

EXPERIMENTAL PAPER

Antimicrobial activity of *Capparis spinosa* as its usages in traditional medicine

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S u m m a r y

Capparis spinosa roots and fruits are traditionally used for the treatment of diseases such as diarrhea and hemorrhoids. In the 10th century, farmers also used aqueous extract from its roots for disinfecting their plants' seeds. Due to widespread use of this plant in traditional medicine, we evaluated different extracts (aqueous, methanol, ethanol and ethyl acetate) of fruits and roots of this plant against bacteria and fungi causing infections in plants or humans. The total phenolic and flavonoid content of extracts from fruits and roots and the antioxidant activity were evaluated. The antimicrobial activity was evaluated by microdilution assay in fluid medium. Among different extracts from roots and fruits, root ethyl acetate and fruit methanol extracts had higher total phenolic content, while fruit ethyl acetate extract contained higher total flavonoid content. The higher antioxidant activity was shown for roots ethanol extract (IC₅₀=88 µg/ml). As its traditional uses, roots aqueous extract from *C. spinosa* exhibited inhibitory effect against bacteria and fungi with the exception of *Pseudomonas aeruginosa*. With the exception of fruit aqueous extract, other extracts (methanol, ethanol and ethyl acetate) from fruit and root exhibited good activity against microorganisms, especially fungi. This study reveals the traditional uses of roots and fruit extracts as antimicrobials agent.

Key words: *Capparis spinosa*, fruits, roots, antimicrobial activity, IC₅₀, extract

INTRODUCTION

Emergence of antibiotic resistant pathogens is a growing problem. The side effects of some chemical antibiotics have also created high demands for medicinal plants as new alternatives.

Capparis spinosa (Capparidaceae) with nutritional value is a plant traditionally used for treatment of many ailments. It grows in dry to cooler regions of west or central Asia. In Iran, *C. spinosa* or “Kavar” fruits or roots are commonly used to treat hemorrhoids. In addition, roots are highly valued as anti-diarrhea [1]. In traditional agriculture, *C. spinosa* roots are soaked in water for night, and then the aqueous extract is dispersed on plants seeds. These seeds are protected from every pest all over the year [2].

We hypothesize that *C. spinosa* fruits or roots may act as antimicrobial agent. Though its antimicrobial activity has been examined in comparison with other plants extracts [3-5]. There is no literature on the traditional prescription of this plant extracts as natural antibiotics. Other pharmacological effects such as protective effect of *C. spinosa* flowering buds, methanol extract on chondrocytes [6], anti-quorum sensing and anti-biofilm activity of dried fruits *C. spinosa* methanol extract [7], anti-proliferative activity against tumor cells and inhibitory effect of fresh *C. spinosa* melons on HIV-1 reverse transcriptase [8] were confirmed.

This study evaluates the antimicrobial activity of aqueous, ethanol, methanol and ethyl acetate extracts from fruits and roots of *C. spinosa* as traditional uses against a variety of microorganisms causing infectious diseases in human and the fungal plant pathogens. We also evaluated their antioxidant activities, total phenolic and flavonoid contents.

MATERIALS AND METHODS

Plant materials and extraction

Dried fruits and roots of *Capparis spinosa* were collected from Mashad-E-Ardehal, Kashan, Iran in April and May 2012, and were authenticated under number 221-1. Extraction was performed with water, methanol, ethyl acetate and ethanol-water (70:30, v/v). The dried roots and fruits powders were separately mixed with solvent at the ratio of 1:10 (w/v) for 24 h at ambient temperature. The mixture was then filtered through filter paper (Whatman No. 2). The residue was rinsed with the same solvent and the extract was dried under vacuum.

