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Phytochemical, Characteristic Analysis and Biological Activity for *Capparis spinosa* L. Fruit extract

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ABSTRACT

Alternative medicine and herbal treatment are among the methods inherited by the people of the Middle East. In the current study, we evaluated the chemical composition of the fruit of *Capparis spinosa* L. (*C. spinosea* L.), as well as the antibacterial, and potential inhibitory effects of the dehydrogenase enzyme (LDH). The presence of the active chemicals in the extract was confirmed by phytochemical screening and characterization techniques such as gas chromatography-mass spectrometry analysis (GC-MS), ultraviolet (UV-Vis) spectroscopy, and infrared spectroscopy (FTIR). The outcomes demonstrated the presence of alkaloids, flavonoids, polyphenols, tannins, and vitamins in the aqueous extract. Moreover, the extract exhibited anti-bacterial activity, especially against gram-negative bacteria. In contrast, the concentrations of the aqueous extract possessed the activity of inhibiting the dehydrogenase enzyme at a concentration of 0.13 mmo l/L. The study concluded that *C.spinosea* L. fruit aqueous extract contains biologically active compounds that could be used in the inhibition processes of both antibacterial and as well as inhibitors of lactate dehydrogenase (LDH).

Keywords: *Capparis spinosa* L, Phytochemical, Enzymes Inhibitor, lactate dehydrogenase.

INTRODUCTION

A major problem is the emergence of bacteria that are resistant to antibiotics. The negative effects of some chemical antibiotics have also increased interest in medicinal plants as an innovative alternative (Anand *et al*., 2019). Recent studies have concentrated on medicinal plant extracts and their biological applications for industrial, pharmaceutical, and environmental objectives, due to their safety, low cost-effectiveness, and few side effects (Al-Musawi *et al*., 2020; Al-Kaabi *et al*., 2021; Al-Musawi *et al*., 2022; Al-Musawi *et al*., 2023; Alyamani *et al*., 2023, Alzubaidi *et al*., 2023). *C. spinosae* L. known as Caper is a perennial shrub of the family Capparaceae, endemic to circum-Mediterranean countries, Iran, and recently in the south of Iraq (Zokian, 2015). *C. spinosae* L. is a famous medicinal herb; it has traditional use, as that possesses a nutritional value and obvious benefit. It is distinguished by containing vitamins and antioxidant compounds such as flavonoids and alkaloids (Tlili *et al*., 2010) and also has several properties: anti-bacterial, antifungal, anti-inflammatory, and anti-oxidant actions (Vahid *et al*., 2017; Neamah *et al.*, 2023). Furthermore, this herb is valued for its anti-diarrhea properties (Abdulridha *et al*., 2023). Reports refer to that the (ether, methanol, ethanol, hexane, and aqueous) extracts of aerial parts of *C. spinosae* L have antifungal, antibacterial, and antiviral activities (Lam *et al*., 2009; Boga *et al*., 2011) Additionally, the fact that these extracts have no adverse environmental effects makes them a suitable choice for use as insecticides against plant diseases that have a significant impact on environment and human health (Pattnaik *et al*., 2021, Abdelmigid *et al*., 2022). Added to this, essential oils in the composition of such plants act as antifungals and antivirals (Matthäus and Özcan, 2005, Rhyaf *et al*., 2023). Caper leaf and fruit extracts significantly reduce liver damage by raising the levels of phase I enzymes for detoxification like cytochrome P450 enzymes (CYP) and phase II detoxification enzymes like glutathione S-transferase (GST), quinone reductase (QR), UDP-glucuronosyl transferase (UGT), and amino acid oxidase (Zhu *et al*., 2022). Other enzyme levels released by the liver in response to injury or illness, such as ALT, AST, ALP, -glutamyltransferase (-GGT), and lactate dehydrogenase (LDH), could be reduced by *C.spinosa* (Annaz *et al*., 2022). Identification of natural bioactive compounds from traditional remedies or dietary components gives a considerable opportunity for developing new drugs or nutritional supplements to treat inflammatory illnesses (Panico *et al*., 2005, Kalaivani *et al*., 2023). The literature indicates that most studies focus on the leaves and roots. So, in this study, we aimed to highlight the phytochemical content of *C. spinosa* fruit extract and its potential effects against gram-positive and gram-negative bacteria, as well as measure the levels of other enzymes like lactate dehydrogenase (LDH).

