

## Antimicrobial activity of leaves and bark of Libyan *Capparis spinosa* subsp *orientalis* (Duh.) Jafri.

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This study aimed to investigate the antimicrobial activity of *Capparis spinosa* bark and leaves against different standard strains and clinical isolates. Soxhlet apparatus and rotary evaporator were used for extraction and evaporation of organic solvents extracts. Maceration and freeze drying used for extraction and drying of water extracts. Nutrient agar, Mueller Hinton agar and Sabouraud dextrose agar were used for bacterial and fungal culture and McFarland 0.5 solution used for calibration. Disc diffusion method and dilution agar plate assays were used to evaluate antimicrobial activities and minimum inhibitory concentrations. Six different antibiotics discs were used as references. According to results *Capparis spinosa* leaves and bark extracts with different solvents revealed variable *in vitro* growth inhibition activity against tested standard strains and clinical isolates with highly significant differences;  $P < 0.01$ . The higher plant activities were obtained from chloroform bark extract and methanol leaves extract with equal minimum inhibitory concentration of 100mg/ml against both standard *Staphylococcus aureus* ATCC 25923 and clinical methicillin resistant *Staphylococcus aureus*, and these activities may due to the high presence of the phytochemical constituents such as flavonoids, tannins, steroids and triterpines. Chloroform bark extract very effective against standard and clinical isolates of *Staphylococcus aureus* and methanol leaves extract is effective against the standard organism. In conclusion, *Capparis spinosa* subsp *orientalis* (Duh. Jafri) leaves and bark extracts have a pronouncing antibacterial activity against *Staphylococcus aureus* especially with the limited treatment choices.

**Key words:** Capparidaceae, *Capparis spinosa* parts extracts, Antimicrobial, Phytochemical constituents, Minimum inhibitory concentration.

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## 1. Introduction:

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Therefore, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years, especially due to the constant emergence of microorganisms resistant to conventional antimicrobials. Natural remedies are preferred over synthetic drugs, which can be harmful or cause undesirable side effects (Hani *et al.*, 2017). Natural products are traditionally widely used for treatment because they are available and cheap compared to the synthetic drugs.

Microorganisms include bacteria, fungi, certain algae and protozoa may be either harmless, disease producing or extremely harmful. Various bacteriological agents that are pathogenic to man and cause many infectious diseases include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus mirabilis*. Some of these organisms form normal flora of man but may become opportunistic pathogens when the host is immunocompromised or when they are coincidentally transmitted to areas where they are not normally resident (Mbata, 2017). The non-motile, facultative anaerobe *Staphylococcus aureus* bacteria is commonly inhabit the nasal cavities and skin of healthy people, but if it enters the body via a cut or medical devices, it can cause either local or serious infections due to its ability to form biofilm (Eliyadet *al.*, 2012). The Gram negative *Pseudomonas aeruginosa* and the Gram positive methicillin resistant *Staphylococcus aureus* are major pathogens that cause nosocomial infection and considered as a community pathogen causing morbidity and mortality. The methicillin resistant *Staphylococcus aureus* is a multi -drug resistant bacteria that resists all penicillins, so the option antibiotics for treatment of its infection are limited to few antibiotics such as Vancomycin, Linezolid, Tigecycline and Mupirocin. Vancomycin is the most common used, but by time the pattern of antimicrobial susceptibility of the bacteria has been changed worldwide and it has been reported that Vancomycin and Mupirocin increasingly become less effective in settings with extensive use of these agents (Simoret *al.*, 2007).

In sense of the fact says bacteria have the genetic ability to acquire and transmit resistance to other organisms and due to the miss use of antimicrobial drugs, the development of antimicrobial resistance by microorganisms has increased (Idresset *al.*, 2015), the matter which has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). In addition to this problem, the synthetic antibiotics used currently are usually expensive and sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal

