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# Anti-microbial and Anti-biofilm activity of leaf fruit and stem *Capparis spinose* extract against *Acinetobacter baumannii.*

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## Abstract

**Background: Now-a-days, the increase in drug resistance among *Acinetobacter* is a major concern all over the world. The aim of this study was to investigate the antimicrobial and anti-biofilm activity of ethanolic extract and ethyl acetate of Leaf, Fruit and Stem *Capparis spinose* extract against *Acinetobacter baumannii*.**

**Methods: Twelve strains of *A. baumannii* were isolated from patients who referred to hospitals in Zabol. Antibiotic resistance pattern was determined by Kirby-Bauer method. Minimum inhibitory concentration and minimum bactericide concentration were determined by microdilution method.**

**Results: The results of the antibiotic resistance pattern showed that 100% of the samples were susceptible to CZ (Cefazolin)-GM (Gentamycin) - AZM (Azithromycin) antibiotics and only 16.6% of the samples were resistant to AM (Ampicillin) antibiotics. The results of this study showed that the lowest inhibitory concentration of the *C. spinose* ethanolic extract against *A. baumannii* was 25 ppm in which two bacterial strains were inhibited while the highest inhibitory concentration was 50 ppm and the highest concentration of fecundity was equal to 100 ppm.**

**Conclusion: Results of this study show the antimicrobial effects of good *C. spinose* biofilm on *A. baumannii,* which can be used as an effective treatment.**

**Keywords:** *Acinetobacter baumannii*, Antibiotic resistance, Plant extract, *Capparis spinosa*.

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## Introduction

Acinetobacterium is a gram negative bacterium and negative lactose that is increasingly recognized as an important factor in hospital infections such as the bacteremia and pneumonia associated with ventilator, surgical infections, secondary meningitis and urinary tract infections, especially in patients admitted to Special care departments. Its colony size is between 1 mm to 2 mm, no pigment and smooth to mucoid [1]. Blood agar usually causes hemolysis and grows at 44 ºC. In the growth stage, the logarithmic form has a basil shape, but in the stagnant phase it is seen as coco-basil. It is a non-fermented bacterial group [2].

Various species of this bacterium are widely distributed in nature and can be separated from water, soil, human skin, food and sewage [3]. The genus of *Acintobacteria* consists of 23 species with a valid name and 10 anonymous species [4].

*Acinetobacter baumannii* has the ability to bind to all biological and non-biological levels, and form biofilms. This ability is an important pathologic property in many bacteria and promotes the development of antibiotic resistance and invasion of host immune system [5]. Being a community of microbial cells that are firmly attached to the surface, they are called biofilms with a microbe-derived polysaccharide matrix. Basically, bacteria create biofilms that resist antimicrobial resistance to the host immune system and maintain the physical and chemical conditions suitable for growth, which makes biofilms resistant to adverse conditions. In the meanwhile, the synergistic and interphase relationships of biofilm bacteria are effective in their resistance to adverse conditions [6].

Populations of a species or different species of bacteria are adhered to each other or to living and non-living surfaces in the field of biofilm extracellular polysaccharides. Biofilm development is a complex process that requires bacterial communicative behaviours and, in contrast to single life, has many benefits for bacteria [7]. The formation of biofilms involves the reversible and irreversible initial binding to the surfaces, the formation of microcells, and, ultimately, the microcrystalline maturity associated with the formation of exopolysaccharides [8]. The spread of biofilm is the main cause of hospital infections because the bacteria that are attached to the surface and protected by external polysaccharide against various types of antibiotics and other environmental stresses, and biofilms increase the bacterial dispersion and bacterial pathology. Since these bacteria are resistant to various antibiotics, they now use plant extracts to eliminate them [9].

*Capparis spinosa* (locally called 'Al-Kabara') is one such plant stable to have highly diverse economic and pharmaceutical

value in various system of pharmaceutical like in Unani, Chinese, Ayurvedic and Greco-Arabi System of pharmaceutical. *C. spinosa* is well recognized with its usual name 'Capers' in various countries. In Iran, *C. spinosa* fruits and roots are generally apply to treat hemorrhoids [10,11].

Although the first snake grass habitat is not well known, today the range of herbs has expanded in a variety of Mediterranean and tropical climates to the Himalayan heights. In Iran, snake grass is found in most of the southern half of the country. Some species of the genus *Capparis* have been used in traditional medicine. It is used as a seasoning for peanuts, and its fruit is widely used in different cultures, which is one of the most important uses for pickles. Of course, the products of this plant may become susceptible in some people [12].