MATERIALS AND METHODS

Fruits Extraction

C. spinosae L. was collected from Ali Al-Gharbi sub-district of Missan Governorate, Iraq, from April to June 2022; professors' specialists in plant taxonomy at the University of Maysan have classified the plant. We collected the fresh fruits of C. *spinosae* L. cleaned them well, dried the fruit samples, and crushed them to obtain a fine powder. The hot aqueous extract of *C. spinosae* L. fruits were prepared by adding 100 ml of deionized water to 10 g of dried fruit powder according to a recent study conducted by (Kdimy *et al.*, 2022) with some modifications. At about 70 degrees Celsius for two hours, stirred using a magnetic stirrer. The extract solution was filtered using sterilized filter paper (Whatman No. 1), after cooling to room temperature and being maintained at 4 °C for more analysis.

Content Estimation of *C. spinosa* L.

The crude extract of C. *spinosa* L. was examined for its phytochemical components, including Saponosides, phenols, flavonoids, tannins, alkaloids, sterols, and triterpenes. (2 ml each) were used independently for each analysis in the same way as the presence of the phytochemicals above is indicated by the formation of a precipitate, a change in color, or foaming (Kahdim *et al*., 2023).

Characterization of Aqueous Extract

Visible and Ultraviolet Spectroscopy

UV-Vis Spectrophotometer was used for analyzing aqueous fruit extract (UV-1800 UV-Vis Spectrophotometer, Shimadzu, Tokyo, Japan) in the 200–800 nm range (Jihad *et al.*, 2021).

FTIR

Fourier transforms IR spectroscopy analysis FTIR instrument (a Shimadzu Instrument, Japan) was utilized to examine the samples over the wavelength range of 400–4000 cm^1 to determine the nature and structure of different functional groups of the bioactive compounds present in the crude extract (Batool *et al.*, 2019).

Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

The samples were subjected to pyrolysis coupled with gas chromatography and mass spectrometry. The analyses were carried out with a CDS analytical ion source 200 ◦C, detector 250 ◦C (YL6900GC/MS, Korea). The temperature of the samples was raised to 600 ◦C for 15 s with a heating rate of 20 ◦C/ms. The effluents produced are driven out of the interface by a carrier gas (helium) to GC; the solvent used is deionized water.

Bioactivity of *C. spinosa* L. Fruit extract.

An inhibitor effect on Lactate dehydrogenase

Estimation of LDH enzyme activity was evaluated following the method described by the instructions from manufacturers (BIOLABO SAS, France) using tris pH 7.2 (80mmol/L) as a buffer, pyruvate (1.6 mmol\L) as a substrate NADH (0.2 mmol\L) as coenzyme (Adler *et al.*, 2019). Enzyme activity is specificated as the amount of enzyme that produces 1 µM of lactate per minute at 37 °C. Diluted solutions (75, 100, 125, 160, 210) ppm of *C. spinose L.* fruit extract, were added as inhibitors of LDH enzyme according to (Rosado *et al.*,1969). 1 ml of the substrate was put in a water bath for 5 min at 37 C, then 20 µl was added from both the inhibitor and pool of serum (from patients with hereditary hemolytic diseases). The activity was measured after 30 seconds, 1 and 2min at 340 nm, Residual activity was estimated by the equation (1).

$$UI\backslash L = \left(\frac{\Delta Abs}{min}\right) x\ 8095 \qquad \text{.....(1)}$$

While the % inhibition was measured by equation (2).

$$\%\ \text{inhibition} = 100 - (\text{Activity UI\L with inhibitors} / \text{Activity UI\L without inhibitors}\ \ x\ 100$$

…….. (2) (Hernández-Meza *et al.*, 2018)

Anti-bacterial Activity Testing

In this study, two types of standard bacterial strains were used, *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25923) as positive and negative gram stains respectively which were provided by the American Type Culture Collection (ATCC). The culture media was prepared with nutrient agar (NA), nutrient broth (NB), and agar Müller-Hinton agar (MHA) using the wells method. Preparation of bacterial colonization in one colony whose age is not above 24 hours, grown on brain heart infusion agar, transported by a loop from nutrient agar to the plate, the plates were placed in the Incubator at (37°C) for 24 hours. The sterile tips (5 mm) in diameter were used to make 2 wells placed on Muller Hinton Agar (MHA). Plates were spread of one colony carried by swab from "fresh overnight cultures" to the Muller Hinton Agar (MHA) plate by a loop. 4 holes with a diameter of 6 mm were filled with 200, 100, and 50 µg mL-1 of C. spinosa fruit extract. A blank well was carried out by adding solvent alone (distilled water) to act as a negative control. After an incubation period under 37 °C for 24 hr., growth inhibition zones were measured. All of the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Phytochemical Screening

The preliminary examination data of *C. spinosa* L aqueous fruit extract was shown in (Table 1). We proceeded to a qualitative chemical screening to identify potential bio-active chemical classes present in C. spinosa L aqueous fruit extract. As depicted in (Table 1), we found that our extract contained: tannins, sterols, alkaloids, polyphenols, and flavonoids.