microorganisms, immunosuppressant and allergic reactions (Firas and Mohammed, 2007). Therefore, alternative antimicrobial agents of herbal origin become of interest (Abdoulraoufet *et al.*, 2015). A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases. According to the World Health Organization 1993, 80% of the world population depends chiefly on the traditional use of plant extracts or their constituents for the treatment of infectious diseases. Medicinal herbs represent a rich source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (Firas and Mohammed, 2007). Plant derived compounds have a potential for many biological activities include antimicrobial activity. From previous research studies, several plants secondary metabolites have been revealed as potential antibacterial agents (Savoia, 2012). Alkaloids, flavonoids, tannins, terpenes, quinins and resins are plant's secondary metabolites contributed to many antibacterial effects against different Gram negative and positive bacteria (Compean and Ynalves, 2014, Benziane *et al.*, 2012, Oliver and Herbert, 1999). *Capparis spinosa* was first used for medicinal purposes by the Sumerians in 2000 BC and ancient Romans and Greeks also used it in medicine field. It belongs to Capparidaceae family, it grows wild on walls or in rocky coastal areas throughout the Mediterranean region. The plant has been used in gout, as diuretics, astringents and tonics in traditional Iranian medicine (Basma, 2011). Furthermore it has been reported that *Capparis spinosa* bark and roots used as analgesic, anthelmintic, expectorant, tonic and vasoconstrictive. It is used internally in the treatment of gastrointestinal infections, diarrhea and also in rheumatism. Externally, it is used to treat skin conditions and capillary weakness (Ramin and Nastaran, 2016, Manikandaselviet *et al.*, 2016).

Orooba, 2012 tested the flowers extract against some isolated bacteria from skin infection; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antimicrobial susceptibility test showed that the *Capparis spinosa* was 100% effective against Gram positive isolates and 10% activity against Gram negative isolates. Many studies done to screen the antimicrobial activity of *Capparis spinosa* roots and flowers but little carried about the plant bark and leaves. In Libyan folklore medicine the plant bark and leaves are common used as anti-cancer and for wound infection treatment. This study aimed to evaluate the antimicrobial activity of extracts of leaves and bark of *Capparis spinosa* with different solvents against different organisms.

## 2. Materials and Methods

### 2.1 Plant Material

*Capparis spinosa* was collected in August 2016 from Shahat region, located in Al Jabal Al Akhdar, Northeast of Libya. Plant was identified and classified by Dr. Hussein Altajouri at Botany department, faculty of Science, Benghazi University, Libya. Plant leaves and bark were cleaned with tap water, air dried at room temperature and then powdered. The dried leaves and bark powder were kept in separate colored bottles, ready for extraction process.

### 2.2 Bacteria

Standard Gram positives bacteria, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 8236 and standard Gram negatives, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and standard fungi; *Candida albicans* ATCC 7590 were obtained from Medicinal and Aromatic Plant and Traditional Medicine Research Institute, National Center for Research, Sudan. . One hundred clinical isolates were collected from different samples (blood, sputum, wound, semen) from patients attending to Benghazi Medical Center, Libya. The clinical isolates were methicillin resistant *Staphylococcus aureus*, *Acinet obacterbaumani*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiela pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

### 2.3 Reference Antibiotics Discs

Amoxicillin 20µg + Clavulanic acid 10µg (AMC), Cefprozidime 30µg (CAZ), Ceftriaxone 30 µg (CTX), Ciprofloxacin 30 µg (CIP), Gentamicin 10 µg (CN) and Vancomycin 30 µg (VA) are standard antibiotics discs used as references in this study. They were bought from Bioanalyse<sup>®</sup> YSE TibbiMalzemeler San..Expire dates were 10 - 18 months valid after the date of the assay.

### 2.4 Preparation of plant extract

One hundred gram of each of leaves and bark powder was thoroughly extracted for enough time (6-10 hours) with enough quantities (250-300ml) of four different solvents; Chloroform, Methanol, Ethanol and water respectively. Soxhlet apparatus and rotary evaporator were used for extraction with organic solvents and evaporation. Maceration and freeze drying used for extraction and drying of water extracts. The yields were air dried, weighed and kept in well labeled colored tight closed bottles in a fridge at 4C°. In the day of the antimicrobial assay, fresh solutions of concentrations of 100mg/ml of each extract were prepared by dissolving 0.2g in 2ml solvent. Water used as solvent for aqueous extracts, mixture of petroleum ether and methanol (1:2) was used as solvent for chloroform extracts and methanol was used as solvent for methanol and ethanol extracts.

## 2.5 Preparation of bacterial and fungal suspension

An overnight nutrient agar slant growth of each of the five standard organisms strains and of each of the 100 clinical bacterial isolates were washed with sterile normal saline 0.9% and brought to a solution of  $10^8$  C.F.U/ ml by calibration with McFarland 0.5 solution and each kept in labelled sterile capped test tubes. Nutrient agar and Sabouraud dextrose agar were used for bacterial and fungal culture, respectively and Muller Hinton agar (MHA) media was used for sensitivity tests.

