Annals of Biology **39** (1) : 15-22, 2023

Impact Score : 0.32 (Scopus)

Isolation and Identification of Alkaloid Compounds from *Capparis spinosa* and to Study its Biological Activity in Misan, Iraq

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(Received: May 17, 2022; Accepted: June 19, 2022)

ABSTRACT

The current study included the preparation of alcoholic (methanol and ethanol) and aqueous extracts of indigenous medicinal plant in Iraq. The leaves, stems and roots of the *Capparis spinosa* were used for studying the chemical components of these extracts by using appropriate reagents**.** This study also included the separation, purification and identification of the alkaloid compounds separated from the roots of the *C. spinosa* plant. The separated alkaloids were diagnosed through the use of some physical and chemical tests, which represented the degree of melting point and solubility, as well as gas chromatography-mass spectroscpy, thin layer chromatography, ultraviolet spectrometry (UV) and fourier transform infrared spectrometry (FT-IR). Antibacterial activity of the isolated alkaloid compounds against harmful bacteria was demonstrated (*Staphylococcus aureus* and *Escherichia coli*). Bacterial strains were isolated from inflammation of the urinary tract (UTI) samples, and normal bacteriological procedures were used to evaluate each bacterial isolate's biochemical values. Using the Vitek-2 method and molecular anlysis, the isolates were further identified.

Key words: *Capparis spinosa*, *Staphylococcus aureus*, *Escherichia coli*, Vitek-2 method

INTRODUCTION

Capparis spinosa L. is ordinarily known as Caper is quite possibly the most widely recognized fragrant plant filling in load up scope of climatic conditions, differing from desert to cooler heights of mountains. It is a lasting winter deciduous animal category that bears adjusted, plump leaves and huge white to pinkish blossoms (Swaminathan and Kochhar, 2019). Different pieces of Caper plant can be utilized as medication, beautifying agents and nourishments. Additionally, it can be utilized as preparing for a lot of oil and rich nutrient E (Calderón *et al*., 2021). Various segments have been distinguished in *C. spinosa*, including alkaloids, flavonoids, unstable oils and unsaturated fats.

Medicinal plants have many active chemical compounds from secondary metabolism. These chemical compounds are abundant in natural families which are represented by essential oils, phenols, glycosides, alkaloids, steroids, saponins and terpenes (Alamgir, 2018). The World Health Organization (WHO) has set up that out of 119 plant-inferred drug prescriptions, about 74% are utilized in present-day medication in manners that relate straight forwardly with their conventional uses as plant medication by local people groups (Heinrich *et al*., 2017).

In recent years, the major modification of medicinal herbs in traditional herbal sciences of nations such as Iran, China, India and Japan have got a lot of attention (Sen and Chakraborty, 2017). Traditional medicine, which is mostly based on herbs, is still used for primary healthcare by more people around the world than conventional or western medicine (Zhang *et al*,. 2015), and it is culturally acceptable and widely available even in the current period (Shahrajabian *et al*., 2021).

Alkaloids are heterocyclic nitrogenous mixes that are generally present in the plant realm and they have high significant impact to treat different infections, and these mixes have antibacterial, antifungal, antiparasitic against malignant growth, and are hostile to tumor activities. Run of the mill alkaloids are from plant source, and are fundamental mixes containing at least one nitrogen iotas (for the most part in the heterocyclic ring). Like-wise alkaloid particles must contain nitrogen associated with at any rate two carbon molecules and have at any rate on ring (Dey *et al.,* 2020).

MATERIALS AND METHODS

Pure cultures of *S. aureus* and *E.coli* were obtained from cultures of pathogenic bacteria from Al-Sadr General Hospital in Misan Governorate Center. The bacteria were activated and cloned three times in nutrient agar before being kept at 4°C on nutrient agar slants.

The plants of *C. spinosa* were collected from the center of Misan city in October 2020 and were identified by Dr. Abdullah. H.AL-Tamimi, Biology Department, Faculty of Sciences/ Basrah University. Each piece of plant was cleaned with tap water, dried at room temperature, then ground into powder using an electronic processor (Cremades *et al.*, 2018). The powdered pieces were stored in plastic cylinders in the refrigerator at 4°C until they were needed.