Total phenolic content (TPC) and total flavonoid content (TFC)

Total phenolic contents of crude extracts were determined by a spectrophotometer using the Folin-Ciocalteu's reagent [9]. Each dry extract (10 mg) was

dissolved in 10 ml of its own solvent (1 mg/ml). 0.2 ml of extract was transferred into a 5 ml volumetric flask and swirled with 3 ml of water. 0.25 ml of Folin-Ciocalteu's reagent was added and swirled. After 3 min, 0.75 ml of 20% (w/v) sodium carbonate solution was added and mixed. This was recorded as time zero. Deionized water was added to make up the volume to 5 ml precisely. The solution was mixed thoroughly and allowed to stand at ambient temperature for 2 h until the characteristic blue color was developed. Quantification of TPC was based on a standard curve generated with gallic acid (GAC) at 760 nm using the following equation:

$$\text{Abs} = 0.0054w + 0.015,$$

where Abs is absorbance and w is the weight (μg). All tests were conducted in triplicate and averaged. The results were expressed as mg of TPC per 100 mg of dry extract as GAC equivalents.

The aluminum chloride colorimetric method was used to estimate TFC in crude extracts. 50 mg of dry extract was dissolved in 10 ml of its own solvent (5 mg/ml). 0.5 ml of extract was mixed with 2 ml of appropriate solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and diluted to the mark with distilled water in a 5 ml volumetric flask. The absorbance was measured at 415 nm after 30 min. TFC in the extracts was determined using a standard curve established with quercetin (QE) (25-100 $\mu\text{g}/\text{ml}$) and the results were expressed as mg of QE per mg of dry extract [10].

Radical scavenging capacity of extracts by DPPH assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of the ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) [11, 12]. Tests were carried out in triplicate. 1 mM BHT (Sigma, USA) was used as positive control. Briefly, 50 μl of 1:5 concentrations of extract were added to 5 ml of a 0.004% methanol solution of DPPH. After a 70 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated in following way:

$$I\% = \left[\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

Microbial strains

Staphylococcus aureus ATCC 25923, *Staphylococcus epidermidis* ATCC 14490, *Staphylococcus saprophyticus* ATCC 15305, *Streptococcus pyogenes* ATCC 8668, *Streptococcus mutans* ATCC 35668, *Bacillus cereus* ATCC 1247, *Bacillus subtilis* ATCC 6051, *Klebsiella*

pneumoniae ATCC 10031, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Shigella dysenteriae* PTCC 1188, *Shigella flexneri* PTCC 1234, *Pseudomonas aeruginosa* ATCC 9027, and fungi *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, *Aspergillus flavus*, *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* ATCC 15517 were used. Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 CFU/ml using standard routine spectrophometric methods. Suspensions of fungi (10^6 CFU/ml) were made in Sabouraud dextrose broth. Subsequent dilutions were made from the above suspensions, which were then used in tests.

Evaluation of antimicrobial activity

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of extracts were determined by micro broth dilution assay. The extracts were dissolved in the solvent originally used, and then they were twofold serially diluted in distilled water containing 51.2–0.05 mg/ml of each extract. After shaking, 100 μ l of each extract dilutions was added to each well of 96 well micro titer plates. The above microbial suspension was diluted (1×10^6 CFU/ml for bacteria; 1×10^4 CFU/ml for fungi) in suitable broth media for each microorganism and then 100 μ l of this suspension was added to each well and incubated at $37 \pm 2^\circ\text{C}$. MIC was defined as the lowest concentration of extract dilution that inhibits bacteria and fungi after 24, 48 h, respectively. MLC values were the first well that showing no growth on suitable agar medium [13].

RESULTS

TPC and TFC of extracts

Evaluation of TPC in different extracts from *C. spinosa* revealed that root ethyl acetate extract (37.2 mg GAC/g) had the highest TPC followed by fruit methanol extract (34.2 mg GAC/g), fruit ethanol extract (31.7 mg GAC/g) and fruit ethyl acetate extract (30 mg GAC/g). Roots aqueous extract (15.4 mg GAC/g) and fruits aqueous extract (17.2 mg GAC/g) contain lower content of TPC than the roots ethanol extract from *C. spinosa* (22.4 mg GAC/g).

The assessment of the TFC in extracts showed that fruit and roots ethyl acetate extracts had higher content of flavonoids (95.5 and 18.1 mg QE/g), followed by fruits methanol extract (17.1 mg QE/g), roots and fruit ethanol extract (1.6 and 1.4 mg QE/g, respectively). Aqueous extract from roots and fruits contain lower content of flavonoids (0 and 0.06 mg QE/g).