Other parts of this plant are used in the manufacture of medicines and cosmetics. Over the past four decades, snake grass has been considered for its economic importance as a crop in some European countries such as Greece and Italy [13]. Also, fruit and grass are used to treat diabetes, fungal infections, digestive tract infections and skin diseases caused by parasites, either orally or septa. Sometimes this plant is used to treat dryness and inflammation of the skin and to increase the blood flow to the skin as an ointment [14].

## Materials and Methods

### *Separation of A. baumannii*

Different strains of *A. baumannii* used in this research were isolated of urine specimens from hospitalized patients in Amiralmomenin Hospital of Zabol (Zabol-Iran). The 50 samples were cultured on agar Blood agar, BHI and agar Nitrite. To detect different species of Acinetobacter, the biochemical tests of urea-azcatalase and oxidase were performed.

Antibiotic susceptibility test was performed using a disk diffusion method in accordance with laboratory and clinical standards on the Mueller-Hinton agar medium. Streaking culture of bacterial suspensions of 0.5 McFarland were applied to the Muller Hinton Agar medium. In addition, the antibiotic discs were used including CZ (Cefazolin)-GM (Gentamycin)-AZM (Azithromycin)- AM (Ampicillin) (Mast, UK) [15].

### *Plant materials*

The leaves, fruit, stem of *C. spinosa* were collected from the sistan region of Iran and they were dried at 25°C. 10 g grinded powders was soaked in 100 mL ethanol 95% and ethyl acetate, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered. Then the filtrates were evaporated using rotary evaporator [16]. The broth microdilution method was used to determine MIC and MBC. Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 6.25 ppm to 100ppm.

### *Biofilm formation assay in presence of the biocides*

At first, the OD (Optical Density) was measured (600 nm) by using an automated ELISA counter, then, the content of each well of the microplate was aspirated and each well was washed three times with 250 µL of sterile physiological saline. The remaining attached bacteria were fixed with 200 µL of 99% methanol per well and after 15 min all of the wells were emptied and left to dry. Then, each well was stained for 5 minute with 0.2 mL of 2% crystal violet. Excess stain was rinsed off by washing the plate slowly with distilled water. After the plate was air dried, the dye bound to the adherent cells was resolubilized with 160 mL of 33% (v/v) glacial acetic acid per well. The OD of each well was measured at 492 nm by using an automated ELISA counter.

### *Statistical analyses*

The growth was compared at each experiment using analysis of variance (ANOVA) repeated measures (SPSS-25.0 for Windows). The level of statistical significance was set at $P<0.01$.

## Results

The results of the antibiotic resistance pattern showed that 100% of the samples were susceptible to CZ (Cefazolin)-GM (Gentamycin)-AZM (Azithromycin) antibiotics and only 20% of the samples were resistant to AM (Ampicillin) antibiotics (Table 1). The results of this study showed that the lowest inhibitory concentration of the *C. spinose* ethanol extract against *A. baumannii* was 25 ppm, in which two bacterial strains were inhibited, while the highest inhibitory concentration was 50 ppm and the highest concentration of fecundity is equal to 100 ppm (Table 2). The results of the study of *C. spinose* ethanol extract showed that the lowest inhibitory concentration was 25 ppm, while the highest inhibitory concentration was 100 ppm, which was inhibited by a strain of bacteria.

***Table 1.*** *Patten antibiotic of A. baumannii.*

| Antibiotic | Resistant (%) | Intermediate (%) | Sensitive (%) |
|---|---|---|---|
| AMC | 1 (10%) | 1 (10%) | 8 (80%) |
| AM | 2 (20%) | 3 (30%) | 5 (50%) |
| GM | 0 (0%) | 0 (0%) | 0 (100%) |
| CZ | 0 (0%) | 0 (0%) | 0 (100%) |
| AZM | 0 (0%) | 0 (0%) | 0 (100%) |

The results of this study showed that the lowest inhibitory concentration of ethanolic extract of grass snake was 12.5 ppm, two strains were inhibited in this concentration, while the highest inhibitory concentration of ethanolic extract was 50 ppm, 5 of which in this concentration of inhibition.

The lowest inhibitory concentration of the gum extract ethyl acetate was 6.25 ppm. Two strains were inhibited at this

concentration, while the highest germination concentration of the ethyl acetate extract was 100 ppm; one strain was inhibited in this concentration (Table 3).

