S. aureus* with lowest MIC values against *S. aureus, S. epidermidis, E. coli, K. pneumonia, P. aeroginosa, B. subtilis* with concentration of 6.25, 25, 25, 25, 50 and 100 mg/ml, respectively. The methanolic extract of *S. ebulus*

Table 1. Antibacterial activity of methanolic extracts of *C. spinosa*, A. capillus-veneris and *S. ebulus*[a].

Conc.(mg/ml) microorganism	Plant											
	C. spinosa				*A. capillus-veneris*				*S. ebulus*			
	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	0	0	9.0 ± 0.1	11.3 ± 0.2	10.5 ± 0.3	13 ± 0.2	15.0 ± 0.1	17.1 ± 0.1	8.6 ± 0.2	11.3 ± 0.1	14.3 ± 0.1	16. 3 ± 0.3
S. epidermidis	0	0	0	10.0 ± 0.1	0	0	8.0 ± 0.1	9.3 ± 0.4	8.3 ± 0.2	10.3 ± 0.1	14.0 ± 0.1	16.6 ± 0.2
B. subtilis	0	0	0	0	0	0	0	9.0 ± 0.1	0	9.3 ± 0.1	13.0 ± 0.3	15.6 ± 0.1
E. coli	0	0	0	10.0 ± 0.2	0	0	0	11.3 ± 0.2	0	0	8.6 ± 0.2	11. 3 ± 0.2
K. pneumonia	0	0	0	0	0	0	0	8.0 ± 0.2	0	0	8.3 ± 0.3	10.3 ± 0.1
P .aeroginosa	0	0	10.3 ± 0.1	11.3 ± 0.2	0	8.2 ± 0.4	10.1 ± 0.3	12 .0 ± 0.1	0	9.0 ± 0.3	12.3 ± 0.1	14.3 ± 0.4

[a] Zone of inhibition, including the diameter of the well (6 mm); mean value of three independent experiments.

Table 2. Antibacterial activity of aqueous extracts of *C. spinosa*, A. capillus-veneris and *S. ebulus*[a].

Conc.(mg/ml) microorganism	Plant											
	C. spinosa				*A. capillus-veneris*				*S. ebulus*			
	50	100	200	400	50	100	200	400	50	100	200	400
S. aureus	0	9.0 ± 0.1	10.6 ± 0.2	12.7 ± 0.2	14.5 ± 0.1	16 ± 0.3	18.3 ± 0.1	19.3±0.4	16 ± 0.1	21.0 ± 0.2	24.3 ± 0.3	27.6 ± 0.3
S. epidermidis	0	0	0	0	0	0	0	9.0±00	0	0	0	9.6 ± 0.2
B. subtilis	0	0	0	0	0	0	0	0	0	0	8.6 ± 0.3	11.3 ± 0.4
E. coli	0	9.3 ± 0.2	11.3 ± 0.3	13.4 ± 0.4	0	0	0	0	0	0	12 ± 0.5	13.6 ± 0.2
K. pneumonia	0	0	-	9.0 ± 0.2	0	0	0	0	0	0	0	8.6 ± 0.2
P .aeroginosa	0	9.3 ± 0.2	9.7 ± 0.1	11.7 ± 0.1	0	0	0	10.1±0.1	0	1 1.5 ± 0.1	13.6 ± 0.8	17.3 ± 0.3

[a] Zone of inhibition, including the diameter of the well (6 mm); mean value of three independent experiments.

had the best MIC values against *S. epidermidis, S. aureus, B. subtilis, K. pneumonia, E. coli and P. aeroginosa* (25, 50, 100, 100, 200 and 200 mg/ml, respectively) and the largest diameter of inhibition zones (16.6 mm) was shown against *S. epidermidis.* The methanolic extract of *A. capillus-veneris* showed the best MIC values against *S. aureus* and *S. epidermidis* (12.5 and 50 mg/ml) and showed the largest diameter of inhibition zones (17.1 mm) by *S. aureus*. The methanolic extract of *C. spinosa* showed the best MIC value against *E. coli* (100 mg/ml).

DISCUSSION

In this study the antibacterial activity of three famous medicinal plants used as antibacterial agents in Iranian folk medicine were evaluated. The results showed that aqueous extract of *S. ebulus* had the best antibacterial effect on the tested microorganisms particularly *S. aureus* with MIC values 6.25 mg/ml. Both extracts of *A. capillus-veneris* are active against *S. aureus* and had MIC values equal to 12.5 and 25 mg/ml, respectively. The antimicrobial effect of aqueous extract of *C. spinosa* was better than methanolic extract and showed the best effects on *S. aureus* and *S. epidermidis*. This study emphasizes the potential of antibacterial effect of *S. ebulus* leaves

Table 3. Minimum inhibitory concentration (MIC) of C. spinosa, A. capillus-veneris and *S. ebulus*[a].

Microorganisms	MBC (mg/ml)					
	C. spinosa		*A. capillus-veneris*		*S. ebulus*	
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract
S. aureus	200	50	12.5	25	50	6.25
S. epidermidis	200	50	50	200	25	25
B. subtilis	200	200	400	400	100	100
E. coli	100	100	200	200	200	25
K. pneumonia	200	100	400	200	100	25
P. aeroginosa	200	100	100	200	200	50

[a]All determinations were done in triplicate.

Table 4. Minimum bactericidal concentration (MBC) of *C. spinosa, A. capillus-veneris and S. ebulus*[a].

Microorganisms	MBC (mg/ml)					
	C. spinosa		*A. capillus-veneris*		*S. ebulus*	
	Methanol extract	Aqueous extract	Methanol extract	Aqueous extract	Methanol extract	extract extract
S. aureus	400	200	25	100	50	25
S. epidermidis	400	200	200	400	100	200
B. subtilis	400	400	400	400	200	400
E. coli	200	400	400	200	200	50
K. pneumonia	400	400	400	400	200	100
P. aeroginosa	400	200	200	400	400	25

[a]All determinations were done in triplicate.

extracts which may be employed as a new antibacterial plant. The commonly known phytochemical compounds from *S. ebulus* is flavonoids, steroids, tannins, glycosides, cardiac glycosides, caffeic acid derivatives, ebulitins, ebulin 1 and volatile substances (Shokrzadeh et al., 2010). The flavonoid part could be the most effective antibacterial compound of the extracts. Also some of the higher phenols like ellagic acid and gallic acid have been reported for *A. capillus-veneris* (Singh et al., 2008) which could be responsible for its antimicrobial activity.

The richness of *C. spinosa* with total phenolic compounds, rutin, tocopherols, carotenoids and vitamin C in leaves and flower buds (Tlili et al., 2010) could be the main factor in its antimicrobial effects. Presence of these compounds in these plants may be related to their antibacterial activity on various bacterial strains. Also based on the results of this study, further in vivo and ex vivo confirmatory tests for aqueous extract of *S. ebulus* leaves are recommended. Conclusively, plants are valuable sources for new compounds and should receive special attention in research strategies to develop new antimicrobials urgently required in the near future.

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