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GC/MS ANALYSIS PRODUCTS AND THEIR RELATION WITH THE ANTIMICROBIAL ACTIVITY OF LEAVES AND BARK OF CAPPARIS SPINOSA SUBSP ORIENTALIS (DUH.) JAFRI

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ABSTRACT

This study aimed to analyze the phytochemical constituents and investigate the antimicrobial activity of *Capparis spinose* crude bark and leaves extracts against different microorganisms. **Methods:** Gas Chromatography/ Mass Spectra (GC/MS), Disc diffusion method and dilution agar plate assays were used for analysis and to evaluate the antimicrobial activities and minimum inhibitory concentrations. **Results**: Extracts of *Capparis spinosa* leaves and bark from different solvents showed variable *in – vitro* activity against tested standard strains and clinical isolates with highly significant differences; $P< 0.01$. The highest activities were shown from chloroform bark extract and methanol leaves extract with equal minimum inhibitory concentration of 100mg/ml against standard *Staphylococcus aureus* ATCC 25923 and clinical methicillin resistant *Staphylococcus aureus*. GC/MS analysis revealed 33 and 25 compounds from extracts of the plant leaves and bark, respectively and the study suggested that the antimicrobial activity shown is referred to these phytochemical constituents such as flavonoids, tannins, steroids and triterpines. **Conclusion:** *Capparis spinose* subsp *orientalis* (Duh. Jafri leaves and bark extracts composed of flavonoids, tannins, steroids and triterpines which have the ability to reduce surface colonization and to control the penicillin binding proteins of Staphylococcus aureus, and this make the plant have a pronouncing anti *Staphylococcus aureus* especially with the limited treatment choices presented for treatment of infections caused by this bacterium.

KEYWORDS: C*apparis spinosa*, GC/MS analysis, phytochemical constituents, *Staphylococcus aureus.*

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. Various bacteriological agents are considered pathogenic to man and cause many infectious diseases include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis.* 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 *Staphylococcu 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 it has been reported that Vancomycin and Mupirocin increasingly become less effective in settings with extensive use of these agents (Simor *et al*., 2007, Eliyad *et al*., 2012). 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, the matter which has created immense clinical problem in the treatment of infectious diseases. In addition to this problem, the

synthetic antibiotics used currently are usually expensive and sometimes associated with adverse effects on host including hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immunosuppressant and allergic reactions. Therefore, alternative antimicrobial agents of herbal origin become of interest (Davis, 1994, Firas and Mohammed, 2007, Idress *et al*., 2015, Abdoulraouf *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). *Capparis spinose* 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 and used in treatment of gastrointestinal infections, diarrhea and also in rheumatism. (Ramin and Nastaran, 2016, Manikandaselvi *et al*., 2016).

The extract of *Capparis spinosa* flowers had been tested against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa and Escherichia coli* isolates had been isolated from skin infection. The result showed that the *Capparis spinosa* was 100% effective against Gram positive isolates and 10% activity against Gram negative isolates (Orooba, 2012). 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 the leaves for wound infection treatment. This study aimed to evaluate the antimicrobial activity of extracts of leaves and bark of *Capparis spinosa* against different organisms.

1. MATERIALS AND METHODS

2.1 Plant Material

Capparis spinosa was collected in August 2016 from Shahat region, around Al-Bayda city, 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.

2.1 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 fugus; *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*, *Acinetobacter baumanii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiela pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa*.

2.3 Tested Antibiotics Discs

Amoxicillin 20µg + Clavulanic acid 10µg (AMC), Ceftazidime30µg (CAZ), Ceftriaxone 30 µg (CTX), Ciprofloxacin 30 µg (CIP), Gentamicin 10 µg (CN) and Vancomycin 30 µg (VA) are antibiotics discs used as references in this study. They were bought from Bioanalyse[@] YSE Tibbi Malzemeler San. Expire dates were 10 - 18 months valid after the date of the assay.

2.4 Preparation of plant extract

Each one hundred gram of each of leaves and bark powder was thoroughly successively 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 for 72 hours 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 4Cº. 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 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 labeled 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 µl of a solution of 100mg/ml *Capparis spinosa* extracts were placed on Mueller-Hinton agar petri dish had been surface spread with 100 µl 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 references 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 3.125 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, 3.125 and 1.56mg/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 (0.01ml), 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. 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

Standard basic methods described by Martinez *et al*. (2003), Sofowora,1993 and Wall *et al.* (1952) with few modifications and Gas Chromatography Mass Spectra (GC-MS) techniques were used in this study to evaluate the Phytochemical constituents of each of chloroform and methanol extracts of bark and leaves of *Capparis spinosa*.

2.9 Statistical Analysis

Data were expressed as mean ± 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 ≥ 0.05, ≤ 0.05 and ≤ 0.01 were considered as not significant, significant and highly significant values respectively.