***Table 2.*** *Minimum inhibitory concentration and minimum bactericide concentration of C. spinose leaves against Acinetobacter (ppm).*

| Bacterium sample | Ethanol extract leaf | | Ethyl acetate extract leaf | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| 1 | 50 | 25 | 50 | 25 |
| 2 | 50 | 25 | 50 | 25 |
| 3 | 100 | 50 | 100 | 50 |
| 4 | 100 | 50 | 50 | 25 |
| 5 | 100 | 50 | 50 | 25 |
| 6 | 100 | 50 | 50 | 25 |
| 7 | 100 | 50 | 50 | 25 |
| 8 | 100 | 50 | 50 | 25 |
| 9 | 100 | 50 | 50 | 25 |
| 10 | 100 | 50 | 50 | 25 |
| 11 | 100 | 50 | 50 | 25 |
| 12 | 100 | 50 | 100 | 100 |

***Table 3.*** *Minimum inhibitory concentration and minimum bactericide concentration of C. spinose fruit against Acinetobacter (ppm).*

| Bacterium samples | Ethanol extract fruit | | Ethyl acetate extract fruit | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| 1 | 25 | 12.5 | 50 | 25 |
| 2 | 50 | 25 | 25 | 12.5 |
| 3 | 25 | 12.5 | 50 | 25 |
| 4 | 100 | 50 | 50 | 25 |
| 5 | 50 | 25 | 12.5 | 6.25 |
| 6 | 100 | 50 | 50 | 25 |
| 7 | 50 | 25 | 50 | 25 |
| 8 | 100 | 50 | 25 | 12.5 |
| 9 | 100 | 50 | 50 | 25 |
| 10 | 50 | 25 | 12.5 | 6.25 |
| 11 | 100 | 50 | 50 | 25 |
| 12 | 50 | 25 | 100 | 50 |

The results showed that the minimum inhibitory concentration of ethanolic extract of root extract was 50 ppm; all 12 strains were inhibited in this concentration, while the lowest inhibitory concentration of root extract acetate extract was 25 ppm, of which 7 strains were inhibited in this concentration (Table 4).

***Table 4.*** *Minimum inhibitory concentration and minimum bactericide concentration of C. spinose stem against Acinetobacter (ppm).*

| Ethyl acetate extract stem | | Ethanol extract stem | | Bacterium sample |
|---|---|---|---|---|
| MIC | MBC | MIC | MBC | |
| 50 | 25 | 50 | 50 | 1 |
| 50 | 25 | 50 | 50 | 2 |
| 100 | 50 | 100 | 50 | 3 |
| 100 | 50 | 100 | 50 | 4 |
| 50 | 25 | 100 | 50 | 5 |
| 50 | 25 | 100 | 50 | 6 |
| 50 | 50 | 100 | 50 | 7 |
| 50 | 25 | 100 | 50 | 8 |
| 50 | 25 | 100 | 50 | 9 |
| 50 | 50 | 100 | 50 | 10 |
| 50 | 25 | 100 | 50 | 11 |
| 100 | 100 | 100 | 50 | 12 |

The results of the OD results for biofilm formation showed that at higher concentrations, the inhibitory effect of biofilm formation was lower (Tables 5 and 6).

***Table 5.*** *Results of biofilm OD at different concentrations of ethanol extract of leaf (ppm).*

| Variables | Concentration (ppm) | | | | | | Bacterium sample |
|---|---|---|---|---|---|---|---|
| Control biofilm any extract | 3.1 | 6.25 | 12.5 | 25 | 50 | 100 | |
| 1.256 | 0.93 | 1.021 | 1.553 | 0.165 | 0.236 | 0.221 | 1 |
| 1.562 | 1.266 | 1.24 | 1.513 | 0.727 | 0.201 | 0.109 | 2 |
| 1.195 | 1.087 | 1.185 | 0.98 | 1.38 | 0.202 | 0.248 | 3 |
| 1.428 | 1.307 | 1.252 | 1.087 | 0.343 | 0.299 | 0.158 | 4 |
| 1.346 | 1.197 | 1.192 | 0.902 | 0.55 | 0.167 | 0.134 | 5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1.452 | 1.11 | 1.263 | 1.213 | 0.462 | 0.238 | 0.194 | 6 |
| 1.325 | 1.199 | 1.264 | 0.897 | 0.345 | 0.198 | 0.165 | 7 |
| 1.121 | 0.957 | 1.462 | 0.993 | 0.354 | 0.18 | 0.203 | 8 |
| 1.248 | 0.98 | 1.144 | 0.571 | 0.436 | 0.186 | 0.157 | 9 |
| 1.359 | 1.166 | 0.902 | 1.124 | 0.36 | 0.246 | 0.168 | 10 |
| 1.526 | 1.19 | 0.907 | 0.732 | 0.278 | 0.204 | 0.153 | 11 |
| 1.369 | 1.127 | 1.153 | 0.566 | 0.697 | 0.637 | 0.148 | 12 |