Antioxidant activity evaluation

The evaluation of antioxidant activities of extracts by DPPH assay showed that the lower IC_{50} belong to root ethanol extract (88 $\mu\text{g/ml}$) followed by methanol fruit extract (340 $\mu\text{g/ml}$) and aqueous fruit extract (500 $\mu\text{g/ml}$). The antioxidant activity of fruit aqueous extract and root ethyl acetate extract were similar (IC_{50} were 560 and 570 $\mu\text{g/ml}$). Methanol root extract ($IC_{50} = 1450 \mu\text{g/ml}$), aqueous root extract and ethyl acetate fruit extract ($IC_{50} > 2000 \mu\text{g/ml}$) exhibited a weak antioxidant activity (tab. 1). All the IC_{50} for extracts were higher than that of BHT (20 $\mu\text{g/ml}$).

Table 1.

Chemical attributes of *C. spinosa*

Extracts	TPC [mg/g]		TFC [mg/g]		IC_{50} [$\mu\text{g/ml}$]	
	Fruits	Roots	Fruits	Roots	Fruits	Roots
Aqueous	17.2	15.4	0.06	0	500	>2000
Ethanol	31.7	22.4	1.4	1.6	560	88
Methanol	34.2	21.3	17.1	0.47	340	1450
Ethyl acetate	30	37.2	96.5	18.1	>2000	570

TPC – Total phenolic content

TFC – Total flavonoid content

Antimicrobial evaluations

The antimicrobial activity of root aqueous extract against a variety of microorganisms (tab. 2) was higher than the fruit aqueous extract (The MIC and MLC values were higher than 51.2 mg/ml). This antimicrobial effect was almost inhibitory effect (tab. 2). The antifungal activity of roots ethanolic extract was higher than fruit ethanolic extract. Different extracts of root from *C. spinosa* showed inhibitory activity against *Staphylococcus* sp.

The antifungal activity of roots aqueous extract against *A. niger*, *A. parasiticus* and *A. flavus* showed the inhibitory effect. *C. albicans* was more sensitive to roots ethanolic extract, followed by roots ethyl acetate extract. The antimicrobial activity of roots extract was higher than fruit extract with exception of fruits ethanolic extract against strains of *S. pyogenes*, *S. mutans*, *E. coli*, *K. pneumonia* and *P. aeruginosa*. Root ethanolic extract had higher antimicrobial activity against strains from the genera of *Streptococcus* or Gram-negative ones.

DISCUSSION

Total phenolic content of extract often determines its pharmacological effects such as antioxidant activity. Based on the results of this study, we propose that

Table 2.