## 2.6 Antimicrobial screening assay

The disc diffusion method was used for the determination of the antibacterial activity ([Mukhtar and Ghori, 2012](#)). Duplicate sterile Discs, 6 mm in diameter (Wattman paper N°1 - Selecta, Germany), after soaked with 20  $\mu$ l of a solution of *Capparis spinosa* extracts were placed on Mueller-Hinton agar petri dish had been surface spread with 100  $\mu$ l of the organism suspension which freshly adjusted to a  $10^8$  CFU/ml. The Petri dishes were then incubated for 18 hours at 37°C. The diameters of the inhibition zone were measured to assess the *in-vitro* antibacterial activity. Discs impregnated with methanol were used as a negative control. Antibiotics discs were used as positive reference for bacteria to compare the sensitivity. The same method as for bacteria was adopted for fungi, where incubation was at 25°C for two days for *Candida albicans*.

## 2.7 Determination of minimum inhibitory concentration (MIC):

[Andrews, \(2006\)](#) agar dilution method was adopted in this study with little modification. The agar plate dilution method was used to determine the minimum inhibitory concentration of the extract which can inhibit the growth of the seeded bacteria on the Mueller-Hinton agar media. Serial dilutions were prepared for each extract in decreasing concentrations in the following order: 200, 100, 50, 25, 12.5, 6.25, and 3.13 mg/ml. In sterile covered glass bottles, 5ml Melted double strength Mueller-Hinton agar cooled to 45°C were mixed with 5ml of each dilution of the tested plant extract to get a final serial dilution of 100, 50, 25, 12.5, 6.25, 3.13 and 1.65 mg/ml of each extract. The mixture was poured to sterile small petri dishes, left to solidify and then the bottom of each plate was marked off into segments, one segment designed for the standard strain and the others designed for the clinical strains. By using of a standard loop (0.01 ml), a loop full of each of tested bacterial fresh suspension adjusted with McFarland 0.5 solution was spotted onto the surface of each segment. The inoculum allowed to be absorbed into the agar before incubation and then the plates incubated at 37°C for 18 hours. After the incubation period the least concentration mg/ml of the plant extract that inhibits the growth of organism was considered as the end point (MIC).

## 2.8 Phytochemical screening

Phytochemical screening for the active constituents; Alkaloids, Anthraquins, Coumarins, Flavonoids, Saponins, Steroids, Tannins and Triterpines, was carried out using the methods described by [Martinez et al., 2003](#), [Sofowora, 1993](#) and [Wall et al., 1952](#), with many few modifications.

## 2.9 Statistical Analysis

Data were expressed as mean  $\pm$  SD. Statistical examination was performed utilizing SPSS version 20, One-way analysis of variance (ANOVA) followed by the LSD Post Hoc test. The P values more than 0.05, less than  $\leq 0.05$  and  $\leq$  less than 0.01 were considered as not significant, significant and highly significant values respectively.

## 3. Results and Discussion:

Little research was carried out for *Capparis spinosa* species in general and on its leaves and bark in specific. The bark and leaves chloroform, methanol, ethanol and aqueous extracts of *Capparis spinosa* were screened for their antimicrobial activity against Gram positive, Gram negative bacteria and fungi. No effect shown with the aqueous extracts.

### 3.1 Antimicrobial screening against standard organisms

The statistical analysis showed that there was a highly significant differences between the effects of different plant parts extracts against different standard organisms (Table 1). The same table showed that although both chloroform and methanol extracts of *Capparis spinosa* were showed antibacterial activity against *Staphylococcus aureus*, it was clear that the chloroform bark extract was more effective against the bacteria, it gave  $30 \pm 0.01$  mm inhibition zone compare with  $20 \text{ mm} \pm 0.01$  revealed by methanol leaves extract. *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* showed sensitivity towards both methanol extracts of the leaves and bark. On the other hand *Candida albicans* revealed inhibition zones of  $16 \text{ mm} \pm 0.71$  with methanol extract of both bark and leaves, but the highest was 18 mm from ethanol bark extract. [AbdRazik, \(2011\)](#) claimed that methanol extract of *capparis spinosa* flowers was more active than hexane extract The traditional medicinal uses of *Capparis spinosa* in Libya are well known but the supporting scientific data available is very scanty. Methanol leaves extract effectiveness was screened against 100 clinical bacterial isolates of seven different genera; methicillin resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. The results proved that all of the clinical isolates devoid of any

susceptibility except for methicillin resistant *Staphylococcus aureus* isolates which showed weak susceptibility with mean inhibition zone of  $5\text{mm} \pm 6.9$  (Figure 1). After that the antibacterial activity for chloroform bark extract was screened against the clinical methicillin resistant *Staphylococcus aureus* isolates and it revealed active growth inhibitory effect with mean zone diameter of  $13 \pm 11$  (Table 2).

