

## Antibacterial Activity from Different Parts of *Capparis spinosa* L.

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( ) *Capparis spinosa*  
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*Proteus vulgaris* *Pseudomonas aeruginosa* *pneumoniae*

MIC

MBC

### Abstract

In the present study ethanol and chloroform extracts from different parts of *Capparis spinosa* (flowers, fruits, leaves and roots) were screened for antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus vulgaris*, ethanol and chloroform extracts from *C. spinosa* roots showed good inhibitory effects against tested bacteria compared with standard antibiotics, meanwhile no clear activity was detected using both extracts from flowers, fruits and leaves against the test bacteria. Minimum inhibitory concentrations MIC and minimum bactericidal concentrations MBC of root extracts were determined against the tested bacteria.

## Introduction

A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. Several hundred genera are used medicinally, mainly as herbal preparations in the indigenous systems of medicine in different countries and are sources of very potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them. The World Health Organization (WHO) reported that 80% of the worlds population rely chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents [1]. Due to the indiscriminate use of antimicrobial drugs the microorganisms have developed resistance to many antibiotics. This has created immense clinical problem in the treatment of infectious diseases [2]. In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppressant and allergic reactions [3]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained.

*Capparis spinosa* L. (caper, caper berry, caper bush) belongs to *Capparidaceae* family, a biennial spiny shrub that bears rounded, rather fleshy leaves and big pinkish-white flowers, it grows wild on walls or in rocky coastal areas throughout the Mediterranean region. It is best known for its edible buds and fruit which are usually consumed pickled [4]. Capers have a sharp piquant flavor and add pungency, a peculiar aroma and saltiness to comestibles such as pasta sauces, pizza, fish, meats and salads. The flavor of caper may be described as being similar to that of mustard and black pepper. The capers strong flavor comes from mustard oil: methyl isothiocyanate (released from glucocapparin molecules) arising from crushed plant tissues [5].

*C. spinosa* cortex and leaves contain stachydrine and 3-hydroxystachydrine, the root contains glucobrassicin, neoglucobrassicin and 4-methoxy-glucobrassicin, the crude extract of the flower buds contain 162 volatile constituents of which isothiocyanates, thiocyanates, sulphides and their oxidative products have been identified as the major components, the seeds and leaves contain glucocapparin and glucocleomin, the root bark contains stachydrine, rutic acid and a volatile substance with garlic oil [6], in addition the root barks are used as analgesic, anthelmintic, aperients, depurative, diuretic, emmenagogue, expectorant, tonic and vasoconstrictive it is used internally in the

treatment of gastrointestinal infections, diarrhea, gout and rheumatism. Externally, it is used to treat skin conditions, capillary weakness [7].

### **Materials and methods**

#### **Plant material**

*Capparis spinosa* was collected from Al-Rashidea region, Nineveh province and was identified at Department of Biology, the plant parts used (flowers, fruits, leaves and roots) were washed with distilled water and were dried at room temperature in dark then grinded to powder using an electrical blender.

#### **Preparation of extracts**

Extract preparation was carried out using two methods including all the plant parts for each method.

#### **Ethanol extracts**

Ethanol extracts were accomplished according to [8] which is modified from the basic method set by [9], and includes dissolving 40gm of sample in 400ml ethanol (concentration 95%) then soaking for 24 hours, followed by filtration through several layers of gauze, the resulting filtrate was evaporated under reduced pressure using a rotary vacuum evaporator at 40°C, the dried extract was stored in sterile bottles until further use.

#### **Chloroform extracts**

Dried and powdered samples (40-50gm) from each plant part used (flowers, fruits, leaves and roots) were extracted with chloroform 500ml using a soxhlet extractor for 15 hours continuously until the used solvent turned pure and colorless [10], the solvent was removed using a rotary vacuum evaporator at 40°C to give concentrated extract which was frozen and freeze-dried until use.