Fifty grams of the *C. spinosa* powder (the root, stem and leaves) was taken in a 1000 ml conical flask with 400 ml distilled water. It was acidified with 4% sulfuric acid. The mixture was left with continuous stirring on the magnetic stirrer device for 24 h at room temperature. The solution was filtered with Whattman No.1 filter paper. The filtrate was transferred to a separating funnel. To every 10 ml solution, 30 ml of chloroform was added and the solution was gently shakened to settle for some time until the solution separated into two layers. The bottom layer was collected and placed in a Petri dish and left to dry at room temperature. The dry substance was washed with acetone to get rid of unwanted materials by performing TLC test to determine the relative effect of the compound, as well as some chemical and physical tests to guarantee its purity.

To evaluate the purity of isolated chemicals and their ratio to front (Rf), thin-layer chromatography was used for 30 and 5 min. TLC plates were used on glass panes (2 x 10 cm) and progressive chromatography was utilized on glass tanks. The sample was spotted or lined with a capillary tube, left to dry, and then placed in the glass chromatography tank with the solvent pre-positioned to a depth of 10 mm at the bottom of the tank (Cseke *et al.*, 2016). Rf (relative flue) was calculated using the equation below:

Rf=Distance travelled by the compound/ forward distance travelled by the solvent

The melting point of insulated compounds was determined using the thermoelectric melting point. The melting points of the compounds were determined in the Electrothermal 9100 device's open capillary tubes, which were not linked. The UV spectra of the purified single compounds were obtained using chloroform as a solvent on a Shimadzu UV-1601PC spectrophotometer. The separated compounds were subjected to UV spectroscopy Department of Chemistry/College of Science at Misan University (Abdel-Raouf *et al.*, 2019).

To detect the functional groups of pure single compounds, the spectra of separated compounds were recorded using FT-IR. The Department of Chemistry/College of the Science/University of Misan employed an infrared spectrophotometer FTIR-8400S (Shimadzu) to analyze the FT-IR spectra recorded in KBr.

The concentrated alkaloid sample was injected into a gas chromatograph with standard improvements (GC-Mass: Agilent Technoloies GC 7890A GC system). Different peaks were separated and recorded at different retention times. The separate chemical compounds were then identified using mass spectrometry. Five hundred mg of dried concentrate was dissolved in 1 ml of distilled water to prepare different concentrates (62.5, 125, 250 mg/ml; Gezahegn *et al.*, 2015).

The agar diffusion method was used to determine the antibacterial activity of the extracts, replacing the antibiotic disc with filter paper discs with a diameter of 6 mm. The aqueous extracts were dissolved in water as: 1, 250, 125, 62.5 and 30 mg/ml. The methanolic extracts were dissolved in methanol alcohol with: 250, 125, 62.5 and 30 mg/ml. The ethanolic extracts were dissolved in ethanol alcohol with: 250, 125, 62.5 and 30 mg/ml. The bacterial strains were subcultured by the streak method. Incubation at 37°C for 18 to 24 h in order to obtain a young culture and isolated colonies. The well isolated colony was mixed with 5 ml of sterile distilled water in a test tube in order to have an initial cell density or a turbidity close to 0.5 Mc Farland (Abs: 0.08-0.1 at 625 nm). Inoculation was carried out by using a swab dipped in the inoculum, and spread over the entire surface of the Mueller-Hinton agar. The operation was repeated two more times by turning the box 60°C each time to ensure a homogeneous

distribution of the inoculum. Finally, the swab was passed around the edge of the agar surface. Then 6 mm diameters of Whatman filter paper No. 3 discs were prepared and impregnated with 30 µl of the different concentrations of extracts of *C. spinosa.* The impregnated discs were then gently deposited by sterile forceps on the surface of the inoculated agar. A disc impregnated with alcohol/distilled water was also deposited as a negative control, as well as a ready-to-use disc serving as a positive control. The petri dishes were incubated in an oven at 37°C between 18 and 24 h. The diameter of inhibition zone was measured by transparent ruler to nearest mm. Inhibition zone with less than 12 mm diameter was considered as having no antibacterial activity; diameter between 12 and 16 mm was considered moderately active, and those with > 16 mm were considered highly active.