Antimicrobial evaluation of *C. spinosa* extracts by micro broth dilution assay

Extracts	Aqueous			Ethanol			Ethyl acetate			Methanol			Antibiotic			
	Roots		Fruits	Roots		Fruits	Roots		Fruits	Roots		Fruits	Antibiotic			
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC		
<i>S. aureus</i>	0.1	*	*	6.4	12.8	12.8	0.1	12.8	6.4	12.8	0.1	12.8	12.8	25.6	0.25	0.25 ^a
<i>S. saprophyticus</i>	0.1	*	*	12.8	6.4	12.8	0.1	12.8	6.4	12.8	0.1	6.4	12.8	25.6	0.25	0.5
<i>S. epidermidis</i>	0.1	12.8	*	12.8	6.4	12.8	0.1	6.4	12.8	12.8	0.1	12.8	6.4	12.8	0.25	0.5
<i>S. pyogenes</i>	12.8	*	*	25.6	25.6	1.6	3.2	6.4	12.8	3.2	6.4	12.8	25.6	3.2	0.12	0.25
<i>S. mutans</i>	51.2	*	*	25.6	25.6	3.2	3.2	6.4	12.8	12.8	6.4	6.4	3.2	3.2	0.25	0.5
<i>E. coli</i>	51.2	*	*	25.6	25.6	6.4	25.6	6.4	6.4	6.4	6.4	6.4	12.8	25.6	0.25	0.5 ^b
<i>S. typhimurium</i>	51.2	*	*	25.6	25.6	6.4	25.6	6.4	12.8	6.4	25.6	3.2	6.4	12.8	1	2
<i>S. dysenteriae</i>	25.6	*	*	25.6	25.6	6.4	12.8	3.2	6.4	12.8	25.6	0.8	3.2	6.4	25.6	0.5
<i>S. flexneri</i>	51.2	*	*	25.6	25.6	6.4	12.8	6.4	12.8	6.4	12.8	3.2	12.8	6.4	25.6	0.5
<i>K. pneumoniae</i>	51.2	*	*	25.6	25.6	6.4	12.8	6.4	12.8	6.4	25.6	6.4	12.8	6.4	12.8	0.25
<i>P. aeruginosa</i>	*	*	*	25.6	25.6	3.2	6.4	6.4	12.8	6.4	12.8	6.4	6.4	6.4	12.8	0.5
<i>B. subtilis</i>	12.8	*	*	1.6	6.4	12.8	1.6	1.6	6.4	12.8	6.4	12.8	6.4	12.8	0.12	0.25 ^a
<i>B. cereus</i>	12.8	12.8	*	3.2	12.8	6.4	51.2	1.6	3.2	6.4	12.8	3.2	6.4	3.2	51.2	8
<i>C. albicans</i>	6.4	12.8	*	1.6	1.6	3.2	6.4	1.6	3.2	3.2	6.4	3.2	6.4	6.4	6.4	0.12
<i>C. glabrata</i>	25.6	*	*	3.2	6.4	6.4	6.4	6.4	6.4	6.4	6.4	3.2	6.4	6.4	6.4	0.25
<i>A. flavus</i>	6.4	*	*	3.2	6.4	3.2	51.2	3.2	6.4	6.4	12.8	3.2	6.4	3.2	51.2	0.5
<i>A. parasiticus</i>	3.2	6.4	*	1.6	6.4	6.4	12.8	3.2	6.4	6.4	12.8	3.2	6.4	3.2	25.6	0.5
<i>A. niger</i>	0.1	25.6	*	1.6	6.4	3.2	12.8	3.2	6.4	6.4	6.4	3.2	6.4	1.6	12.8	0.5

MIC – Minimal Inhibitory Concentration [mg/ml]

MLC – Minimal Lethal Concentration [mg/ml]

Antibiotics: a – vancomycin

B – gentamycin

C – amphotericin B [μ g/ml]

(*) – higher than 51.2 mg/ml

root ethyl acetate extract, with higher total phenolic and flavonoid content exhibited higher antioxidant activity, followed by fruit methanol extract while the higher antioxidant activity was in root ethanol extract followed by fruit methanol extract and root ethyl acetate extract. Therefore, components other than phenolic or flavonoid contents may play important role in its antioxidant activity.

Phytochemical analysis of roots and fruits of *C. spinosa* showed the different profile of components. The presence of alkaloid, such as stachydrine, cadabicine, capparispine, capparispine 26-O- β -d-glucoside, cadabicine 26-O- β -d-glucoside hydrochloride was demonstrated in fruit and roots, but the alkaloid content of *C. spinosa* root was higher than that of its fruits [14-17] and quercetin content was higher in fruits than in roots [18]. Indoleglucosinolates such as glucobrassicin, neoglucolorassicin and methoxyglucobrassicin were isolated from roots [19]. Sinigrin and glucocleomin were reported in fruits [20]. Regardless of different compounds in fruit or root, solvent can affect phytochemical composition of each extract. The presence of alkaloid, steroids, flavonoids, tannins, phenols and saponin are reported in aqueous extract from aerial parts of *C. spinosa* while its ethanolic extract contains terpenoids. Steroids, flavonoid and saponines are absent in ethanolic extract [21]. Thus, the complexity of components in each extract and the amount of each component make the conclusion about the relation between total phenolic or total flavonoid content and its antioxidant activity difficult.