*Table 6. Results of biofilm OD at different concentrations of ethyl acetate extracts of leaf (ppm).*

| Control biofilm any extract | Concentration (ppm) | | | | | | Bacterium sample |
|---|---|---|---|---|---|---|---|
| | 3.1 | 6.25 | 12.5 | 25 | 50 | 100 | |
| 1.489 | 1.338 | 1.309 | 1.196 | 0.262 | 0.21 | 0.202 | 1 |
| 1.925 | 1.708 | 1.585 | 1.313 | 0.5 | 0.268 | 0.169 | 2 |
| 1.759 | 1.55 | 1.479 | 1.143 | 0.407 | 0.299 | 0.181 | 3 |
| 1.895 | 1.581 | 1.573 | 0.901 | 0.397 | 0.283 | 0.177 | 4 |
| 1.855 | 1.664 | 1.552 | 1.47 | 0.513 | 0.28 | 0.274 | 5 |
| 1.639 | 1.568 | 1.5 | 1.449 | 0.729 | 0.249 | 0.359 | 6 |
| 1.728 | 1.68 | 1.3 | 1.088 | 0.394 | 0.369 | 0.196 | 7 |
| 1.658 | 1.529 | 1.339 | 1.276 | 0.186 | 0.393 | 0.208 | 8 |
| 1.432 | 1.137 | 1.36 | 1.54 | 0.45 | 0.282 | 0.225 | 9 |
| 1.563 | 1.335 | 1.475 | 1.336 | 0.434 | 0.276 | 0.175 | 10 |
| 1.697 | 1.379 | 1.446 | 0.995 | 0.295 | 0.242 | 0.204 | 11 |
| 1.658 | 1.317 | 0.768 | 0.935 | 0.215 | 0.263 | 0.192 | 12 |

## Discussion

The results of the antibiotic resistance pattern showed that 100% of the samples were susceptible to CZ-GM (Gentamycin)-AZM antibiotics and only 16.6% of the samples were resistant to AM (Ampicillin) antibiotics.

In the study of Goodarzi et al. [17] on antibiotic resistance pattern of *A. baumannii* in Tehran, the results showed that the resistance strains to antibiotics were: 103 (95.4%) to ceftazidime, 108 (100%), 100 (6/92%) to ciprofloxacin, 103 (95.4%) to piperacillin-tazobactam, 44 (40.7%) to gentamicin, 87 (80.6%) to tetracycline and 1 (0.9%) to clostin.

The study of Jafari et al. [18] examined the antibiotic resistance pattern of *A. baumannii* in Fars province and the results showed that the percentages of *Acinetobacter* isolates of amphenem, moropenem and ciprofloxacin in serial dilution method were 40.9%, 60%, and 77.7%; respectively. In a study by Simhon et al. [19] sensitivity to ampicemin decreased from 98.1% in 1990 to 64.1% in 2000, and the sensitivity to ciprofloxacin decreased from 50.5% to 13.1%. A study in UK was conducted by Caroline and colleagues in 2002, and results showed that 46% of isolates were resistant to ciprofloxacin and 2% of isolates were resistant to ampipenem [20].

In a study conducted in Tehran in 2009 by Boromand et al., 53.4% of the samples were resistant to ciprofloxacin and 24.6% of them were resistant to ampipenem [21]. Antibiotic resistance patterns among hospital pathogens may vary widely from one country to another or in different regions of a country. Previous reports from around the world suggest that 30-83.9% of Acetone is resistant to multiple medicines [22]. On the other hand, Ferreira et al. found that 68% were resistant to carbapenems [23].

Peymani et al. [24] found that in 81% of isolates in antibiotic classes beta-lactam, aminoglycosides and quinolones, there were complete or intermediate drug resistance in 2012, and their percentage of resistance to ampicemin and maropenem were 68% and 69%, respectively. The results of the OD results for biofilm formation showed that at higher concentrations, the inhibitory effect of biofilm formation was lower.

