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Extracted chemical compounds from Capparis spinosa leaves and their antibacterial activity on pathogenic bacteria.

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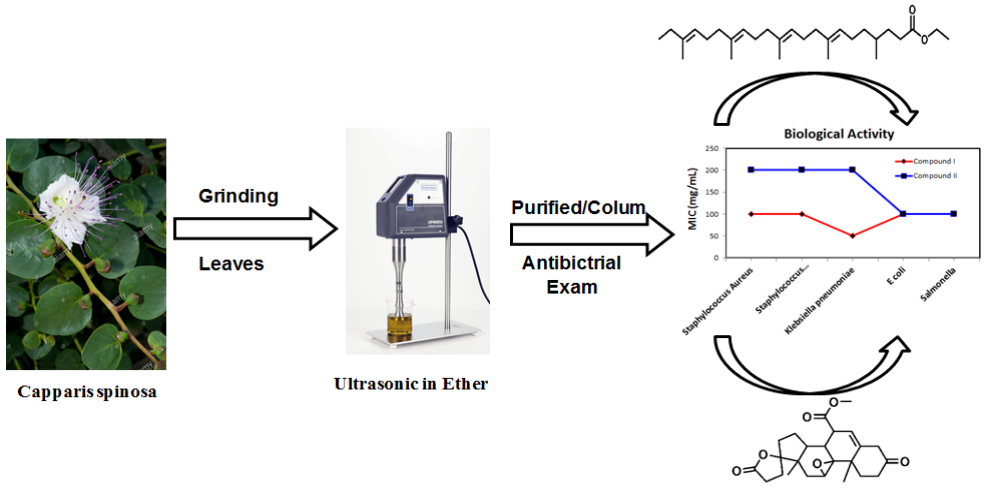
Abstract:

The antibacterial activity of the extracted compounds of Capparis spinosa has been evaluated on some gram-positive and gram-negative bacteria. Two compounds have been isolated and purified by column chromatography (silica gel). The extracted compounds were identified by FT-IR, ^{1}H, ^{13}C NMR and Mass techniques. In this study, the results showed that the two compounds have a significant difference at $p \leq 0.05$ between Capparis spinosa extract concentration and control for *Staphylococcus epidermidis*, *Staphylococcus aureus*, *E. coli*, *Salmonella Typhi* and *Klebsiella pneumoniae*. The minimum inhibitory concentration (MIC) of the pathogenic bacteria was 100 mg/ml concentration for the compound I and 200 mg/ml for the compound II on *S. epidermidis* and *S. aureus* respectively, while the MIC was 100 mg/ml concentration for the compounds I and II on *E. Coli* and *Salmonella* . The MIC was 50 mg/ml concentration for compound I, and 200 mg/ml concentration for compound II on Klebsiella pneumoniae. The compound I are more effective compared with the compound II.

Keywords: Capparis spinosa, leave, antibictrial, pathogenic bacteria.



The scheme represent the evaluation of the antibacterial activity of the two isolated compounds from Capparis spinosa leaves aginast gram positive and negative pathogenic bacteria

INTRODUCTION:

Capparis spinosa is one of the medicinal plants (1,2). These plants were considered a source of biomedical compounds which have continued to play an important role in the care of human health during the past times. According to the world health organization, the plant extracts or their bioactive components are used as the treatment in traditional therapies of 80% of the world's inhabitance. More than 50% of all modern drugs are of the natural sources (3). During the past two decades, much attention has been paid to the pharmacological effects of C. spinosa because it has a large number of bioactive components, special polyphenolic, steroids, flavonoids compounds, etc..(4–6). Moreover, the treatment of infectious diseases with antimicrobials agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics (7).

On other hands, the Phytochemical analysis showed there are different parts of C. spinosa have rich sources of polyphenols. For this reason, the study has been focused on the health-promoting effects of this plant and its bioactive components (6). Later, there has been much scientific evidence showing that C. spinosa have various pharmacological effects including antioxidant, hepatoprotective and anticancer effects (8–10).