#### **Preparation of extract concentrations**

1gm of each extract (ethanol and chloroform) was dissolved in 5ml DMSO (Dimethylsulfoxide) to give an extract 200mg/ml and this was used as a standard concentration in providing next extenuations (100, 50, 25, 12.5, 6.25 and 3.12mg/ml), then were sterilized by pasturalization at 62°C.

## **Test bacteria**

The microorganisms used was *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* which all had been obtained and identified from Department of Biology, College of Science, University of Mosul.

## **Screening for antibacterial activity**

The disc diffusion method [11] was used to evaluate the antibacterial activity. Nutrient agar was prepared in the plates as the media for the test microorganisms. One hundred filter paper discs Whatman No.1 (6mm in diameter) were sterilized at 140°C for one hour then impregnated with 1ml of each extract concentration [12]. The bacterial inoculum ( $10^8$  cfu /ml compared with McFarland tube No. 1), was spread evenly on to the surface of the Nutrient agar plates using a sterile cotton bud before the extract discs were positioned on the inoculated agar surface, antibiotic discs (Bioanalyse) 6mm in diameter of Chloramphenicol 30µg, Cephalexin 30 µg, Tetracycline 30 µg and Gentamycin 10 µg were used as positive controls. All the plates were incubated for 24 hours at 37°C, at the end of the period, inhibition zones formed were evaluated in mm.

## **Determination of MIC and MBC values**

Minimum Inhibitory Concentrations (MIC) was determined using turbidity test by adding 0.1ml of each extract concentration into tubes containing 9.8ml sterile Nutrient Broth, tubes were inoculated with 0.1ml of bacterial suspension, each test was done in triplicate. Tubes were incubated at 37°C for 24 hours O.D. was determined using a SERIES CECIL 1021, 1000 spectrophotometer at 595nm. The MIC values were taken as the lowest concentration of the extracts that showed no turbidity after 24 hours of incubation at 37°C [13] by comparing with the control tube which includes 9.8ml Nutrient Broth and 0.1ml of bacterial suspension in addition to 0.1ml from each extract concentrations.

The Minimum Bactericidal Concentration (MBC) was determined by subculture of the tubes showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value [14].

### Result and Discussion

The results of the present study showed that (leaves, fruits and flowers) of *Capparis spinosa* had no antibacterial activity using ethanol and chloroform extracts, these plant parts were eliminated and the study concentrated on the roots only which showed high antibacterial properties using ethanol and chloroform extracts, Gram positive bacteria was more sensitive towards extracts than Gram negative (Table 1), this may be attributed to the fact that Gram-negative cell wall is a multilayer structure and quite complex [15], and the role of secreted exoenzyme in defending bacteria against plant extracts [16]. Our results disagreed with [17] who reported that the aerial parts of *C. spinosa* had antibacterial activities against Gram positive and Gram negative bacteria using water, ethanol and butanol as solvents, this may be consequence of different solvents, different types of strain, isolation area, and different assay methods, our results also disagreed with what was reported by [18] who revealed antibacterial activity of aerial parts from *C. spinosa* against Gram negative bacteria, meanwhile our study agreed with [19] who reported no inhibitory effects of *C. spinosa* aerial parts against Gram positive and negative bacteria. Our results indicated that ethanol extract of *C. spinosa* roots was more active against all test bacteria compared with chloroform extract, and *Staph. aureus* was more sensitive towards plant extracts than all other tested bacteria, even the least ethanol extract concentration 3.12mg/ml was active against it, meanwhile *Kleb. pneumoniae* showed less susceptibility towards the extracts and was inhibited only using high concentrations of both extracts ethanol (200, 100 and 50mg/ml) and chloroform (200 and 100mg/ml) as in (Table 1, Figure 1 and 2). The main antibacterial cause of *C. spinosa* roots is due to the existence of compounds such as indole, aliphatic glucosionlates, polyprenols, flavonoids and alkaloids [20], and most of these compounds can be dissolved in ethanol better than chloroform, this may expound the suitability of ethanol extract in achieving antibacterial effects more than chloroform extract. From (Table 1) it can be seen that *Staph aureus* showed high sensitivity when treated with the used antibiotics (Chloramphenicol, Cefalexin, Tetracycline and Gentamycin) meanwhile *Kleb. pneumoniae* was less susceptible among test bacteria, generally *C. spinosa* root extracts achieved better inhibition zones against test bacteria compared with the antibiotic Gentamycin and a moderate effect compared with Cefalexin and Tetracycline, meanwhile a weak effect was obtained compared with Chloramphenicol.

