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Phytochemical, antibacterial and antioxidant activities of *Capparis spinosa* L. Cultivated in Iraq

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The *Capparis spinosa* L. is a species has a great interest in the field of traditional medicine for its pharmacological properties with many bioactive compounds. Our study is aiming at the recovery of this species through a phytochemical analysis and an evaluation of antibacterial and antioxidant activities of leaves of *Capparis spinosa* L. collected from natural habitats within the region of Al-Jadriya, Baghdad, Iraq. Phytochemical investigation demonstrated the presence of flavonoids, phenols, alkaloids, tannins, and glycosides in the methanolic extract of leaves. The quantitative analysis of total phenolic contents is being performed by Folin-Ciocalteu method and expressed in terms of gallic acid equivalents. *C. spinosa* exhibited progressive phenolic content in methanolic extract which was 21.62, 24.81 and 29.54 mg/g in concentration 8, 10 and 12 mg/ml, respectively. The antioxidant activity is determined by the DPPH test, showed that the radical scavenging capacity (EC_{50}) of methanolic extract was found to be (7.1 mg/ml), while the (EC_{50}) of vitamin C and BHT was (1 and 1.4 mg/ml) respectively. The antibacterial activity evaluated against pathogenic strains such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* revealed the effectiveness of methanolic extract against the most tested isolates at 100 mg/ml while *P. aeruginosa* exhibited resistance against extract. Minimal inhibitory concentrations (MICs) results revealed the activity of methanolic extract against *S. aureus* at 75 mg/ml, while the MIC of *P. aeruginosa* reached to 250 mg/ml. On the basis of the above findings, it can be concluded that *C. spinosa* possesses obvious antioxidant and antimicrobial potential can be used as a natural medicinal agent.

Keywords: *Capparis spinosa*, antibacterial, antioxidant, phytochemical analysis.

INTRODUCTION

Capparis species belonging to family Capparaceae are common plants with medicinal attributes, out of which *C. spinosa* which grows wild in dry regions around the Mediterranean basin and have been reported for their traditional uses because of therapeutic characteristics (Hamed et al., 2007; Musallum et al., 2011). Previous studies investigated the phytochemical components of capers extracts and showed the presence of many bioactive compounds from different chemical families, such as phenolic acids, flavonoids, alkaloids, fatty acids, aldehydes, esters, vitamins and glucosinolates (Romeo et

al., 2007; Ma and Zhang, 2017). Many studies have indicated to the bioactive compounds of different parts of *C. spinosa* such as leaves (Ramezani et al., 2008; Mollica et al., 2017). In the species *C. spinosa*, Polyphenols, phenolics and flavonoides and glycosides were demonstrated to possess strong antioxidant activity with free radical scavenging effectiveness and have received considerable attention to their pharmacological functions as antitumor, antimutagenic and antioxidant activities (Gunjan et al., 2015). The aerial parts of *C. spinosa* has been reported as a potential source of antibacterial compounds and the extracts of *C.*

spinosa parts were reported to be effective to inhibit the growth of different bacterial strains especially those which have acquired resistance to antibiotics (Mahboubi and Mahboubi, 2014; Gull et al., 2015). So, the present study was designed aiming at exploring the phytochemicals of the methanolic extract of *C. spinosa* leaves and determine the antioxidant activity, also the antibacterial potential of this extract against Gram positive and negative bacteria.

MATERIALS AND METHODS

Plant material

Fresh leaves of *C. spinosa* were procured from natural habitats of Al-Jadriya city, Baghdad, Iraq. Authentication and identification of the plant was carried out by the specialist, Department of Biology, College of Science, University of Baghdad.

Extract preparation

Methanolic extract was prepared according to N'Guessan et al., (2007), the leaves of plant was cleaned, dried under shade at room temperature and powdered. 100g was extracted with methanol using Soxhlet apparatus for 6 hours. The extracts were filtered and concentrated by a rotary evaporator under reduced pressure at approximately 40°C.

Primary qualitative analysis

Phenolic compounds test was carrying out by using (1%) ferric chloride (Waterman and Mole, 1994). Flavonoids test was achieved by using (5N) alcoholic potassium hydroxide (Francesca et al., 2016). Tannins were achieved by using (1%)

lead acetate (Molan et al., 1997). Carbohydrates test was done by using Molish's reagent. Glycosides test was carried out by using Benedict's reagent. Alkaloids test was done by using Wagner's reagent (Harborne, 1984).

Determination of total phenolic contents

Total phenolic content of methanolic extract was determined spectrophotometrically using the Folin-Ciocalteu method described by Jayaprakasha et al., (2001), then 0.4 ml of each sample were mixed with 2.0 ml of the Folin-Ciocalteu reagent (diluted 10 times), and 1.6 ml of 7.5% sodium carbonate solution. Total volume was adjusted to 5 ml by adding distilled water. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760 nm by spectrophotometer.