C. spinosa (Caper) belongs to the family called Capparaceae, The Mediterranean is the native region to this plant. C. spinosa grow along the roadside, stony area, on the slopes, and rocky and generally well adapted to dry areas basin. Capparis wild species are found in many countries that surrounding the Mediterranean basin extending to North Africa (Great Sahara) and the dry regions of the Western and Central Asia (11).

The research focus on extraction and purified some of chemical compounds extracted from Capparis spinosa leaves. In additional, the pure extracted compounds were

subjected to prove that C. spinosa has antibacterial activity against gram positive and gram negative bacteria.

MATERIALS AND METHODS:

Plant material.

Capparis spinosa was collected from Samawah city during the month of July 2017. The plant identified by a botanist at Al Muthanna University. The leaves of plant washed and dried at room temperature in the dark for 14 days and then finely ground by using an electric grinder.

Microbiological material.

The bacterial strains were used responsible for various infections such as skin and urinary tract which are a major problem for public health are gram-positive and negative bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, E coli, Salmonella Typhi* and *Klebsiella pneumoniae*). These strains provided by the bacteriology laboratory of Al Hussein Teaching Hospital. These bacteria were isolated from patients and were maintained by subculture on agar media.

Ultrasonic extraction:

Capparis spinosa leaves (10 g) and 100 ml of petroleum ether are introduced into a flask. The mixture was exposed to ultrasound for 1 hour under 60 kHz at room temperature and sheltered from light. The later process has been repeated under same condition for ten times. Afterward, the mixture was filtrated and the final volume (accumulate filtrate) is concentrated in rotary evaporator under reduced pressure (12).

Purification of the compounds using Silica Gel Chromatography:

The petroleum ether extracted (2.00 g) was fractionated on a silica gel column (60-120 mesh) with ratio of eluent (hexane: ethyl acetate) various such as (1:0) and (2:1) To give two fractions (Fr. 1- Fr. 2). Fraction (2) was subjected to further chromatography on a silica gel column (5 cm) with same eluate using a stepwise gradient of hexane and ethyl acetate (2:1) to afford a further one pure compound (Fr.2.1) and mixture of others (13,14). The obtained fraction I and II gives (170 mg and 143 mg respectively).

Antimicrobial susceptibility testing:

The extracted compounds were dissolved in 10% DMSO, then they were two fold serially diluted in a liquid growth medium (MHB). The final concentrations of each of them are ranged from 400 mg/ml to 0.78 mg/ml. After shaking, 100 µl of each extract concentration was added to the different wells of 96 well micro titer plates, except the 12th well (growth control, without the extracted compounds). Then, each well is inoculated with 100 µl of a microbial inoculum (1×106 CFU/ml), except of the 11th well (sterility control, without bacteria), and incubated at 37 ºC. MIC was measured at 620 nm. The plate was measured pre- and post-incubation at 37 °C for 24 hours. According to Banjara, et al (15), calculation of the Bacterial growth inhibition was achieved by the following equation.

$$\text{Percentage growth inhibition} = \frac{\text{OD of control}}{\text{OD of control - OD of test}} \times 100$$

Statistical analysis:

This study designed by Completely randomized design (CRD) that used in the statistically analysis of variance for data of plant extraction yields and the crude and pure compounds activity against bacteria by using one-way ANOVA test, independent t-test and Dunnett's test at a 5% level of significance. Data were processed and analyzed by using statistical program social science (SPSS 22) and the results were expressed as Mean±SD (McDonald, 2014) (16)

RESULTS AND DISCUSSION:

Elucidation the Structure of isolated compounds:

Compound 1: (7,11,15,19) – ethyl 4,8,12,16,20-pentamethyldocosa -7,11,15,19 – tetraenoate is a yellow syrup, R_f value 0.37 (hexane 100%), and was exhibited a molecular formula $C_{29}H_{50}O_2$ as concluded from the FT-IR absorption spectrum of the pure compound I and from the mass spectrum (ESI-MS m/z 430.4) as in Fig. 3. The absorption peaks of IR spectra (cm^{-1}) are: Carbonyl of ester group at 1738, (C=C) 1666, (C-O) 1242. Both ^1H and ^{13}C NMR in Fig. 1 and 2 respectively: ^1H NMR ($CDCl_3$) δ = 5.06 (bs, 3H, CH), 4.51 (t, 2H, OCH_2), 4.47 (m, 1H, CH), 2.7 (t, 2H, COCH2), 1.97 (t, 2H, γ-CH2), 1.69-1.61 (m, 8H, CH3), 1.53-1.23 (m, 10H, CH2), 1.21-1.18 (m, 15H, CH3), 0.79 (dt, 6H, ∞-CH3). ^{13}C NMR ($CDCl_3$) δ = 172.76 (CO), 136.87, 135.77, 135.22 (4x C), 124.67, 124.28 (2x CH), 60.90 (OCH2), 39.74 (∞-2), 37.75 (∞ -1), 32.68, 32.22, 31.41, 30.29 (bulk-CH2), 26.79 (β-CH2), 20.88 (γ-CH2), 16.51, 15.87, 14.11, 11.85 (∞-CH3).

Compound 2: Methyl 2',15'-dimethyl- 5,5'- dioxo- 18'-oxaspiro[oxolane-2,14'-pentacyclo-octadecan]-7'-ene-9'-carboxylate is a light green syrup, R_f value 0.303 (hexane: ethyl acetate 2:1), and was exhibited a molecular formula $C_{24}H_{30}O_6$ as concluded from the FT-IR absorption spectrum (Fig. 4 and Fig. 5) of the purified compound II and from the mass spectrum (ESI-MS m/z 414) as in Fig. 6. The absorption peaks of IR spectra (cm^{-1}) are: Carbonyl of ester group at 1738 and 1715 belong to carbonyl of ketone group, (C=C) 1666. Both ^1H and ^{13}C NMR in Fig. 4 and Fig. 5 respectively: ^1H NMR ($CDCl_3$) δ = 5.55-5.47 (dm, 1H, CH), 5.06 (s, 3H, CH3), 4.07 (dd, 1H, COCH), 2.73 (m, 1H, CH epoxide), 2.02-1.97 (m, 6H, 3x CH2), 1.61-153 (m, 6H, 2x CH, 2x CH2 (4,6,14,8)), 1.2-122 (m, 6H, 3x CH2), 0.81 (dt, 6H, 2x CH3). ^{13}C NMR ($CDCl_3$) δ = 207.61 (CO), 174.56, 168.94 (CO), 141.58 (C), 124.42 (CH), 93.33 (C), 64.59 (C epoxide), 53.38 (CH epoxide), 52.17 (OCH3), 48.38, 45.85 (C, OCH2), 43.33 (C), 37.91, 36.86 (2x CH2), 35.20 (CH), 33.86, 31.49, 29.79 (3x CH2), 28.78 (CH), 25.89, 23.43, 21.49 (3x CH2), 16.00 (CH3).

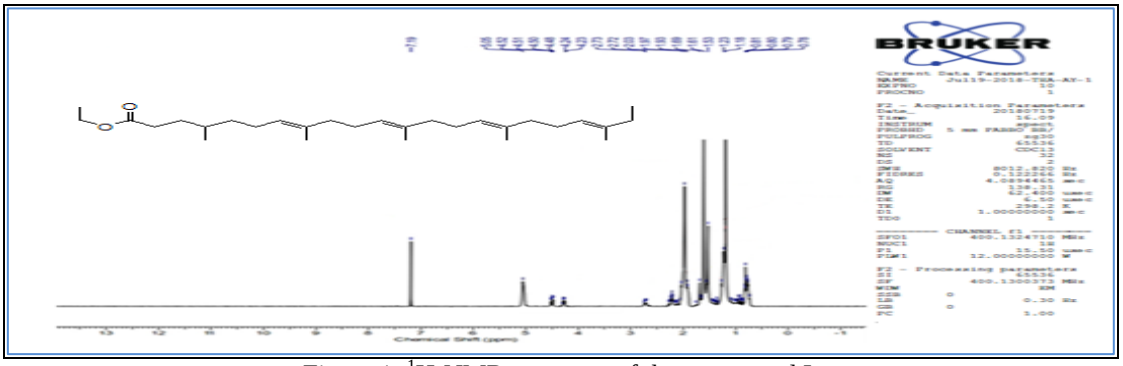


Figure 1: [1]H-NMR spectrum of the compound I.