This study showed high growth inhibition activities from Chloroform bark and methanol leaves extracts against standard *Staphylococcus aureus*. Also it showed good and weak activities from chloroform extract of bark and methanol extract of leaves respectively against the clinical *Staphylococcus aureus* isolates. [Firas and Mohammed, 2007](#) did an antibacterial screening for ethanol extracts of leaves and roots of *Capparis spinosa* and their results disagreed with this study for the clinical *Staphylococcus aureus* which not affected by the plant leaves extract as they reported. While this investigation cleared that the methanol leaves extract have good growth inhibition activity against the *Staphylococcus aureus* strain, and this may contribute to the different solvents used. Even though the leaves extracts screened in [Firas and Mohammed](#) study and this study were from different solvent but both were devoid from any activity against *Pseudomonas aeruginosa* isolates. However, [Orooba, 2012](#) finding was differ where he reported that *Capparis spinosa* plant extract have good antibacterial activity against clinical *Pseudomonas aeruginosa* and *Escherichia coli*, while the methanol leaves extract in the present study did not show any activity against *Escherichia coli* isolates and showed weak activity against standard *Escherichia coli*. The LSD Post Hoc analysis revealed highly significant differences between inhibition zones from different plant parts and from different solvent and also between different organisms. [Farzadet al., 2016](#) were optimized the extracting parameters of crude polysaccharides from the *Capparis spinosa* leaves. They documented that much more antimicrobial activity using this polysaccharide was found against Gram-negative bacteria (*Escherichia coli*, *Shigella sonnei* and *Salmonella typhi*) than Gram-positive bacteria (*Bacillus panis* and *Staphylococcus aureus*). This study used to test crude ethanol leaves extract rather than dealing with polysaccharide specifically and the results disagreed with Farzad results where the crude ethanol extract showed lower activity against the negative *Escherichia coli* strain than that of the positive *Staphylococcus aureus*. [Rahimifardet al., 2015](#) carried a study to screen the antibacterial activity of methanol extract of the aerial part of different plant species; *Capparis scartilaginea* and *Capparis mucronifolia* and they found that the highest antibacterial activity of *Capparis mucronifolia* was against Gram positive *Staphylococcus epidermidis* and they referred this to the flavonoid compound of the plant. Even though [Rahimifardet al., 2015](#) and the present study investigated different plant species but both concerned with the aerial parts and used the same solvent and agreed

in that the plant *Capparis* possess high antibacterial activities against Gram positives *Staphylococcus aureus*.

### 3.2 Minimum inhibitory concentration

The lowest minimum inhibitory concentration in the present study was 50mg/ml revealed from methanol leaves extract against each of standard *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*. Both plants parts extracts showed highly effectiveness against the standard *Staphylococcus aureus* with MIC of 100mg/ml. Methanol leaves extract showed MIC of 100mg/ml against *Pseudomonas aeruginosa* (Table 3).

### 3.3 Comparison of plant extracts effects with reference antibiotics discs against clinical methicillin resistant *Staphylococcus aureus*

When the effectiveness of chloroform extract of *Capparis spinosabark* compared with the effectiveness of methanol extract of the leaves and of reference drugs against methicillin resistant *Staphylococcus aureus*, the results showed that even though chloroform bark extract actively inhibited the clinical isolate but the activity was lower compared to that of Ciprofloxacin, Gentamicin and Vancomycin. Also the figure cleared that chloroform bark extract of the plant was the only effective agent compared to methanol leaves extract and to the beta-lactam antibacterial references; Augmentin, Ceftazidime and Ceftriaxone (Figure 2). This result suggested that chloroform bark extract has anti extended-spectrum  $\beta$ -lactamases activity and can be used as alternative to Augmentin, Ceftazidime and Ceftriaxone. The LSD Post Hoc statistics interpretation cleared that there were high significant differences between the effect of extract of different plant part from different solvents and the effect of tested references drugs (Table 4).