**Table 1:** Antibacterial activity of ethanol and chloroform extracts from *Capparis spinosa* roots.

Microorganisms	Extracts	Extract concentration (mg/ml)							Control			
		200	100	50	25	12.5	6.25	3.12	C	CL	TE	CN
<i>Staph. aureus</i>	E CH	22 18	18 16	16 14	13 12	10 10	9 -	8 -	30	32	30	19
<i>B. subtilis</i>	E CH	18 16	16 14	14 13	12 10	10 -	- -	- -	28	30	27	18
<i>Kleb. pneumoniae</i>	E CH	14 12	12 10	9 -	- -	- -	- -	- -	20	22	28	16
<i>Ps. aeruginosa</i>	E CH	16 14	14 12	12 10	10 -	- -	- -	- -	24	24	32	17
<i>Pr. vulgaris</i>	E CH	18 17	16 15	15 13	12 10	10 -	- -	- -	26	24	27	18

- : No activity, E: Ethanol extract, CH: Chloroform extract, C: Chloramphenicol, CL: Cefalexin, TE, Tetracycline, CN: Gentamycin

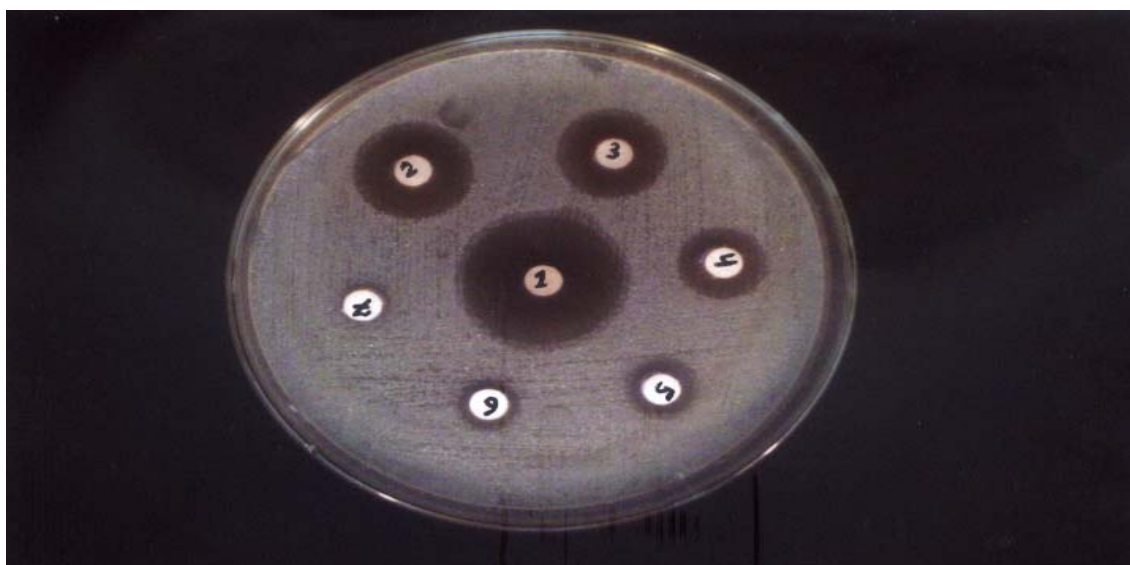


Figure 1: Effect of ethanol extract concentrations from *C. spinosa* roots against *S.aureus* 1(200mg/ml), 2(100mg/ml), 3(50mg/ml), 4(25mg/ml), 5(12.5mg/ml), 6(6.25mg/ml), 7(3.12mg/ml).