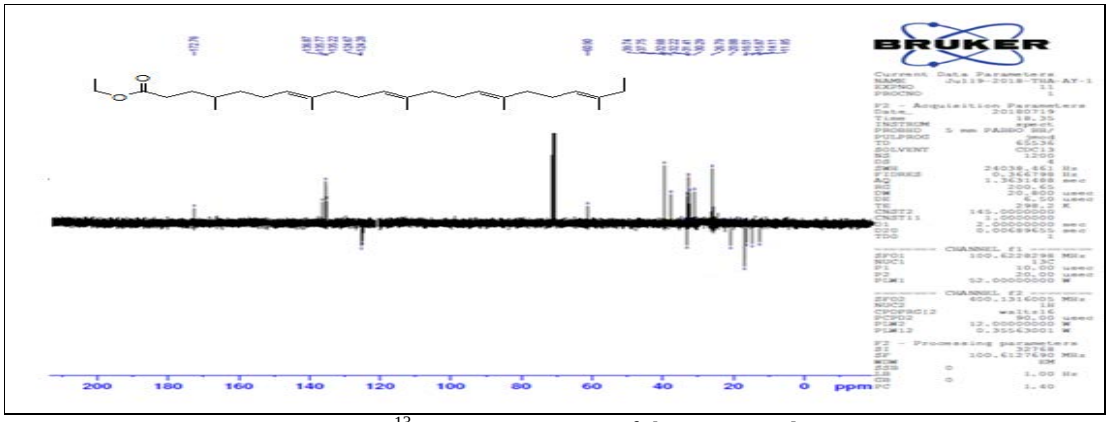


Figure 2: [13] C-NMR spectrum of the compound I.

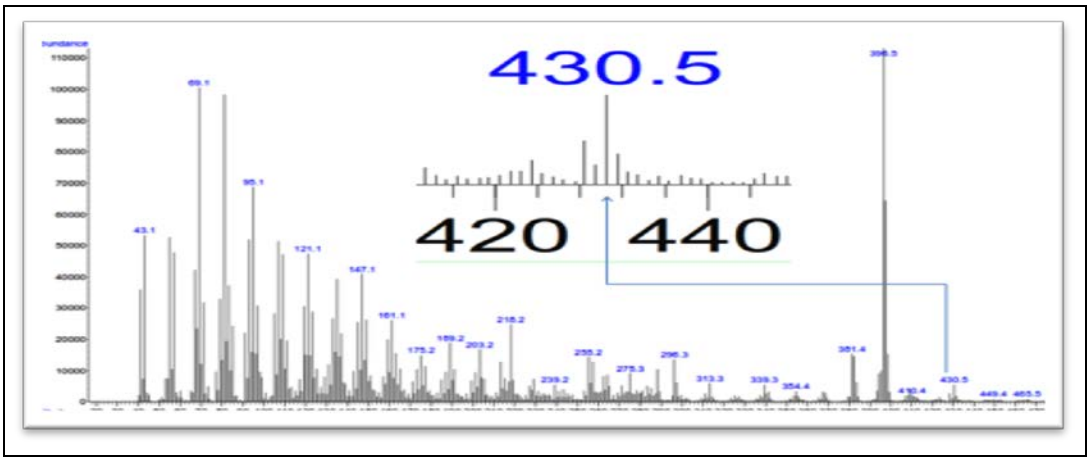


Figure 3: Shows mass spectrum of compound I.