### 3.3 Phytochemical screening

The phytochemical screening of *Capparis spinosaleaves* extract revealed highest yield of 15% from the methanol extract followed by ethanol extract 5%, water extract 3.7% and chloroform extract 3.5%. For bark the yield of methanol extract of bark, 4% was more than that revealed from other solvents used (Table 5). The chloroform bark extract showed the presence of high levels of steroids, triterpenes, and low levels of coumarins while the methanol leaves extract showed the presence of high levels of flavonoids, tannins, moderate levels of steroids and low levels of alkaloids and triterpenes. The presence of these phytochemical constituents may contribute to the effectiveness of the extracts against the tested organism (Table 6). The study indicated that the plant has pronounced growth inhibition activity against the Gram positive *Staphylococcus aureus* and suggested that this high effectiveness referred to the high presence of triterpenes and presence of coumarins in the

chloroform extract. Coumarins exhibit fairly high penetration ability through the cell wall (Oliver and Herbert, 1999). Also the study referred the effectiveness of plant to the tannins offered more from the methanol extract. As the phenomena of the ability of *Staphylococcus aureus* to colonize surfaces and form biofilms (which vary from strain to strain) has been reported (Zuluaga et al., 2006 and Fowler et al., 2005), and management of biofilm infections is extremely difficult due to their inherent resistance to antimicrobial chemotherapies and to the host immune response (Boles and Horswill, 2011). This study suggested that the effectiveness of the plant methanol extract is contributed to the ability of tannins to reduce *Staphylococcus* surface colonization (David et al., 2013). It is in line that the high activity of the plant can justify its uses in folkloric medicine.

### **Conclusion:**

*Capparis spinosa* leaves and bark extracts with different solvents revealed variable *in vitro* growth inhibition activity against tested strains and clinical isolate of *Staphylococcus aureus* with highly significance differences;  $P < 0.01$ . The higher plant activities were from chloroform bark extract against both standard and clinical isolates followed by methanol leaves extract against the standard organism with equal minimum inhibitory concentration of 100mg/ml, and these activities may contributed to the high presence of the phytochemical constituents such as flavonoids and also tannins which has the ability to reduce surface colonization of the Gram positive *Staphylococcus aureus* and then reduce the organism infection incidence. Also this study claimed that chloroform bark extract has anti beta lactamases activity. These findings could be of pharmaceutical interest when we consider the bacterial resistant and the broad-spectrum side effects associated with some well-known commercial anti-bacterial agents.

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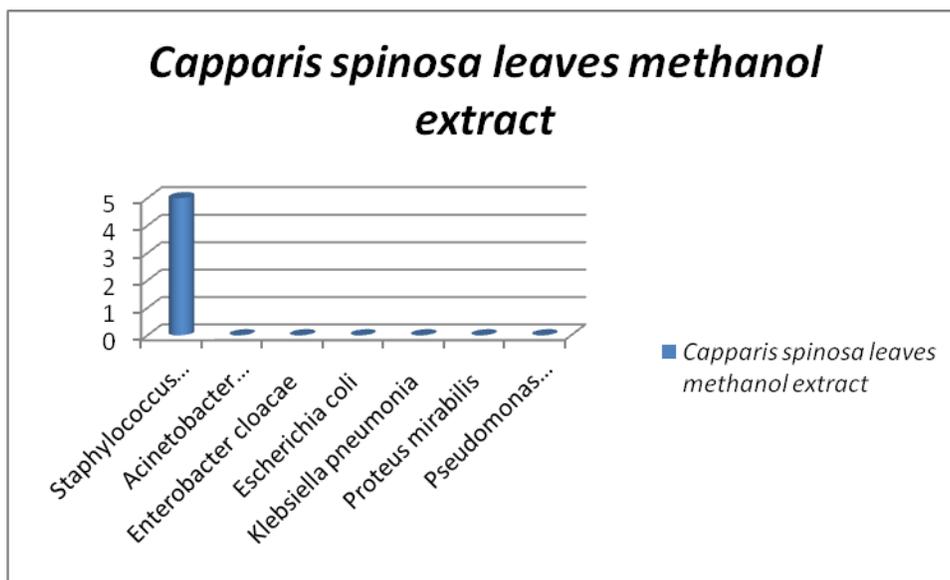
**Table (1):** Means of diameters of Inhibition Zones (MDIZ) in (mm) and Standard Deviation of *Capparis spinosa* parts extracts against Standard Organism