RESULTS AND DISCUSSION

Physical properties of isolated compounds showed that phenol, 2, 6 dimethoxy-, Diethyl Phthalate, n-Hexadecanoic acid, Octadecanoic acid, Pyrrobutamine, 5-Hydroxy-4', 7-dimethoxyflavone, Xylometazoline acetate and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3-one were fairly stable (Table 1). It was a beige gelatinous substance with a brownish colour and higher melting points. At room temperature, the isolated compounds dissolved easily in methanol alcohol and water but were slightly soluble in ethanol, acetone and chloroform. The high solubility of our alkaloid compounds in water was due to the presence of polarized groups in the structures of these compounds. This character encouraged to study the biological activity of these alkaloid. The structures of the isolated compounds were assigned on the basis of their TLC, UV, IR spectra and Gas Chromatography-Mass Spectroscopy (GC-MS).

Table 1. Properties of alkaloid compounds

Biological name	Alkaloids
Molecular formula	C8 H10 O3, C12 H14 O4, C16 H32 O, C18 H36 O, C2 H22 ClN, C17 H14 O5, C16 H24 N2 and C39 H56 O2
Melting point	a221 (deco.)
Weight before isolation (g)	40
Weight after isolation (g)	9.8
Shape and status of isolated compounds	A beige gelatinous substance with a browish colour

The behaviour of isolated compounds was similar to the behaviour of alkaloids (orang, after spraying with dragendroff reagents) and isolated compounds from the roots of *C. spinosa* (Alkaloids) had Rf = 0.25 and Rf = 0.23 (Table 2). The difference in the value of Rf of alkaloids was ascribed to the degree of polarity and functional groups of the each compounds and may be due to their mobile phase. Thin layer chromatography revealed the appearance of a single spot using a chloroform : methanol solvent system, to confirm the purity of the alkaloid (0.5 : 9.5). The peak value of Rf was 0.25. The appearance of one spot was evidenced of the purity of the alkaloid. Small Rf value indicated low dissolvability of compound in mobile phase therefore the compound slowly moved (Motar and AL-Hadad, 2018).

Table 2. TLC results of alkaloid compounds

Properties	Alkailoids
Rf	0.23,0.25
Colour in visible light	Yellow
Colour in dragenddroff Reagent	Orange
Mobile phase	1. NH3OH: CHCl3 (19.5: 0.5) 2. NH3OH:C3H6O: CHCl3 (9:0.5:0.5)
Time of posting	28, 34 min

The qualitative UV spectra profile of isolated alkaloids from root of *C. spinosa* were selected at 200-600 nm wavelength for sharpness of the peaks and proper baseline (Table 3). The electronic absorption (Table 3) and the spectra of compound (Fig. 1) indicated two bands. The shorter band at 255 nm was ascribed to the locallyexcited as $_{TT}\rightarrow_{TT}^{*}$ transition with the double band (C=C). The spectra of long band at 283 nm of alkaloids, was ascribed to the $n\rightarrow_{TT}^{*}$ transitions.

The FT-IR spectrum *C. spinosa* was characterized by five bands corresponding to

Table 3. UV spectral data of compounds by using methanol as solvent, λ max (nm)

No.	0.05 mg/ml			
	Band I		Band II	
	λ max	Absorption	λ max	Absorption
Compounds	283.00	0.2	255.00	0.2

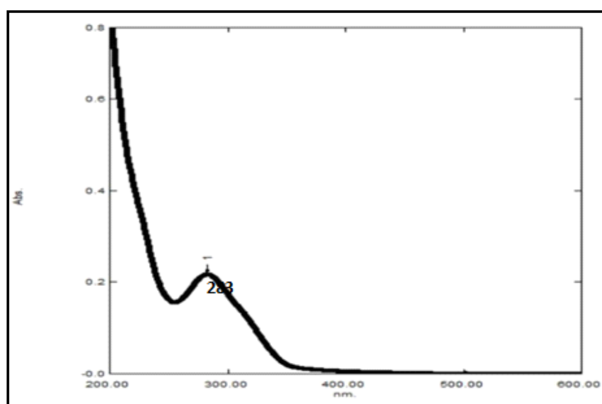

Fig. 1. UV spectrum of compounds *C. spinosa*.