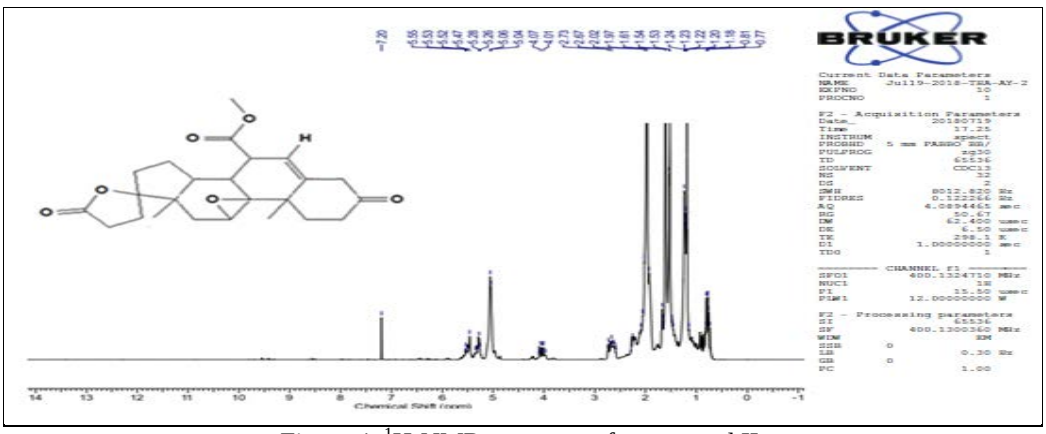


Figure 4: [1]H-NMR spectrum of compound II.

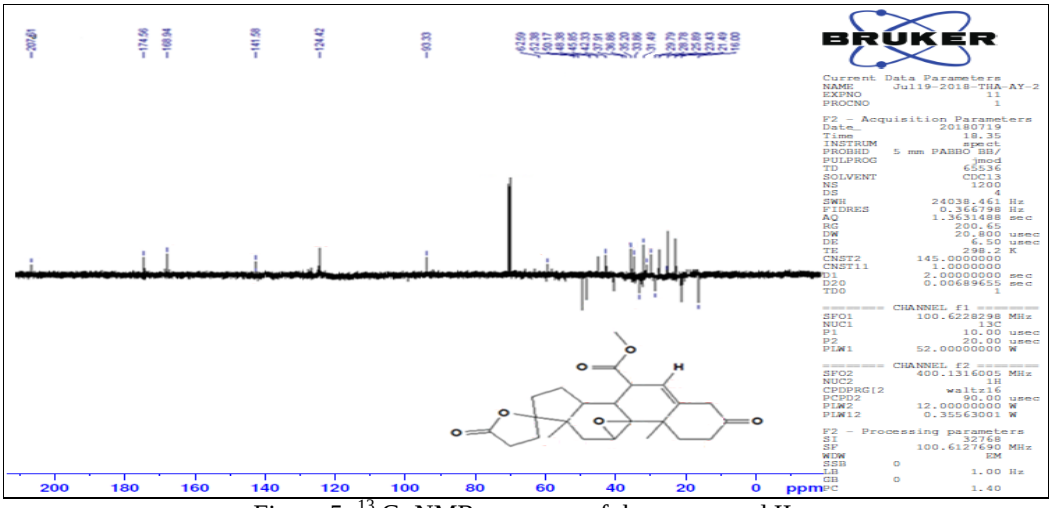


Figure 5: ^{13}C -NMR spectrum of the compound II.