Extracts	MDIZ of <i>Capparis spinosa</i> bark chloroform extract				MDIZ of <i>Capparis spinosa</i> leaves methanol extract			
	CHCl <sub>3</sub> ± SD	MeOH ± SD	EtOH ± SD	H <sub>2</sub> O	CHCl <sub>3</sub> ± SD	MeOH ± SD	EtOH ± SD	H <sub>2</sub> O
<i>Bacillus subtilis</i>	-	14 <sup>b</sup> ±0.71	16 <sup>ab</sup> ±1.4	-	-	11 <sup>d</sup> ±0.71	-	-
<i>Staphylococcus aureus</i>	30 <sup>a</sup> ±.01	12 <sup>c</sup> ±0.71	12 <sup>c</sup> ±0.01	-	-	20 <sup>a</sup> ±0.01	14 <sup>a</sup> ±.00	-
<i>Escherishia coli</i>	-	16 <sup>a</sup> ±0.71	-	-	10 <sup>b</sup> ±.00	13 <sup>c</sup> ±0.71	11 <sup>b</sup> ±1.4	-
<i>Pseudomonas aeruginosa</i>	12 <sup>b</sup> ±.71	14 <sup>b</sup> ±0.71	15 <sup>b</sup> ±0.71	-	13 <sup>a</sup> ±1.4	13 <sup>c</sup> ±0.71	-	-
<i>Candida albicans</i>	10 <sup>c</sup> ±.01	16 <sup>a</sup> ±0.71	18 <sup>a</sup> ±1.4	-	-	16 <sup>b</sup> ±0.71	15 <sup>a</sup> ±0.71	-
<b>Sig.</b>	<b>**</b>							

SD = Standard deviation \*\* = Highly significant Concentration of extract 100mg/ml  
Means with same colored superscript letter are non-significantly different

CHCl<sub>3</sub> = Chloroform MeOH = Methanol EtOH = Ethanol 96% H<sub>2</sub>O = Water

MDIZ (9-12) = partial active MDIZ (13-18) = Active MDIZ (>18 mm) = Very active



**Figure (1):** Means diameters (mm) of inhibition zones of 100mg/ml of methanol extract of *Capparis spinosa* leaves against clinical methicillin resistant *Staphylococcus aureus* isolates

**Table (2):** Means of Inhibition Zones diameter (MDIZ) in (mm) and Standard Deviation of (100mg/ml) methanol leaves and chloroform bark extracts of *Capparis spinosa* against clinical isolates.

Serial No.	Clinical isolates	Number	MDIZ/ MLE (mm) ± SD	MDIZ/CBE (mm) ± SD
1	<i>Staphylococcus aureus</i>	31	5 ± 6.9	13 ± 11
2	<i>Acinetobacter baumannii</i>	10	-	ND
3	<i>Enterobacter cloacae</i>	5	-	ND
4	<i>Escherichia coli</i>	10	-	ND
5	<i>Klebsiella pneumoniae</i>	25	-	ND
6	<i>Proteus mirabilis</i>	5	-	ND
7	<i>Pseudomonas aeruginosa</i>	14	-	ND

SD = Standard deviation (-) = Bacteria resist the extract MLE = Methanol leaves extract  
 ND = Not done MDIZ = Means of diameters of inhibition zones CBE = Chloroform bark extract  
 MDIZ (9-12) = partial active MDIZ (13-18) = Active MDIZ (>18 mm) = Very active

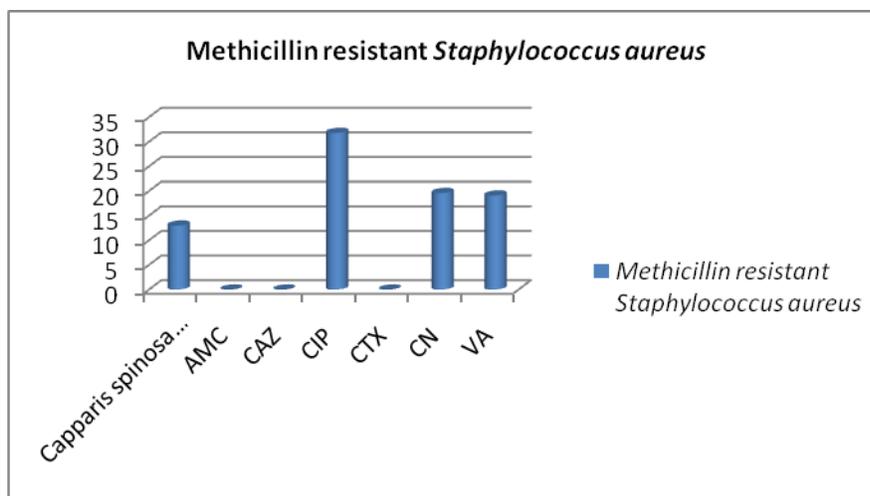
**Table (3):** Minimum inhibitory concentrations of Chloroform bark and Methanol leaves extracts against standard organisms