The tests were carried out in triplicate. Total phenolic content was calculated from a calibration standard curve of gallic acid (Figure 1) and the results were given as mg gallic acid equivalent per gram dry weight.

DPPH assay

In order to obtain an indication of the antioxidant activity of methanolic extract, 5 ml of a freshly prepared (0.004 %) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 µl of different concentration of leaves extract (2 , 4 , 6 , 8 , 10 and 12) mg/ml and the absorbance of each dilution, after 30 minutes, was measured at 517 nm (Kedare and Singh, 2011).

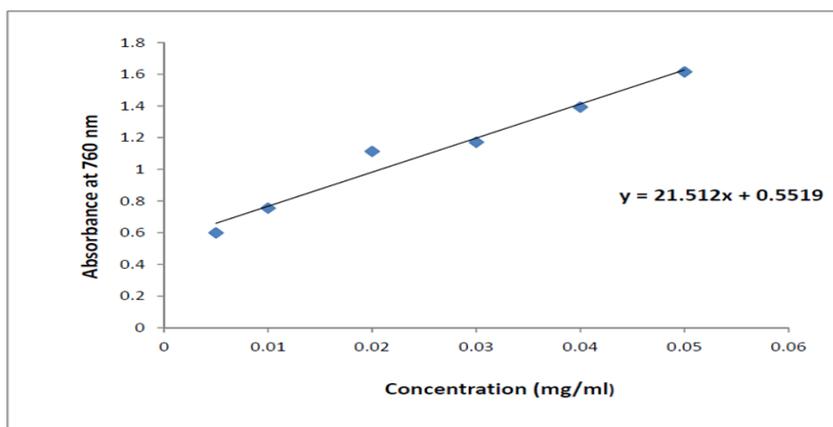


Figure 1. Standard curve of gallic acid (Al-azawi, 2014).

Butylated hydroxytoluene (BHT) and vitamin C were the antioxidants used as positive control. All tests were performed in triplicate and the methanol was used as a blank solution. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

% Reduction = (Abs DPPH – Abs Dil.) /Abs DPPH x 100 Where Abs DPPH = average absorption of the DPPH solution, Abs Dil. = average absorption of the three absorption values of each dilution. With the obtained values, a graphic was made using Microsoft Excel. The EC₅₀ of each extract (concentration of extract or compound at which 50% of DPPH is reduced) was taken from the graphic.

Microorganisms and media

The bacterial isolates *Staphylococcus aureus*, *Klebsilla pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* isolated from patients with urinary tract and wound infections by using specific selective media. The bacteria were obtained from Al-Yarmook Teaching Hospital, Baghdad, Iraq.

Antibacterial activity test of extract

Agar disc diffusion assay was used for this test; 0.2 ml volume of the standard inoculums (10⁷ CFU/ml) of the tested bacterial isolate was spread on Mueller Hinton Agar (MHA) with a sterile swab and allowed to dry. Then 6 mm diameters of What man filter paper (No.3) discs were prepared. Methanolic extract of leaves (12.5, 25, 50 and 100 mg/ml) were saturated into each disc and allowed to dry at room temperature and then introduced to the medium to diffuse the plant extract into medium before incubation at 37 °C for 24 hr. The diameter of inhibition zone was measured by transparent ruler to nearest mm. Cephotoxime (30 µg/ml) (MAST) was used as positive control. Inhibition zone with diameter less than 12 mm. were considered as having no antibacterial activity, diameter between 12 and 16 mm. were considered moderately active, and these with > 16 mm. were considered highly active (Indu et al., 2006).

Minimum inhibitory concentration (MIC) of methanolic extract

The minimum inhibitory concentrations were determined by the macrobroth dilution methods for antibacterial assay, different concentrations of the extract ranging from 12.5 to 300 mg/ml were prepared by serial dilutions in Mueller Hinton broth medium. Different concentrations of Cefotaxime

ranging from 5 and 50 µg/ml used as positive control were also prepared by serial dilution in Mueller Hinton broth medium. The tubes were inoculated with 100 µl of each of the bacterial strain. Blank Mueller Hinton broth was used as negative control. The bacterial containing tubes were incubated aerobically at 37°C for 24 h. The first tube in the series with no visible growth after incubation period was taken as the MIC (Thrupp, 1986).

RESULTS AND DISCUSSION

Phytochemical screening of *C. Spinosa* leave extract

Methanolic extract of *C. Spinosa* leaves showed the presence of Flavonoids, phenols, alkaloids, tannins and glycosides in preliminary phytochemical investigations as in Table 1. Li et al., (2007) mentioned that flavonoids identified in the various parts of the *C. Spinosa* plant, and this corresponds with Zhang and Ma, (2018) who explained that *C. spinosa* exhibited important pharmacological effects because it is rich in many bioactive compounds including flavonoids. Many studies revealed the presence of different bioactive compounds in all parts of *C. spinosa* such as leaves, buds, seeds, roots and buds. However, *C. spinosa* has a wide range of bioactive compounds such as polyphenolics, flavonoids, alkaloids, steroids, terpenoids and tocopherols (Vahid et al., 2017).

