Evaluation of Antibacterial Activity and Flavonoid Content of Two Capparis Species from Iran

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Abstract

Background: Due to multiple drug resistance against antimicrobial drugs for treatment of infectious disease, investigations to discovering new antibacterial compounds from natural sources have been designated.

Objective: According to antimicrobial effects of some Capparis species, this study was carried out to evaluate the antimicrobial activity of the extract and fractions of *Capparis cartilaginea* and *Capparis mucronifolia* against 6 bacterial strains.

Methods: Aerial parts of the Capparis species were extracted by maceration method using methanol and fractionated separately by liquid-liquid fractionation method. The antibacterial activity of the extract and fractions were studied against 6 bacterial strains using MIC microplate method. Total flavonoid content (TFC) of the extracts and fractions was determined using $AlCl_3$ reagent.

Results: The total extract and methanolic fractions of *C. mucronifolia* were the most effective fractions against the bacterial strains. Methanolic fraction of *C. cartilaginea* was the most effective fraction with MIC 10.42 µg/ml against *Salmonella enterica.* The highest antibacterial activity of *C.mucronifolia* was against *Staphylococcus epidermidis* with MIC 7.8 µg/ml. Methanolic fractions of Capparis species showed the highest TFC value in $AlCl_3$ colorimetric assay.

Conclusion: The results of this study indicate that the extracts and fractions of *Capparis cartilaginea* and *Capparis mucronifolia* have antimicrobial effect against 6 gram positive and gram negative strains. According to the high amount of flavonoids in methanolic fraction of *C. mucronifolia*, the antibacterial activity may be related to the flavonoid compounds of this plant.

Keywords: ***Capparis cartilaginea, Capparis mucronifolia*, Antimicrobial activity, Flavonoid, MIC**

Introduction

Plants have been used for treatment of infectious disease for many years. Nowadays multiple drug resistance has developed due to the increasingly use of antimicrobial drugs for treatment of infectious disease [1]. Antibiotic resistance is posing an ever increasing therapeutic problem [2]. So, these problems lead to effort to discover new antibacterial compounds from natural source.

Medicinal plants have been used as effective treatments for numerous human diseases like infectious disease in traditional medicines for thousands of years. In recent decades, researchers have paid attention to identify safer herbs and biologically active compounds isolated from plant species [3]. Antibacterial activity of some plant extracts or their active compounds have been reported in different studies [4, 5].

Genus capparis (Capparaceae) consist of bout 150-200 species in tropical and subtropical countries throughout the world [6]. This Genus has four endemic species in Iran [7]. Capparis species have been used as anti-inflammatory and analgesic (in gout and arthritis rheumatoid), diuretic, antibacterial and anthelmintic agent in Iranian traditional medicine [8]. Some component including Alkaloids, Terpens, Flavonoid and fatty acids were reported from different Capparis species [9]. These plants showed different biological effects including antioxidant, antibacterial, antihyperglycemic, hypolipidemic and analgesic effects in scientific studies [10-13].

Capparis cartilaginea and *Capparis mucronifolia* are two species which are grown in the southern parts of Iran. According to antibacterial effect of the other capparis species like *C. spinosa*, in this study antibacterial activity of the aerial parts of these plants were studied in some strains of gram positive and gram negative bacteria with MIC micro plate method.

Materials and Methods

Plant material

Fresh aerial parts of *C. cartilaginea* and *C. mucronifolia* were collected in May 2013 from Genow protected region in west north of Bandar Abbas and, Ahmadi region of Hadjiabad County respectively, Hormozgan Province, Iran. Specimens were identified by R. Asadpour and vouchers were deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran under code number 1562 and 1564 respectively.

Extraction and isolation

2 kg of the aerial parts of plants were dried, grinded and extracted with methanol by maceration. The extraction was repeated for 3 times. Different fractions of the extracts were obtained by liquid-liquid fractionation using water, petroleum ether, chloroform and methanol. The extracts and fractions obtained were concentrated by rotary evaporator and finally dried and stored in a clean, dark container and cool place. The antibacterial activity was evaluated by determining the MIC microplate method.

Test microorganisms

The bacterial Gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228) and *Enterococcus faecalis* (ATCC 29212), and Gram-negative bacteria including *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Salmonella enterica* (ATCC 13311) were purchased from Mast diagnostic company as lyophilized ampoule.

Culture of microorganisms:

After recovery of the microorganism in TSB (Triptycase soy broth) for 24-48 h at 37 °C, the overnight microorganisms which be used for antimicrobial testing, obtained by subculture on plates containing TSA medium and incubation for 24 h at 37 °C.

Minimum inhibitory concentration (MIC) using microplate:

Micro-dilution assay was used to determine MIC of the extracts against 6 bacterial strains. MIC was determined by testing 8 concentrations of the extract against gram-positive and Gram-negative bacteria, by microplate serial dilution method and using Mueller Hinton broth. The reconstituted extract was diluted to give concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812 µg/ml. The lowest concentration of the extract that could inhibit the bacterial growth was considered as MIC [14]. Chephalexin and gentamicin were used as positive controls.