Conc. mg/ml	Methanol leaves extracts					Chloroform bark extract
	<i>B.subtilis</i>	<i>S.aureus</i>	<i>E.coli</i>	<i>Ps.aeruginosa</i>	<i>C.albicans</i>	<i>S.aureus</i>
100	-	-	-	-	-	-
50	-	+	-	+	-	+
25	+	+	+	+	+	+
12.5	+	+	+	+	+	+
6.25	+	+	+	+	+	+
3.125	+	+	+	+	+	+
1.56	+	+	+	+	+	+

Conc. = Concentration (-) = No growth (+) = Growth

*B.subtilis* = *Bacillus subtilis*      *S.aureus* = *Staphylococcus aureus*

*Escherichia coli* = *E.coli*      *Ps.aeruginosa* = *Pseudomonas aeruginosa*      *C.albicans* = *Candida albicans*



AMC= Amoxicillin+Clavulanic acid (30µg) CAZ= Ceftazidim (30µg) CIP= Ciprofloxacin (30µg)

CN= Gentamicin (10µg) CTX= Ceftriaxone (30µg) VA= Vancomycin (30µg)

**Figure (2):** Susceptibility of tested methicillin resistant *Staphylococcus aureus* to *Capparis spinosa* chloroform bark extract and reference AMC, CAZ, CIP, CN, CTX and VA reference discs

**Table (4):** Comparison of means of inhibition zones (mm) of plant parts extract and antibiotics references against clinical methicillin resistant *Staphylococcus aureus*.

Extracts & standard antibiotics	Number	Means (mm)	± Standard Deviation	Minimum Zones (mm)	Maximum Zones (mm)
<i>Capparis spinosa</i> methanol leaves extract	31	4.6 <sup>d</sup>	± 6.9	0	16
<i>Capparis spinosa</i> chloroform bark extract	31	13 <sup>c</sup>	± 11	0	28.6
AMC 30µg	10	0 <sup>e</sup>	0	0	0
CAZ 30µg	10	0 <sup>e</sup>	0	0	0
CIP 30µg	10	31.8 <sup>a</sup>	± 2.8	28	36
CTX 30µg	10	0 <sup>e</sup>	0		
CN 10µg	10	19.6 <sup>b</sup>	±1.7	17	23
VA 30µg	10	19.1 <sup>b</sup>	±1.5	16	21
<b>Sig.</b>	**				

\*\* = highly significant difference with (P ≤ 0.01)

Means with superscript different letter are significantly differ.

AMC = Amoxicillin 20µg + Clavulanic acid 10µg CAZ = Ceftazidime 30µg CTX = Ceftriaxone 30µg CIP = Ciprofloxacin 30µg CN = Gentamicin 10µg VA = Vancomycin 30µg

**Table (5):** Percentage yields of different extracts of *Capparis spinosa* bark and leaves from different solvents

Yield percentage Solvent extract	Bark yield %	Leaves yield %
Chloroform extract	1.3	3.5
Methanol extract	4	15
Ethanol extract	0.3	5
Water extract	0.18	3.7

**Table (6):** Phytochemical screening of chloroform bark extract and methanol leaves extracts of *Capparis spinosa*

Bioactive secondary metabolites	Chloroform Bark Extract	Occurrence	Methanol Leaves Extract	Presence
Alkaloid	-	Absent	+	Traces
Anthraquin	-	Absent	-	Absent
Coumarins	+	Traces	-	Absent
Flavonoids	-	Absent	+++	High
Saponin	-	Absent	-	Absent
Steroids	++++	Very high	++	Moderate
Tannins	-	Absent	+++	High
Triterpenes	+++	High	+	Low

(-) = Absent (+) = Traces (++) = Moderate presence (+++) = High presence

(++++) = Very high presence

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