Table 1: Chemical components of the *C. spinosa* L. Fruit aqueous extract.

Chemical Tests	Aqueous Extract
Saponosides	+
Phenols	+
Flavonoids	+
Tannins	+
Alkaloids	+
Sterols	+
Triterpens	-

+ = Positive result, - =Negative result.

Characterization of Aqueous Extract

UV–Vis Spectroscopy

The UV-Vis spectrum analysis was recorded in Fig. (1) for the prepared aqueous extract at 200-600 nm to show the electronic transitions in active compounds. It was observed that there is no absorption band in the visible region. At the same time, the U.V spectrum had an absorption band at 290 nm that relates to the $n \rightarrow \pi^*$ related to CH=CHCOOH electronic transitions when ϵ=100 mol\cm.

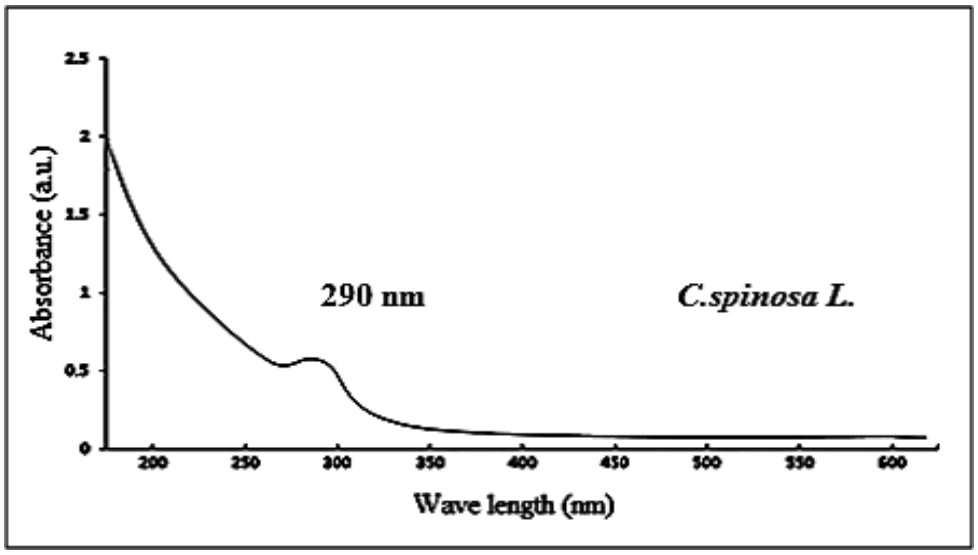

Fig. 1: U.V-Visible spectrum of *C. spinosa* L. aqueous fruit extract [11].

FTIR

The infrared (IR) spectra supported the results obtained from (Table 2), by giving stretching vibration frequency and types of bands in Fig. (2) corresponding to several active Functional groups present in *C. spinosae* L. aqueous fruit extract compounds: as shown in (Table 2).

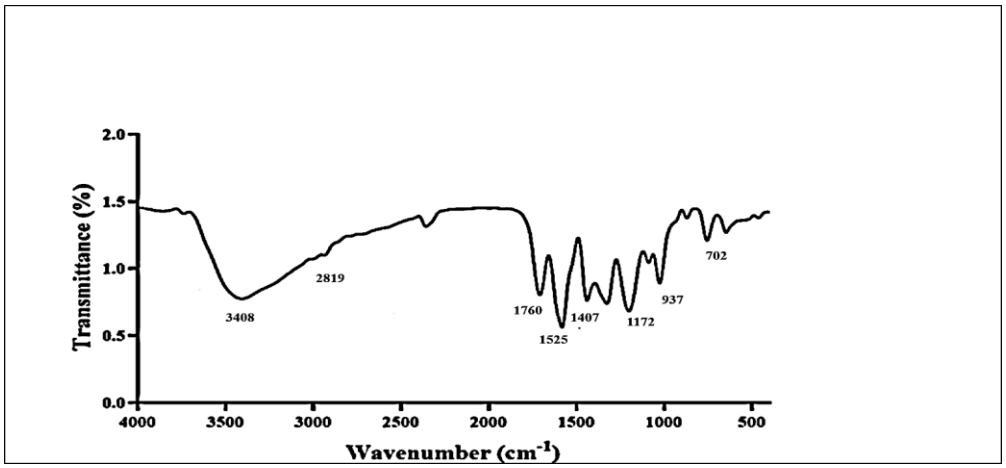

Fig. 2: IR spectrum of *C. spinosa* L. aqueous fruit extract [11].