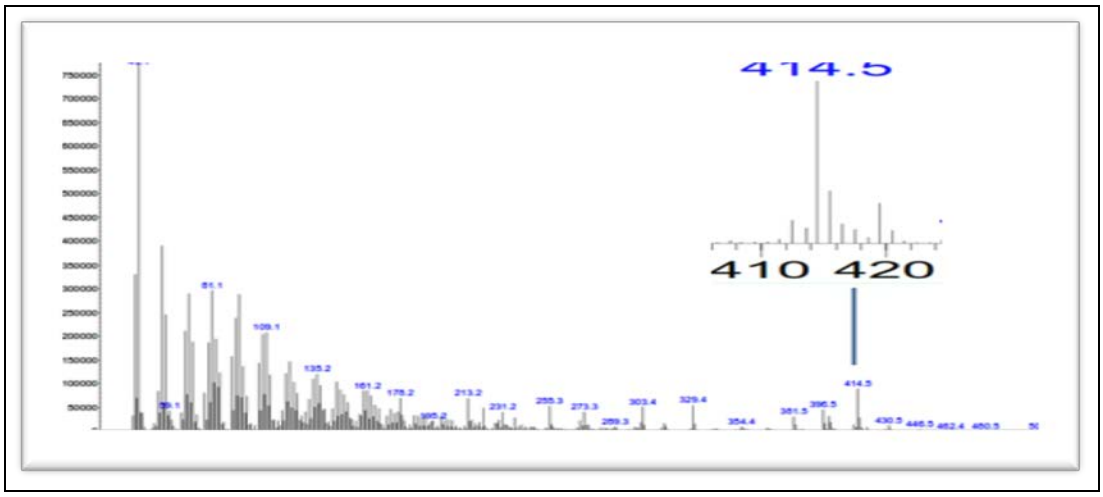


Figure 6: Shows mass spectrum of compound II.

The compounds were isolated identification by FT-IR, ^{1}H and ^{13}C NMR and mass spectrum (17,18).

Activity of extracted compounds on pathogenic bacteria.

The two fractions including compounds I and II were evaluated for their antibacterial activity against five of the pathogenic bacteria by using a spectrophotometric assay as shown. The two compounds show a significant difference at p≤ 0.05 between Capparis spinosa extract concentrations and control for all the bacteria studied. The minimum inhibitory concentrations (MIC) of compound I and II against *S. aureus* and *S. epidermidis* were 100 mg/ml, and 200 mg/ml respectively as shown in Fig. 7. The separated compounds show a significant difference at p≤ 0.05 between Capparis spinosa extract concentrations and control against *E. Coli and Salmonella*. The MIC concentration of compound I and II was 100 mg/ml against both bacteria species.

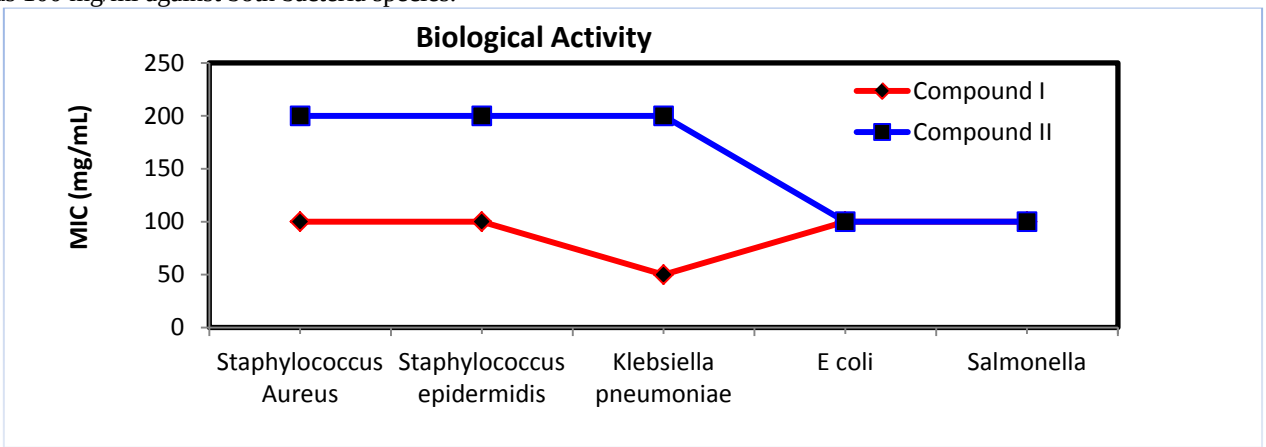


Figure 7: Represent the MIC value for compounds I and II on five pathogenic gram positive and negative bacteria studied.