Total flavonoid content:

The total flavonoid content (TFC) of *C. cartilaginea* and *C. mucronifolia* methanolic extracts and their fractions was determined using $AlCl_3$ reagent [15]. Briefly, 2.5 mL of each sample (and/or quercetin as the standard), previously dissolved in 90% ethanol, was mixed with 2.5 mL of a 2% $AlCl_3$ solution in 90% ethanol. After 40 min, the absorbance of the yellow color produced was measured at 425 nm. The TFC [as µg quercetin equivalents / mg of sample] for the sample was calculated on the basis of a linear calibration curve obtained using quercetin ($y=0.0169x+0.3526$, $r^2=0.995$).

Results

TFC of the Capparis extracts and fractions are presented in Table 1. Extract and fractions of *C. mucronifolia* showed higher amounts of TFC rather than *C. cartilaginea.*

The inhibitory effects of the methanolic extracts and fractions of *C. cartilaginea* and *C. mucronifolia* against different microorganisms are presented in table 2 and table 3 respectively. The extract and fractions of these plant showed different antibacterial activity against gram-positive and gram negative bacteria in microplate method. The total extract and methanolic fraction of *C. mucronifolia* were the most effective fractions of this plant against bacterial strains. The most effective fraction of *C. cartilaginea* was methanolic fraction with MIC 10.42 µg/ml against *S. enterica*. The most effectiveness of *C. mucronifolia* was against *S. epidermidis* with MIC 7.8 µg/ml for total extract and methanolic fraction.

Table 1- TFC of the Capparis extracts and fractions (µg quercetin/mg extract)

Capparis species	Total extract	*Petrolium ether* fraction	*Aqueous* fraction	Methanolic fraction	Chloroformic fraction
C. mucronifolia	36.54±0.5	65.8±0.3	69.13±5.4	76.1±1.74	-
C. cartilaginea	16.72±0.71	16.64±4.2	17.87±1.8	33.77±0.76	38.61±1.4

*Data are mean ± SD, n=3

Table 2- MIC values of extract and fractions of *C. cartilaginea* (µg/ml)

Microorganism	Total extract	*Petrolium ether* fraction	*Aqueous* fraction	*Methanolic* fraction	*Chloroformic* fraction	Chephalexin	Gentamicin
Pseudomonas aeruginosa	52.08	62.5	15.63	31.25	52.08		2.5
Esherichia coli	62.5	62.5	31.25	41.67	52.08		5
Salmonella enterica	31.25	15.63	31.25	10.42	13.02		23.4
Enterococcus faecalis	15.63	52.08	31.25	62.5	31.25	16.6	
Staphylococcus aureus	13.69	31.25	13.02	31.25	31.25	2.6	
Staphylococcus epidermidis	62.5	26.04	26.04	31.25	62.5	5.2	

- Data are mean of three replicate

Table 3- MIC values of extract and fractions of *C. mucronifolia* (µg/ml)

Microorganism	Total extract	*Petrolium ether* fraction	*Aqueous* fraction	*Methanolic* fraction	Chephalexin	Gentamicin
Pseudomonas aeruginosa	>1000*	>1000	>1000	>1000		2.5
Esherichia coli	31.25	250	187.5	46.87		5
Salmonella enterica	46.87	250	125	62.5		23.4
Enterococcus faecalis	250	250	250	250	16.6	
Staphylococcus aureus	7.8	250	46.87	11.7	2.6	
Staphylococcus epidermidis	7.8	250	46.87	7.8	5.2	

- * > 1000= extract or fraction was inactive,
- Data are mean of three replicate.

Discussion

Antibiotic resistance is posing an ever increasing therapeutic problem and these problems lead to effort to discover new antibacterial compounds from natural source [2].

C. cartilaginea and *C. mucronifolia* are two species of capparis genus which are endemic in the south of Iran. Some species of capparis (like *C. spinosa*) showed significant antibacterial activity [12], so in this study antibacterial effect of the extract and fractions of aerial parts of *C. cartilaginea* and *C. mucronifolia* was determined by micro plate MIC method against 6 bacterial strains. The most effective fraction of *C. cartilaginea* was methanolic fraction against *S. enterica*

and total extract and methanolic fraction of *C. mucrpnifolia* showed the best activity against *St. epidermidis*.

Previous antimicrobial study on *C. cartilaginea* showed MIC> 1000 µg/ml for bacterial strains including *Staphylococcus aureus, Bacillus subtilis and Microccocus flavus* [16]. This study showed the antibacterial effect of the extract and different fractions of *C. cartilaginea* against 6 bacterial strains using MIC method. Also, this study is a first report on the antibacterial activity of *C. mucronifolia* against gram positive and gram negative bacterial strains. The tested capparis species showed lower antibacterial activities in comparison to *C. spinosa*, against bacterial strains [17]. Phytochemical investigations of Capparis species showed the presence of flavonoids [18, 19]. In this study, TFC of the extracts and fractions of two Capparis species was determined. The findings showed higher TFC value in *C. mucronifolia* extract and fractions. The methanolic fractions of two Capparis species showed high amounts of total flavonoids in $AlCl_3$ colorimetric assay. Since the methanolic fraction of *C. mucronifolia* showed the best antibacterial activity, flavonoids may be responsible for antibacterial activity of this plant.

Conclusion

The results of this study indicate that the extracts and fractions of *C. cartilaginea* and *C. mucronifolia* have antimicrobial effect and total extract and methanolic fraction of *C. mucronifolia* showed the best antibacterial activity. Certainly further investigations are necessary to determine the active principles which responsible for antibacterial activity.

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