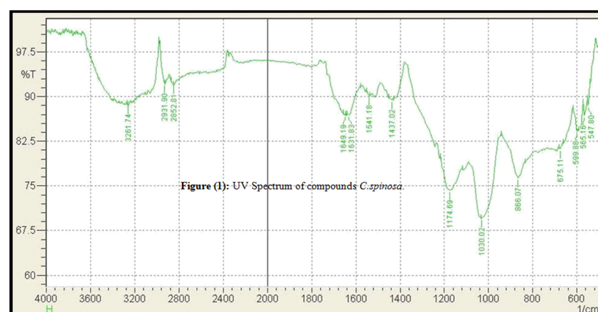

Fig. 2. FT-IR spectrum of compounds *C. spinosa*.

the stretching vibrations of the aromatic NH, OH and aliphatic CH groups, C = C and C = O, which occurred at 3400-3100, 3200-3500, 2809-3000, 1500-1650 and 1650-1800 cm, respectively (Table 4; Fig. 2). The results of the FT-IR functional groups for isolated alkaloid compounds were consistent with the structure (Narmada *et al.,* 2020) according to the alkaloid dictionary. A section of the *C. spinosa* plant was subjected to FT-IR analysis. The findings demonstrated the ability to identify effective functional groups in the chemical components of each part of the caper plant, as well as the ability to distinguish between aromatic and non-aromatic compounds, alkenes, alkanes, esters, ethers, carboxylic acids, and unknown compounds, as each compound had its fingerprint. Further, knowing the chemical bonds such as CH, CO, OH, CF stretching, or others, and measuring the intensities (Altameme, 2015) of each peak of the peak curve and determining the group frequency to confirm the biological activity of each compound made it possible to understand the chemical and physical properties of each compound.

The concentration of eluted chemicals was plotted as a function of retention time in the gas chromatogram (RT). The identified chemical components were shown by the chromatogram peaks. The concentrations of eluted chemical ingredients were reflected by the height of the peaks. A compound's mass spectrum was a graphical representation of ion distribution based on its mass and charge ratio (m/z), which was critical for identifying the chemical structure and its characteristics. The peak area (concentration) of the chemical ingredients discovered was n-hexadecanoic acid, which had a concentration of 0.35%. Linolenic acid, often known as palmitic acid, was a naturally occurring chemical. It was, nevertheless, found in most natural sources and was responsible for its therapeutic properties. n-hexadecanoic acid had anti-inflammatory, antioxidant, anti-androgenic and hypocholesterolemic properties in previous research (Chinnadurai *et al.,*2019). The inhibiting impact of n-hexadecanoic acid on the phospholipase A2 enzyme (Nkadimeng *et al.,* 2020) inhibited the inflammatory process. Palmitic acid inhibited macrophage invasion, which may diminish macrophage buildup in the synovial fluid of arthritic joints (Lohdip *et al.,* 2018). Furthermore, *in-silico* cytotoxicity tests revealed that n-hexadecanoic acid interacted with the DNA topoisomerase-1 enzyme, causing cytotoxic effects that were responsible for its anti-cancer action (Ravi and Krishnan, 2017). These secondary metabolites, on the other hand, were responsible for their pharmacological effects, such as anti-inflammatory, antioxidant and anti-cancer properties (Ravi and Krishnan, 2017; Lohdip *et al.,* 2018; Chinnadurai *et al.,* 2019). Antibacterial and antifungal properties were

Table 4. FT-IR spectral data of compounds recorded as KBr discs/cm

No.	NH2 stretching	OH stretching	Aliphat ic CH stretching	C=C stretching	C=O stretching
Compound	3100-3400 weak	3200-3500 medium Broad beak	2800-3000 weak	1500-1650 weak	1650-1800 weak

also present in octadecanoic acid (Abubakar and Majinda, 2016).