Total phenolic contents

Analysis of phenolic content was done by Folin- Ciocalteu method and expressed in terms of gallic acid equivalents. *C. spinosa* exhibits progressive phenolic content in methanolic extract which was (21.62, 24.81 and 29.54 mg/g in Concentration 8, 10 and 12 mg/ml) respectively.

Table 1. Phytochemical analysis (Qualitative) of methanolic extract of *Capparis spinosa* L. Leaves extract.

Phytochemical compound	Test result
Flavonoids	+
phenols	+
alkaloids	+
Tannins	+
Glycosides	+

+ Positive, - negative.

This result is approach to Azemi et al., (2011) who mentioned that the total phenolic content of leaves was 259.7 mg galic acid per 100 grams powder. Phenolic compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals (Roya and Fatemeh, 2013). Also it was reported that main components of the total amount of phenolic compounds was rutin (quercetin-3-O-rutinoside) followed by kaempferol 3-O-rutinoside (Siracusa et al., 2011).

Radical scavenging activity by DPPH

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. the antioxidant activity is expressed as an effective concentration (EC_{50}), which is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by the linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds (Nahak and Sahu, 2010). The radical scavenging capacity (EC_{50}) of methanolic extract was found to be (7.1 mg/ml), while the (EC_{50}) of vitamin C and BHT was (1 and 1.4)

respectively as shown in (Figure 2).

Effectiveness of antioxidant properties is inversely correlated with EC_{50} values. Lee et al., (2008) reported that if the EC_{50} value of an extract is less than 10 mg/ml it indicates that the extract is an effective antioxidant. The antioxidant activity of the methanolic extract of *C. spinosa* L. buds is attributed to the presence of rutin compound (Germano et al., 2002). Similarly, Inocencio et al., (2000) suggested that *C. spinosa* as a source of antioxidant molecules especially the derivatives of quercetin and kaempferol in the methanolic extract.

Antibacterial activity of methanolic extract

The results of antibacterial and MIC tests were demonstrated in tables (2 and 3), our findings revealed that the concentration 100 mg/ml of methanolic extract of *C. spinosa* had the highest activity against the most tested bacteria especially *S. aureus* with the highest inhibition zone (21 mm) (Figure 3), also 18 mm and 17 mm for *K. pneumonia* and *E. coli*, respectively, while the pathogenic bacteria *P. aeruginosa* exhibited resistance to the antibiotic used as control and to the methanolic extract at concentration 100 mg/ml.

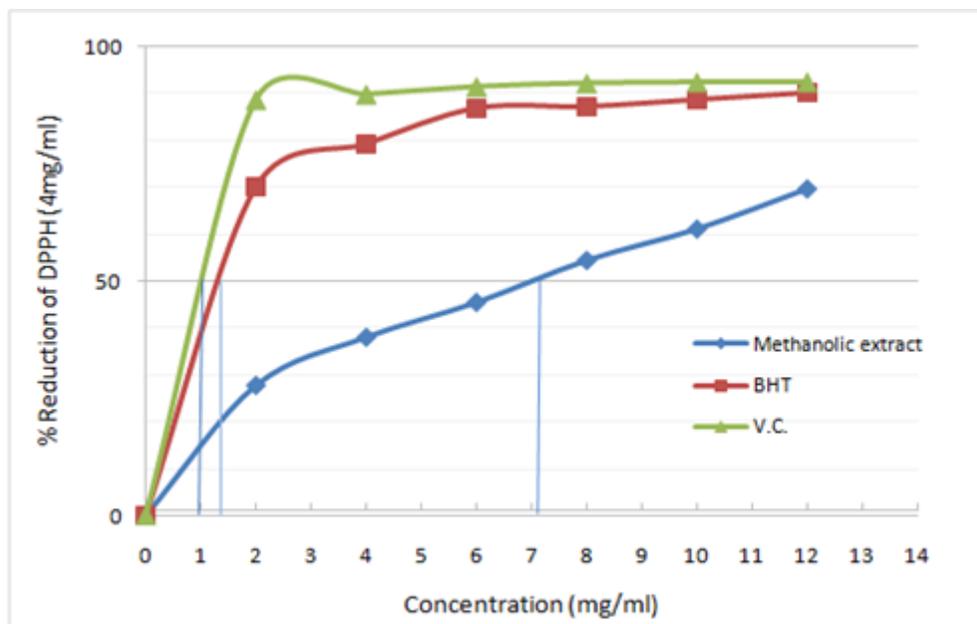


Figure 2. Radical scavenging activity by DPPH.