2. RESULTS AND DISCUSSION

Little research was carried out for *Capparis spinose* species in general and on its leaves and bark methanol extracts in specific. The bark and leaves chloroform, methanol, ethanol and aqueous extracts of *Capparis spinose* were screened for their antimicrobial activity against Gram positive, Gram negative bacteria and fungi. No effect shown with the aqueous extracts. The statistical analysis showed that there was a highly significant differences between the effects of the extracts from different plant parts 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 standard *Staphylococcus aureus*, it was clear that the chloroform bark extract was more effective against the bacteria, it gave 30±0.01mm inhibition zone compare with 20mm± 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 16mm± 0.71 with methanol extract of both bark and leaves, but the highest was 18mm from ethanol bark extract. Abd Razik, (2011) claimed that methanol extract of *capparis spinosa* flowers was more active than hexane extract. The traditional medicinal uses of *Capparis spinose* 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 and the results proved that all of the clinical isolates devoid of any susceptibility except for methicillin resistant *Staphylococcus aureus* isolates which showed less susceptibility with mean inhibition zone of 5mm ±6.9 compared with the standard strain. After that the antibacterial activity of 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±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 spinose* and their results disagreed with this study for the clinical *Staphylococcus aureus* which not inhibited 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.

Farzad *et al.*, 2016 were optimized the antibacterial of ethanol extracted 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 dysenteriae* and *Salmonella typhi*) than Gram-positive bacteria (*Bacillus panis* and *Staphylococcus aureus*). This study concerned with testing of crude ethanol leaves extract rather than dealing specifically with polysaccharide 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*. Rahimifard *et al*., 2015 carried a study to screen the antibacterial activity of methanol extract of the aerial part of different plant species; *Capparis cartilaginea* and *Capparis mucronifolia* and they found that the highest antibacterial activity of *Cappris mucronifolia* was against Gram positive *Staphylococcus epidermidis* and they referred this to the flavonoid compound of the plant. Even though Rahimifard *et 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 plant possess high antibacterial activities against Gram positives *Staphylococcus aureus*. 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 high effectiveness against the standard *Staphylococcus aureus* with equal MIC of 100mg/ml. Methanol leaves extract showed MIC of 100mg/ml against *Pseudomonas aeruginosa* (Table 3).

When the effectiveness of chloroform extract of *Capparis spinosa* bark 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 study 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. This result suggested that chloroform bark extract has anti extended-spectrum *β*-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).

Both basic and GC-MS phytochemical analysis in this study showed that the plant methanol and chloroform extracts comprises of total of 33 and 25 bioactive compounds, respectively (Table 5 and Table 6). Chloroform bark extract in this study 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. The alkaloid bioactive compound, 1-Methyl-pyrrolidinr-2-carboxylic acid was constitutes 41.8% of the total area of methanol leaves extract in this study and this compound is known as anti-*Staphylococcus aureus* agent (Ajani *et al*., 2012). Also Palmitic acid in this study found in the chloroform extract constitute 1.51% of total constituents area and not presented in the methanol extract and the fatty acid Hexadecanoic acid, ethyl ester was found higher within the constituents of chloroform bark extract with area percentages 32.45%. Both Palmitic acid and Hexadecanoic acid, ethyl ester are bioactive compounds have been reported to mainly have selective anti-*Staphylococcus aureus* activity (Agoramoorthy *et al*., 2007, Neumann *et al*, 2015). 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 which 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 too to the ability of tannins to reduce Staphylococcus surface colonization (David *at al.,* 2013). Furthermore, this study claimed that the plant extract possess an effect on the penicillin binding proteins (target site) since the tested methicillin resistant *Staphylococcus aureus* in this study resisted the tested penicillin antibiotic; Augmentin and the 3rd generation extended bet-lacatm antibiotics; Ceftazidime and Ceftriaxon, but it showed susceptibility towards the extract. It is well known that the predominant mechanism of resistance to β-lactams in Gram-negative bacteria is the production of beta-lactamases, whereas resistance to these compounds

in Gram positive organisms is mostly achieved by modifications of their target site, the penicillin-binding proteins (Epand *et al*., 2016). This result is in line with that the high activity of the plant can justify its uses in folkloric medicine.