The antimicrobial activity of each extract is also related to its chemical components. Flavonoids as the major class of phenolic group, show antimicrobial activity by inhibition of nucleic acid synthesis, cytoplasm membrane function and energy metabolism [22]. Fruits aqueous extract with higher total phenolic and total flavonoid content than roots aqueous extract exhibited lower antimicrobial activity. Indeed, the fruit aqueous extract exhibited no antimicrobial activity. Roots aqueous extract showed inhibitory effect against bacteria and fungi as its uses in traditional agriculture. Roots extracts (ethanol, methanol and ethyl acetate) exhibited the higher antimicrobial activity than fruits extracts. Only fruit ethanolic extract had higher activity against strain of *Streptococcus* sp. and Gram-negative bacteria. Thus, as its traditional uses fruits and roots of *C. spinosa* can be used for treatment bacterial infection in hemorrhoids and diarrhea. Like antioxidant activity, components other than phenolics play critical role in its antimicrobial activity.

CONCLUSION

Traditional medicine can play an essential role in the discovering of new compounds for treating different ailments. As traditional uses of *C. spinosa*, root and fruits for inhibition of microbial growth, we plan a study for the evaluation of the antimicrobial and antioxidant activities of *C. spinosa* roots and fruits extracts. At first, we determined total phenolic and total flavonoid content of each extract of fruits and roots.

This investigation showed that traditional medicine is a rich source of information that can be used to improve the human life. Furthermore, primitives used the aqueous extract of *C. spinosa* for safe keeping their seeds from microbial pests. Recent findings have also proved that *C. spinosa* extracts, especially its aqueous extract has antiseptic activity and inhibits the growths of bacteria and fungi. It is essential to evaluate the efficacy of *C. spinosa* aqueous extract on the prevention of the seeds from microbial pests, in farm conditions.

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AKTYWNOŚĆ PRZECIWBAKTERYJNA *CAPPARIS SPINOSA* I JEGO STOSOWANIE W MEDYCYNIE TRADYCYJNEJ

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Streszczenie

Korzenie i owoce *Capparis spinosa* stosuje się tradycyjnie do leczenia takich chorób jak biegunka i hemoroidy. W X wieku rolnicy stosowali także wyciąg z korzenia do dezynfekcji nasion. Z powodu szerokiego zastosowania tej rośliny w medycynie tradycyjnej zbadaliśmy działanie różnych wyciągów (wodnego, metanolowego, etanolowego i otrzymanego przez ekstrakcję octanem etylu) z owoców i korzeni tej rośliny przeciwko bakteriom i grzybom powodującym choroby roślin i ludzi. Oznaczono całkowitą zawartość fenoli i flawonoidów w wyciągach z owoców i korzeni oraz ich aktywność przeciwutleniającą. Działanie przeciwbakteryjne zostało oznaczone za pomocą testu mikrorozcieńczeń w podłożu płynnym. Wśród różnych wyciągów z korzeni i owoców, wyciąg otrzymany za pomocą ekstrakcji octanem etylu z korzenia i wyciąg metanolowy z owoców miał wyższą zawartość całkowitą fenoli, podczas gdy wyciąg z owoców otrzymany za pomocą octanu etylu miał wyższą zawartość flawonoidów. Silniejsze działanie przeciwutleniające wykazano dla wyciągu etanolowego z korzenia ($IC_{50} = 88 \mu\text{g/ml}$). Zgodnie z tradycyjnym zastosowaniem, wyciąg

wodny z korzenia *C. spinosa* wykazał działanie przeciwbakteryjne i przeciwgrzybicze, z wyjątkiem *Pseudomonas aeruginosa*. Poza wyciągiem wodnym z owoców, inne wyciągi (metanolowy, etanolowy i otrzymany za pomocą octanu etylu) z owoców i korzeni wykazywały wysoką aktywność przeciwdrobnoustrojową, szczególnie wobec grzybów. Zaprezentowana praca ukazuje tradycyjne zastosowania wyciągów z owoców i korzeni *Capparis spinosa* jako czynników przeciwdrobnoustrojowych.

Słowa kluczowe: *Capparis spinosa*, owoce, korzenie, działanie przeciwbakteryjne, IC_{50} , wyciąg