In 1394, the effect of alcoholic essence of Satureja khuzestaniea on the expression of the gene associated with Biofilm *A. baumannii* was investigated. Due to the inhibitory

effect of the Khuzestan Sourifolia essential oil on the Biofilm *A. baumannii* gene, it would probably be possible to use this essential oil as a supplement [25]. The results of this study showed that the lowest inhibitory concentration of the *C. spinose* ethanol extract against *A. baumannii* was 25 ppm, in which two bacterial strains were inhibited, while the highest inhibitory concentration was 50 ppm and the highest concentration of fecundity is equal to 100 ppm. The results of the study of *C. spinose* ethanol extract showed that the lowest inhibitory concentration was 25 ppm, while the highest inhibitory concentration was 100 ppm, which was inhibited by a strain of bacteria.

The study of Abd Razik [26], antibacterial effects of different concentrations ranging from (125-1000 mg/ml) of hexane and methanol juice of *C. spinose* flowers was determined by using agar well diffusion method in clinical strains. Methanol juice of *C. spinosa* flowers was the most active than hexane juice. The activity of *C. spinosa* determined by measuring inhibition region as following: *Lactobacillus sp*., *Escherichia coli*, *Streptoccus sp*. and *Staphylococcus aures* demonstrate inhibition region at concentration of 125 -1000 mg/ml. *Klebsiella* sp., *Pseudomonas aeruginosa* and Enterococcus sp. showed inhibition region of 500- 1000 mg/ ml. it was no inhibition region for Proteus sp. Hexane juice of *C. spirosa* flowers was less active than methanol juice against examine bacteria. *Lactobacillus* sp., *S. aureus* and *Streptococcus* sp. demonstrate inhibition region at concentrations 150- 1000 mg/ ml while *Eseherichia coli*, *S. aureas* and *Klebsiella sp*. demonstrate sensitivity at concentration 1000 mg/ml. There was no inhibition region for *P. aeruginosa*, *Enterococcus sp.* [26]. Ibrahim [27] investigated the antimicrobial acting of the flower extract of *C. spinosa*, gel and leaf extract of Aloe vera were examine against isolated bacterial of skin infection *S. aureus*, *S. epidermidis*, *S. pyogenes*, *P. aeruginosa* and *E. coli*. Antimicrobial susceptibility examine demonstrated that the *C. spinosa* was 100% effective versus gram positive isolates and 90% acting against gram negative isolates, A. vera leaf was 20% effective versus the entire tested gram positive as well as 15% effective versus gram-negative isolates. *Capparis spinosa* showed prominent antibacterial activity versus gram negative and positive.

Benakashani et al. [28] studied the antimicrobial effect of nanoparticles produced *C. spinosa* by disc diffusion method against different pathogenic bacteria such as *E. coli*, *Salmonella typhimurium*, *S. aureus* and *Bacillus cereus*. The results showed that synthesized silver nanoparticles have an excellent antimicrobial property and a high antimicrobial acting compared to the ionic silver.

Biofilm is a structural society and a social expression of micro-organisms, which are placed in a space between them by the surface of the enamelled polyester produced by them and connected to one surface. Biofilm formation is an active process involving the initial binding, the formation of microcolony, the production of extracellular polymeric material, followed by growth and maturation, and ultimately thinning and releasing the cell from biofilms [29]. Since their antibiotic resistance is increasing, it requires new treatments.

Bahador et al. [30] studied effect of Satureja khuzestanica essence, on the expression level of bap gene in *A. baumannii*. The results showed by reducing bap gene expression. They hope in future be used it to the clinic with a wider range as a complementary therapy and also for surgery operation.

José Alves et al. [31] reported the *Acinetobacter baumannii* was the microorganism with the lowest susceptibility to mushroom extracts inhibitory effect on biofilm production (highest inhibition—almost 29%, by Russula delica extract). David Alejandro et al. [32] investigated the effect of sub-lethal concentrations of MBC of the extract, by inhibiting the formation of biofilms. The result show that concentrations of 25%, 50% and 75% of the MBC (1.87, 3.74 and 5.61 mg/mL, respectively), caused biofilms inhibitions of 19.5%, 40.8% and 36.0%, respectively.

## Conclusion

The results of this study show the anti-microbial effects of good *C. spinose* biofilm on *A. baumannii,* which can be used as an effective treatment. The results of this study show the good antimicrobial effects of the extract *C. spinosa* on the pathogenic bacteria. At first, we resolved total phenolic and total flavonoid extent of each extract of fruits and roots.

## Conflict of Interests

The authors declare no conflict of interest.

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