On the other hand, one of the things to consider is the type of solution that can be used in the extraction affecting the chemical compounds in analysis, because another study by Altamema (2016), revealed the existence of 53 compounds such as heptadecanoic acid, ethyl ester, 1,2Benzenedicarboxylic acid, monoacid (2-Ethylhexyl ester, 9,12-octadecadie). These findings revealed that Caper roots had a significant role and a big number of chemical components that, when compared to other portions, supported the use of Caper in traditional medicine (Nabavi *et al.,* 2016). Caper had also been found to have certain medicinal and antioxidant characteristics (Yadav and Malpathak, 2016), as well as aromatic plants in Mediterranean cooking, based on the flavour profile (Rosa *et al.,* 2022). However, these findings are still preliminary, and more experimental and clinical research is required. Therefore, these results are shown in Fig. 3 and Table 5.

E. coli, which is already known to be multi-resistant to different antibiotics, inhibited its growth by aqueous, and methanolic extracts of *C. spinosa* roots at a concentration of 250 with an inhibition zone of 20, 5 and 20, respectively. As for *S. aureus* bacteria, its growth was inhibited by the methanol alcohol extract of the root of *C. spinosa* at a concentration of 250 in the inhibition zone 19, as well as the ethanol extract at a concentration of 250 for the roots and leaves of *C. spinosa* in the 11 and 10 inhibition zone, respectively. Such results were very interesting because these bacteria were isolated from the hospital environment. It was very difficult to control it by therapeutic means (Tables 6 and 7). Studies must be conducted on the way these compounds act in the bacteria cell.

The results showed that the aqueous extract had a strong bactericidal effect on the G -ve bacteria, and it did not show any activity on

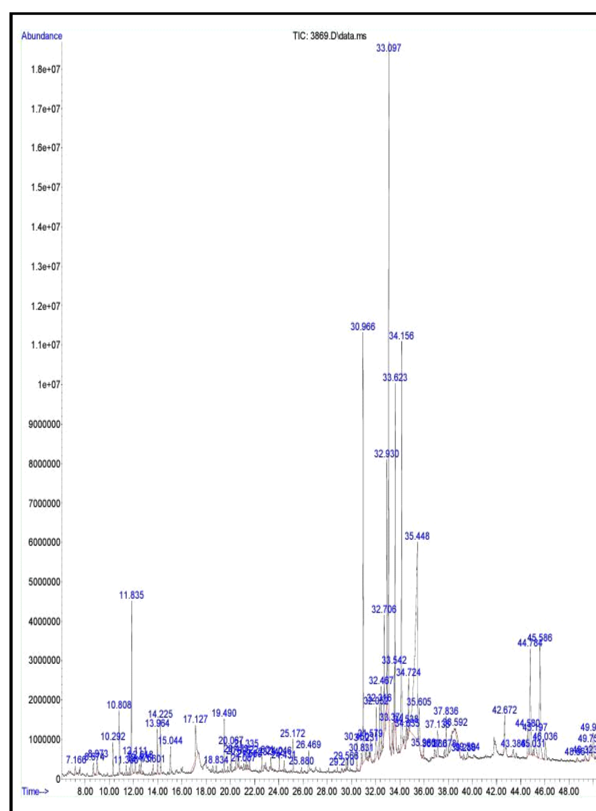

Fig. 3. Chromatogram of *C. spinosa* root extracts by GC-MS.