The two compounds in Fig. 9 show a significant difference at p≤ 0.05 between Capparis spinosa extract concentrations and control against *Klebsiella*. The MIC of compound I was 50 mg/ml and of compound II was 100 mg/ml. This may be due to the unsaturated long chain ester of compound I and also relevant to the structure of cell membrane of the pathogenic bacteria. This is agreed with the previous studies by Agoramoorthy **G.** et al (19,20). Moreover, The second compound (steroids compound) has more MIC concentration in comparison with the first compound. The steroids compounds proposed have antibacterial activity. This result is in agreement with results achieved by Chattopadhyay (21). The first compound has more activity than the second compound, we believe it might be due to the hydrophobicity of the first compound, which is more than of the second compound. In addition to, this maybe relevant to the different structures of the different kinds of bacteria species. Compound I (fatty acid ethyl esters) predicted to be inseted and oligomerized in the cell membranes forming pores. This facilelitated K ions to move from inside cells to outside. This may be lead to the death of cells (22,23). For this reason, fatty acid ethyl esters inhibited the growth of studied bacteria. Desbois & Smith (24) suggested that the first target of the free fatty acid action is the cell membrane, where FFAs disrupted the electron transport chain and oxidative phosphorylation besides the interfering with cellular energy production. FFA action maybe according to impairment of nutrient uptake, generation of peroxidation and auto-oxidation degradation products.

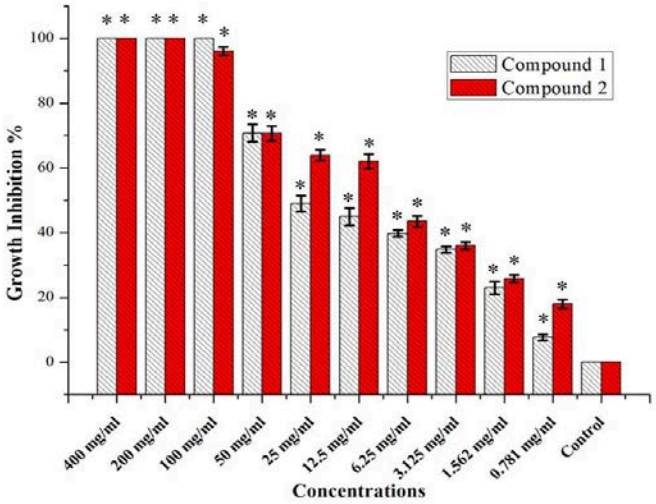


Figure 8: Growth inhibition of *Staphylococcus aureus* caused by different concentration of the leaves extract of *Capparis spinosa* for two pure compounds. * represent a significant difference at p≤0.05 between *Capparis spinosa* extract concentrations and control. Data represent the mean ± SE for three repeated experiment.

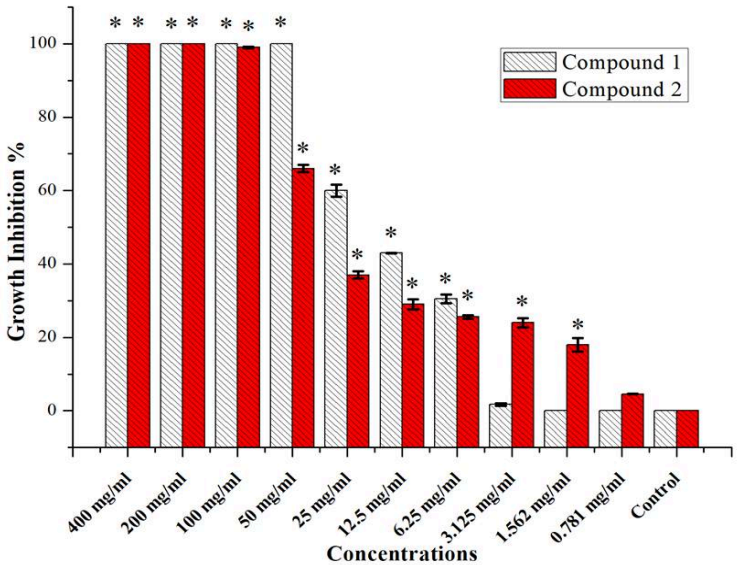


Figure 9: Growth inhibition of *Klebsiella pneumoniae* caused by different concentration of the leaves extract of *Capparis spinosa* for two compounds. * represent a significant difference at p≤0.05 between *Capparis spinosa* extract concentrations and control. Data represent the mean ± SE for three repeated experiment

CONCLUSIONS:

The study was shown that Capparis spinosa leaves which were collected from As Samawah city, were found to be suitable for separation some compounds. the two separated compounds were showed antibacterial activity, so it might be using these compounds as an antibacterial drug with a low cost and available source.

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