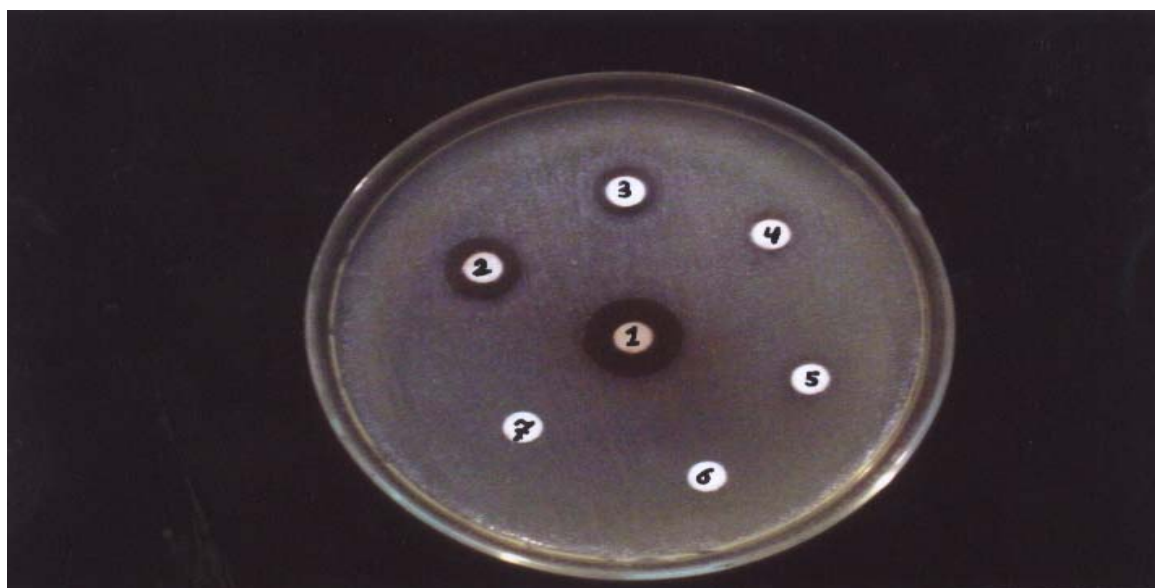


Figure 2: Effect of chloroform extract concentrations from *C. spinosa* roots against *Kleb. pneumoniae* 1(200mg/ml), 2(100mg/ml), 3(50mg/ml), 4(25mg/ml), 5(12.5mg/ml), 6(6.25mg/ml), 7(3.12mg/ml).

The MIC values of the ethanol and chloroform extracts from *C. spinosa* roots against test bacteria are shown in (Table 2). The MIC value of the ethanol extract against Gram positive bacteria was 0.25mg/ml in addition to *Pr. vulgaris*, and 1.0mg/ml against *Kleb. pneumoniae*, meanwhile MBC values of both extracts against *Staph. aureus*, *B. subtilis* and *Pr. vulgaris* were 1.0mg/ml, both extracts were unable to show MBC values against *Kleb. Pneumoniae* at 2.0mg/ml, these results agreed and were very close with what was reported by [21] who studied the MIC values of *C. tomentosa* roots against Gram positive and negative bacteria in addition to *Candida albicans*.



**Table 2:** Minimum Inhibitory Concentration (MIC) & Minimum Bactericidal Concentration (MBC) of *Capparis spinosa* root extracts.

Extract type	Microorganisms									
	<i>Staph. aureus</i>		<i>B. subtilis</i>		<i>K. Pneumoniae</i>		<i>Ps. aeruginosa</i>		<i>Pr. vulgaris</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Ethanol extract	0.25	1.0	0.25	1.0	1.0	-	0.5	2.0	0.25	1.0
Chloroform extract	0.5	1.0	0.5	1.0	2.0	-	0.1	2.0	0.5	1.0

- : No activity at 2.0mg/ml.

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