Table 5. Bioactive compounds and their biological activity

S. No.	RT	Name of the compound	Activity of the compound
1.	15.042	Phenol,2,6 dimethoxy-	Antioxidant, antibacterial, anti-inflamamatory and anthelmintic
2.	20.065	Diethyl Phthalate	Antimicrobial activity
3.	26.468	n-Hexadecanoic acid	Anti-inflammatory, antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5- Alpha reductase inhibitor, potent mosquito larvicide
4.	29.565	Octadecanoic acid	Antimicrobial, anticancer, hepatoprotective, anti-arthritic, Anti-asthama, diuretic
5.	37.68	Pyrrobutamine	Antihistamine
6.	39.595	5-Hydroxy-4',7-dimethoxyflavone	Antifungal
7.	42.672	Xylometazoline acetate	Nasal decongestant
8.	44.784	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4, 4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14, 14a,14b-octadecahydro-2H-picen-3-one	Antisickling

Table 6. The inhibition zone of crude extracts from (leaves, stems and roots) of *C. spinosa* on the growth of standard bacteria *E. coli*

Plant parts	Type of extracts	Concentration (mg/ml)				Control
		30	62.5	125	250	
Leaves of *C. spinosa*	Coh. E	6	6	6	12	6
	Coh. M	6	7	8	9	6
	C. A.	6	8	9	11	6
Stems of *C. spinosa*	Coh. E	6	7	8	11	6
	Coh. M	6	8	8	9	6
	C. A.	6	6	9.66	12.66	6
Roots of *C. spinosa*	Coh. E	6	6	6	7	6
	Coh. M	11	12	13	20	6
	C. A.	6	6	6	20.5	6

L. S. D.(0.05) =2.341 for interaction, Coh. E = Alcoholic extract ethanol, Coh. M = Alcohol extract methanol, C. A. = Cold aqueous.

Table 7. The inhibition zone of crude extracts from (leaves, stems and roots) of *C. spinosa* on the growth of standard bacteria *S. aureus*

Plant parts	Type of extracts	Concentration (mg/ml)				Control
		30	62.5	125	250	
Leaves of *C. spinosa*	Coh. E	6	6	6	10	6
	Coh. M	6	6	6	6	6
	C. A.	6	6	7	8	6
Stems of *C. spinosa*	Coh. E	6	6	6	9	6
	Coh. M	6	6	7	19	6
	C. A.	6	6	6	6	6
Roots of *C. spinosa*	Coh. E	6	6	9	11	6
	Coh. M	6	6	6	7	6
	C.A.	6	6	6	7	6

L. S. D. (0.05) =1.823 for interaction, Coh. E =Alcoholic extract ethanol, Coh. M = Alcohol extract methanol, C. A. = Cold aqueous.

the G +ve bacteria, while the alcoholic extracts had moderate effects on both the types. The alcoholic extract of *C. spinosa* roots bark had antimicrobial and antihelminthic activity, according to Akkari *et al.* (2016). Barka *et al.* (2016) made a similar observation, stating that *C. spinosa* had strong antibacterial activity against both G +ve and G -ve bacteria.

The extracts had a substantial effect on *S. aureus* and *E. coli* bacteria. *C. spinosa* had the potential to be a source of natural antibacterial compounds with therapeutic benefits. *C. spinosa* was a significant plant that produced a large number of active metabolites naturally through the secondary metabolism pathway. Because alkaloid substances had a variety of physiological effects, they were capable of possessing biochemical activities that destroyed harmful microorganisms such as bacteria and fungi. Alkaloids activity extracted from medicinal plants had been shown in certain research because these chemical compounds had physiological impacts and spectacular therapeutic properties for destroying harmful microorganisms such as bacteria (Ikram *et al.*, 2021).

CONCLUSION

The roots, stems and leaves of *C. spinosa* contain active compounds: alkaloids, flavonoids, phenols, glycosides, tannins, triterpene, resin, coumarin, aponine and amino acids which were not detected in other plants. The alcoholic extracts showed anti-bacterial activity, both G +ve and G -ve, and it was more effective than the aqueous extracts. This study showed that the active compounds

present in the extract were responsible for its pharmacological effects. It is worth mentioning that the discovered compounds had anti-inflammatory, anti-microbial, anti-fungal, and anti-oxidant activities for their medicinal uses. There is a need for investigations to evaluate the safety and efficacy of this traditional herbal plant in various disorders. GC-MS of the aqueous extract acidified with sulfuric acid of the roots of *C. spinosa* showed about 20 to 25 compounds.

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