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 *Capparis spinosa* bark chloroform extract				MDIZ of *Capparis spinosa* leaves methanol extract			
Standard Organisms ↓	$CHCl_3$ ± SD	MeOH ± SD	EtOH ± SD	H_2O	$CHCl_3$ ± SD	MeOH ± SD	EtOH ± SD	H_2O
Bacillus subtilis	-	14^{b} ±0.71	16^{ab} ±1.4	-	-	11^{d} ±0.71	-	-
Staphylococcus aureus	30^{a} ±.01	12^{c} ±0.71	12^{c} ±0.01	-	-	20^{a} ±0.01	14^{a} ±.00	-
Escherishia coli	-	16^{a} ±0.71	-	-	10^{b} ±.00	13^{c} ±0.71	11^{b} ±1.4	-
Pseudomonas aeruginosa	12^{b} ±.71	14^{b} ±o.71	15^{b} ±0.71	-	13^{a} ±1.4	13^{c} ±0.71	-	-
Candida albicans	10^{c} ±.01	16^{a} ±0.71	18^{a} ±1.4	-	-	16^{b} ±0.71	15^{a} ±0.71	-
Sig.	**							

SD = Standard deviation ** = Highly significant Concentration of extract 100mg/ml
Means with same colored superscript letter are non-significantly different $CHCl_3$ = Chloroform
MeOH = Methanol EtOH = Ethanol 96% H_2O = Water MDIZ (9-12) = partial active
MDIZ (13-18) = Active MDIZ (>18 mm) = Very active

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	*Staphylococcus aureus*	31	5 ±6.9	13±11
2	*Acinetobacter baumanii*	10	-	ND
3	*Enterobacter cloacae*	5	-	ND
4	*Escherichia coli*	10	-	ND
5	*Klebsiella pneumoniae*	25	-	ND
6	*Proteus mirabilis*	5	-	ND
7	*Pseudomonas aeruginosa*	14	-	ND

SD = Standard deviation (-) = Bacteria resist the extract MLE= Methanol leaves extract
ND = Not CBE = Chloroform bark extract

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

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

Conc. = Concentration (-) = No growth (+) = Growth
B.subtilis = Bacillus subtilis S.aureus = Staphylococcus aureus E.coli = Escherichia coli Ps.aeruginosa = Pseudomonas aeruginosa C.albicans = Candida albicans

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	Means (mm)	± Standard Deviation	Minimum Zones (mm)	Maximum Zones (mm)
***Capparis spinosa* methanol leaves extract**	4.6^{d}	± 6.9	0	16
***Capparis spinosa* chloroform bark extract**	13^{c}	± 11	0	28.6
AMC 30µg	0^{e}	0	0	0
CAZ30µg	0^{e}	0	0	0
CIP 30µg	31.8^{a}	± 2.8	28	36
CTX 30µg	0^{e}	0		
CN 10µg	19.6^{b}	±1.7	17	23
VA 30µg	19.1^{b}	±1.5	16	21
Significance	**			

** = highly significant difference with (P≤ 0.01)
Means with superscript different letter are significantly differ.
AMC = Amoxicillin 20µg + Clavulanic acid 10µg CAZ = Ceftazidime30µg CTX = Ceftriaxone30µg CIP = Ciprofloxacin30µg CN = Gentamicin10µg VA = Vancomycin30µg

Table (5): Main bioactive chemical composition distinguished in methanol leaves extract of *Capparis spinosa.*

Number of compound	Retention time	Area %	Molecular weight	Formula	*Capparis spinosa* methanol leaves extract compounds names
1	3.64	1.74	98	C5H6O2	2-Cyclopenten-1-0ne,2-hydroxy
2	4.06	3.02	110	C6H6O2	2-furancarboxaldhyde, 5-methyl
3	4.47	0.56	128	C6H8O3	Alpha-Acetobutyrolactone
4	5.11	2.29	244	C14H28O3	1-butoxypropan-2-yl heptanoate
5	5.22	11.29	115	C6H13NO	N-Metheyl-L-prolinol
6	5.76	0.79	170	C10H18O2	1-Octen-3-yl-acetate
7	6.72	1.89	144	C6H8O4	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP)
8	7.07	0.50	122	C7H6O2	Benzoic acid
9	7.67	2.55	129	C6H11NO2	2-Methyl-pyrolidine-2-carboxylic acid
10	7.84	3.94	120	C8H8O	Benzofuran, 2,3-dihydrobenzofuran
11	8.95	41.80	129	C6H11NO2	1-Methyl-pyrrolidinr-2-carboxylic acid
12	12.05	9.80	162	C6H10O5	Beta-D-Glucopyranose, 1,6 - anhydro
13	12.53	0.48	180	C10H12O3	3,5,dimethoxyacetophenone
14	13.34	1.07	162	C6H10O5	1,6-Anhydro-Alpha-d-galactofuranose
15	14.19	0.19	476	C26H36O8	1H-Cyclopropa(3,4)benz(1,2-e)azulene-5,7
16	14.25	0.23	180	C11H16O2	3-acetyl-2,4,4-triethylcyclohex-2-en-1-o
17	15.15	0.34	238	C14H22O3	Acetic acid,2-(2,2,6-trimethyl-7-oxa-bieye
18	15.32	1.14	222	C13H18O3	2-Cyclohexane-1-one, 4-hydroxy-3,5,5-trim
19	15.55	0.64	296	C20H40O	3,7,11,15-tetramethyl-2-hexadecen-l-ol
20	15.67	1.13	198	C9H10O5	3-hydroxy-4,5-dimethoxybenzoic acid
21	15.82	1.56	254	C16H30O2	10-methyl-E-11-tridece-1-ol acetate
22	16.02	0.43	278	C20H38	9-Eicosyne
23	16.49	0.86	270	C17H34O2	Hexadecadienoic acid, methyl ester
24	16.91	6.80	256	C16H32O2	n- Hexadecadienoic acid
25	18.24	0.17	294	C19H34O2	9,12- octadecadienoic acid, methyl ester
26	18.30	0.12	296	C19H36O2	9- octadecadienoic acid (Z),methyl ester
27	18.32	0.54	278	C18H30O2	9,12,15- octadecatrienoic acid, methyl ester
28	18.43	0.28	296	C20H40O	Phytol
29	18.51	0.16	298	C19H38O2	Methyl stearate
30	18.66	0.41	308	C20H36O2	Linoleic acid ethyl ester
31	18.70	0.45	282	C18H34O2	Oleic acid
32	18.73	2.06	278	C18H30O2	Gamolenic acid
33	18.89	0.77	284	C18H36O2	Octadecanoic acid