Table 2: Infrared spectrum results for *C. spinosa* L aqueous fruit extract, its absorption bands, and their functional group.

Functional group	Bond	Bond shape	Bond Frequency cm^{-1}
Phenolic, alcoholic, Amines	O-H, N-H	B.	3408
C-H sympatric of aliphatic CH_3	C-H	w-Sh	2819
C=O stretch Carbonyl group	C=O	m-Sh	1760
Ring stretch, sharp band	Benzene ring in aromatic compounds	S-Sh	1525
In-plane O-H bending, N-H bending	O-H in carboxylic acid derivatives, N-H aromatic compensators, N-H heterocyclic	m-sh	1407
C-O-C stretch in Asy.	Ar-O in alkyl aryl ether	m-sh	1172
C-O-C stretch in sym.	C-O-C	m-sh	937
O-H deformation	Ar-OH in phenol	w-sh	702

B-Broad; m-medium; S-strong; W-weak; Sh-sharp

GC-MS

The results obtained from (Table 1) agree with those given during the mass spectrum analysis in (Table 3) and Fig. (3). The availability of compounds with chemical plant origin, chemical name, Peak, Retention time, and Area percentage were shown, each according to the molecular weight during the test.

Table 3: The main chemical composition of *C. spinosa* L. aqueous fruit extract

NO.	Name	RT	Area%	M. wt	Chemical formula	Classification
1	N-Nitroso pi peridine	3.548	34.24	114	C5H10N2O	Alkaloid
2	3-Methyl-1-butanol	3.749	35.01	88	C5H12O	Alcohol
3	2,2'-Di hydroxyl-4',6'-di methoxy chalcone	4.102	45.75	300	C17H16O5	Poly phenols
4	Antibiotic k25 2b	4.952	7.88	453	C26H19N3O5	Alkaloid
5	2-Docosa hexaenoyl-1-stearoyl-sn-glycero-3-pho	5.091	7.55	791	C45H78NO8P	Sterols
6	Glafenin	5.208	11.61	372	C19H17ClN2O4	An anthranilic acid derivative
7	Colchicine	5.658	6.25	399	C22H25NO5	Alkaloid
8	Acetildenafil	6.038	5.18	466	C25H34N6O3	Alkaloid
9	7,8-Dihydro-1-Biopterin	6.848	4.34	239	C9H13N5O3	Alkaloid
10	Rhodamine 6G Cation	7.491	13.45	443	C28H31N2O3	Alkaloid
11	Buprenorphine glucuronide	7.761	5.35	643	C35H49NO10	Alkaloid
12	Hepta Carboxy porphyrin 1	9.172	32.82	786	C39H38N4O14	Alkaloid
13	(6S)-5-Methyl tetra-hydro folic acid	10.078	3.92	459	C20H25N7O6	Alkaloid
14	Lutein	10.85	29.04	568	C40H56O2	Flavonoids
15	6,7-Di Methyl tetra hydropterin	11.95	10.78	195	C8H13N2O	Alkaloid