Table 2. Antibacterial activity of *C. spinosa* methanolic extract against some of pathogenic bacteria.

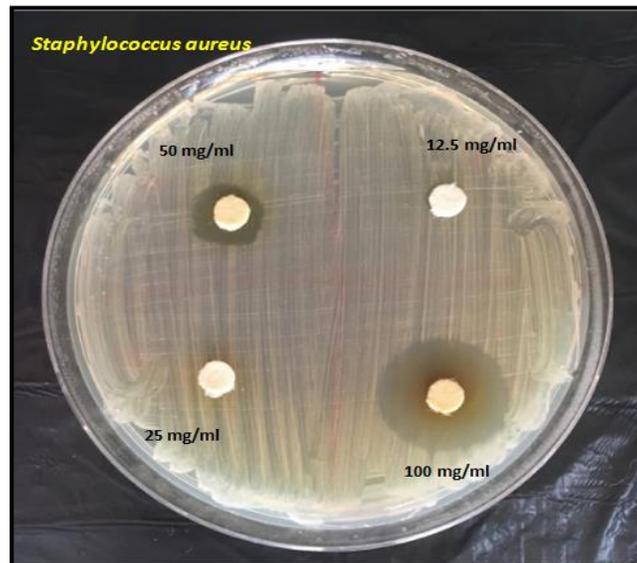
Tested bacterial isolates	Inhibition zone (mm)*		
	Methanolic extract (50 mg/ml)	Methanolic extract (100 mg/ml)	Control (Cefotaxime)
<i>Staphylococcus aureus</i>	11	21	12
<i>Escherichia coli</i>	-	17	11
<i>Klebsiella pneumonia</i>	14	18	9
<i>Pseudomonas aeruginosa</i>	-	-	-

*The result is the average of 3 tested isolates.

Table 3. MIC of methanolic extract of *C. spinosa* against tested bacteria.

Tested bacterial isolates	Minimum inhibitory concentration (MIC)* (mg/ml)
<i>Staphylococcus aureus</i>	75
<i>Escherichia coli</i>	100
<i>Klebsiella pneumonia</i>	100
<i>Pseudomonas aeruginosa</i>	200-250

*The result is the average of 3 tested isolates.

**Figure 3. Antibacterial activity of methanolic extract of *C. spinosa* against *S. aureus* at different concentrations by Disc diffusion method.**

The concentration 50 mg/ml showed moderate activity against *K. pneumonia* and *S. aureus*, only. The results of MIC in broth medium matched with the findings of disc diffusion test on the agar medium, where the MIC of *S. aureus* was 75 mg/ml in comparison with the MIC of *P. aeruginosa* (200-250 mg/ml) (Table 3). Generally, the tested Gram positive bacteria were found to be more sensitive to the extract than Gram negative bacteria.

The growth of three bacterial isolates was significantly inhibited by the application of *C. spinosa* methanolic extract in comparison with

antibiotic i.e., Cefotaxime. The results of Gull et al., (2015) revealed that the methanolic extracts were found to be more effective in comparison with ethanol and acetone extracts against Gram positive and negative bacteria for different parts of *C. spinosa*. The previous study of Mahasneh (2002) indicated to the inhibitory effect of aerial parts of *C. spinosa* plants against Gram-positive and negative bacteria. The sensitivity of Gram positive bacteria in contrast with gram positive might be due to cell membrane permeability of the outer phospholipidic membrane in Gram negative bacteria, which makes the wall impermeable to

chemical compound (Sharma et al., 2010).

The inhibitor effect methanolic extracts against bacterial growth might be due to bioactive compounds like phenolics and flavonoids, where higher phenolic and flavonoid compounds found in different parts of *Capparis* species (Pourmorad et al., 2006; Imran et al., 2014).

Another study reported that the antibacterial activity against some of pathogenic bacteria (*E. coli*, *Shigella dysenteriae* and *Salmonella typhi*) might be due to the polysaccharides of *C. spinosa* leaves (Mazarei et al., 2017).

CONCLUSION

Our results showed that the methanolic extract of leaves of *C. spinosa* L. contain many of bioactive compounds have high potency in scavenging of free radicals, also the high antibacterial potential indicated to the positive correlation between these compounds and the inhibitory effect of *C. spinosa* extract against antibiotic resistant bacterial strains. More attention has been focused on the therapeutic and protective functions of naturally antioxidants in aerial parts of *C. spinosa* and on the mechanism of their antibacterial action.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AHA and KKG contributed to the design of the experiments and performed the experimental work. All authors carried out laboratory tests. KKG wrote the manuscript, all authors revised and approval the final version.

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