Table (6): Main bioactive chemical composition distinguished in Chloroform bark extract of C*apparis spinosa.*

Number of compound	Retention time	Area %	Molecular weight	Formula	*Capparis spinosa* chloroform bark extract compounds names
1	7.22	6.85	115	C6H13NO	2-Pyrolidinemethanol, 1-methyl-
2	12.22	0.71	180	C11H16O2	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro
3	14.73	064	242	C15H30O2	Pentadecanoic acid
4	15.13	2.02	168	C10H16O2	7-Oxabicyclo(4,1,0)heptane,1-methyl1-4(2
5	15.30	1.35	182	C11H18O2	2,6,8-Rimethylbicyclo(4,2,0)oct-2-ene-1,8
6	15.54	1.44	296	C20H40O	3,7,11,15-tetramethyl-2-hexadecen-l-ol
7	15.63	0.87	268	C18H36O	2-Pentadecanone,6,10,14-trimethyl-
8	15.79	1.24	238	C14H22O3	1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyc
9	16.02	0.56	278	C20H38	9-Eicosyne
10	16.48	1.35	270	C17H34O2	Hexadecadienoic acid, methyl ester
11	16.93	32.45	256	C16H32O2	n- Hexadecadienoic acid
12	17.18	1.51	284	C18H36O2	Hexadecadienoic acid, ethyl ester
13	18.23	0.74	280	C18H32O2	9,12- octadecadienoic acid, methyl ester
14	18.28	0.38	296	C19H36O2	9- octadecadienoic acid (Z),methyl ester
15	18.31	0.64	278	C18H30O2	9,12,15- octadecatrienoic acid, methyl ester
16	18.42	8.87	296	C20H40O	Phytol
17	18.70	5.83	308	C20H36O2	Linoleic acid ethyl ester
18	18.74	2.46	282	C18H34O2	Oleic acid
19	18.75	11.6	278	C18H30O2	Gamolenic acid
20	18.89	2.53	284	C18H36O2	Octadecanoic acid
21	21.79	0.91	380	C19H40O3S2	d-Ribose,2-deoxy-bis(thioheptyl)-dithioac
22	22.01	1.20	330	C19H38O4	Hexadecanoic acid, 2-hydroxy-1-(hydroxy
23	23.09	5.03	380	C27H56	2-methylhexacosane
24	24.23	6.68	414	C29H50O	.gamma.-Sitosterol
25	24.535	1.94	206	C15H26	(-)-Neoclovene-(1), dihydr-

3. CONCLUSION

Capparis spinosa leaves and bark extracts with different solvents revealed variable *in – vitro* growth inhibition activity against tested *Staphylococcus aureus* strain and clinical methicillin resistant *Staphylococcus aureus* with highly significance differences; P< 0.01. The higher plant activities were from chloroform extract of the plant park against both standard and clinical isolates followed by methanol extract of the leaves against the standard organism with equal minimum inhibitory concentration of 100mg/ml, and this activity suggested to be contributed to the high presence of the bioactive constituents such as flavonoids in addition to the presence of tannins which has the ability to reduce surface colonization of the Gram positive *Staphylococcus aureus* and then reduce the organism infection incidence. Furthermore, the study suggests that the extract has an effect on the penicillin binding proteins of the bacteria. 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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