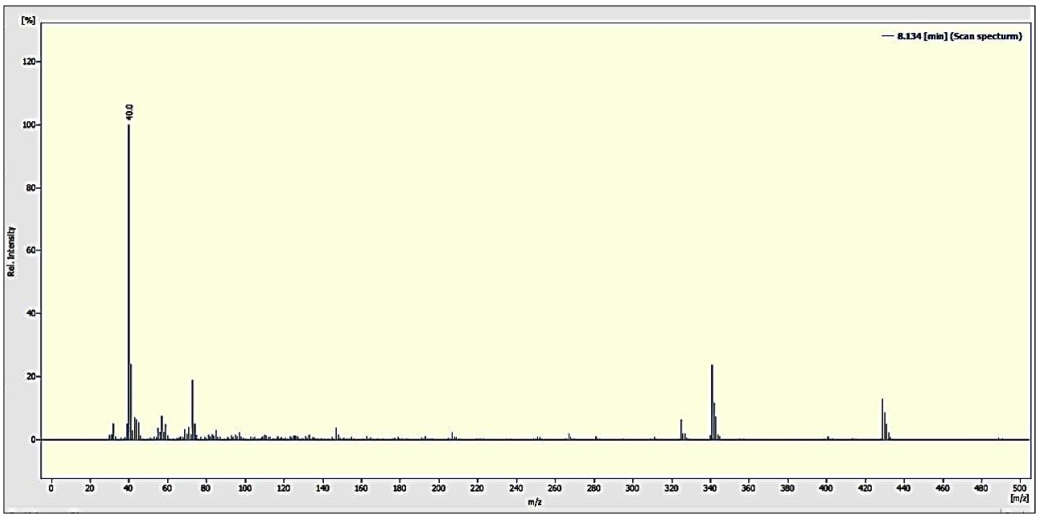

Fig. 3: GC-MS spectrum of *C. spinosa* L. aqueous fruit extract

Bioactivity of *C. spinosa* L**. Fruit Extract.**

An inhibitor effect on Lactate dehydrogenase enzyme

Fig. (4) shows the % inhibition activity of (LDH) enzyme estimated with 371UI\L using an effect of C. spinosa L. Fruit extract at five diluted concentrations. In addition, Fig. (5) shows a drawing of the line Weaver Burk plot) the equation to LDH enzyme in the serum of hereditary hemolytic patients. The values of both V_{max} and k_m without inhibitor effect were (333.3, 0.16) respectively, while the values of V_{max} and k_m with inhibitor effect of *C. spinosa* L. Fruit extract were (333.3, 0.13). Therefore, these data lead us to the type of inhibitor: competitive inhibition because of hydrogen bonding between the active site and a functional group in aqueous fruit, extract compounds.

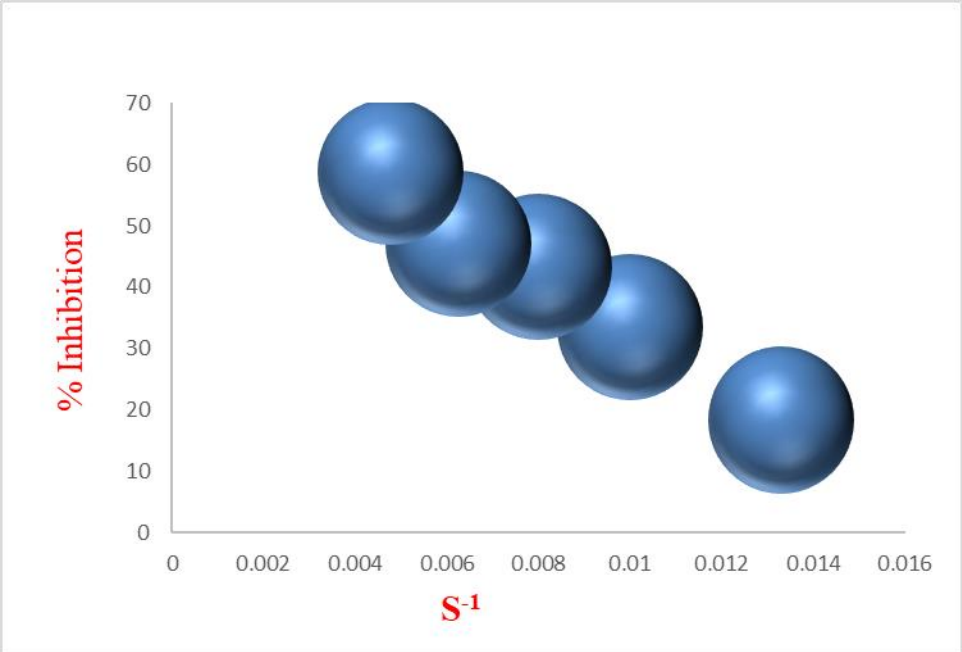

Fig. 4: Effective LDH- inhibition by *C. spinosa* L. Fruit extract.

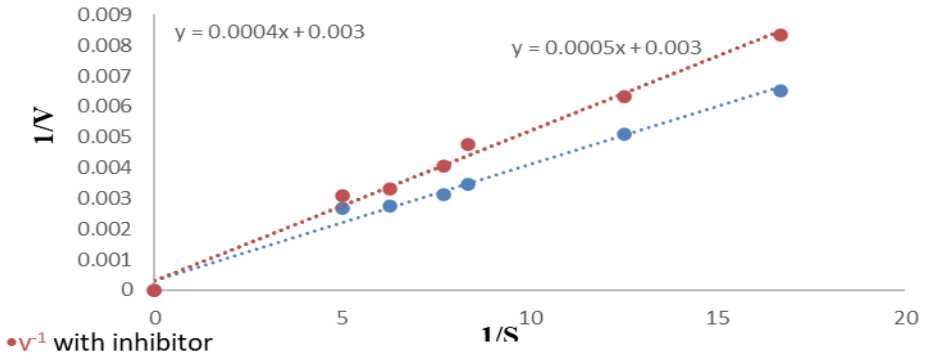

Fig. 5: Line Weaver Burk plot equation of LDH with and without the inhibitory effect of *C. spinosa* L. Fruit extract.

Anti-bacterial activity of *C. spinosa* L. Fruit extract

Biological activity against bacterial growth was assessed by measuring the diameter of the growth-inhibition zone, using *C. spinosa* L. extract. The results of an inhibition effect on tested bacteria are illustrated in (Table 4). A better zone of inhibition was recorded for the extract of *C. spinosa* L. against *S. aureus* (17.1 mm at 200 mg/ml concentration). On the other hand, the growth of *E. coli* was also negatively inhibited by extract, (15.2 mm at 200 mg/ml concentration). The findings suggest that *C. spinosa* L. fruit extract can be utilized to avoid the spread of bacteria including *S. aureus* and *E. coli*, which are associated with serious health hazards and cause foodborne illnesses. The findings agree with those of the antibacterial activity of the Abelmoschus esculentus Pods Aqueous Extract, which was previously reported by (Khan *et al.*, 2022). In a related study, the maximum antibacterial activity of an aqueous extract of *C. spinosa* L. against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* was evaluated (Adwan *et al.*, 2021). Most studies and research focus on the roots, leaves, and seeds of *C. spinosa* L. extracts, and there are a few studies on the biological effect of its fruit. This plant has many medicinal uses such as antirheumatic, diuretic, astringent, tonic, antidiarrheal, febrifuge, gout, sciatica, epilepsy, feminine sterility, dysmenorrheal, toothache, headache, ulcers, scrofula, ganglions, expectorant, hemorrhoids, chest, and spleen disease (Rahnavard *et al.*,2017). *C. spinosa* is rich in nutrients, alkaloids, and flavonoids such as Lutein which increase efficacyAntioxidant enzymes also reduce the effectiveness of liver enzymes (Zhang *et al.*, 2018; Tlili *et al.*, 2010) and it is important in different metabolic processes in the human body.

The possession of the *C. spinosa* L. Fruit extract has an effect on killing germs, due to it possessing phenolic compounds and tannins, the ability of tannins to form strong complexes with proteins is the most important aspect of their nutritional and toxicological effects that precipitate the proteins of the microorganism due to the formation of hydrogen bonds between the aromatic hydroxyl groups and proteins, which will lead to the inhibition of enzymes necessary for the metabolism of microorganisms (Jihad *et al.*, 2021; Alyamani *et al.*,2021; Al-Taei *et al.*, 2023) Besides the presence of the active compounds and their active groups allowed the antibacterial activities and inhibition enzyme taken in the current study. Also, it is known that alkaloid compounds have a scavenging effect on bacterial growth (Boga *et al.*,2011; Shareef *et al.*,2021).

Table 4: Antimicrobial activity of crude extract of c. myxa fruit

Bacteria	**Zone of Inhibition (diameter in mm)**		
	***C.spinosa* L. fruit Extract (mg/mL)**		
	50	100	200
S. aureus	14.6 ± 1.0	16.2 ± 1.5	17.1 ± 1.0
E. coli	12.8 ± 1.5	13.5 ± 0.5	15.2 ± 1.0

CONCLUSION

The fruit of *C. spinosa* L. was successfully extracted, and its phytochemical components were assessed using GC-MASS analysis, LDH enzyme inhibitors, and antimicrobial investigation of the extract. The conventional method also detected the presence of phytochemicals, and GCMSS evaluated the aqueous extract of *C. spinosa* L. fruit's chemical composition. 15 phytochemicals in the aqueous extract of *C.spinosa* fruits cultivated in the southern parts of Iraq were identified using GC-MS. The inclusion of active substances like polyphenols or their analogs may be the cause of the aqueous extract's apparent antibacterial effects.

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التحاليل اللونية الكيميائية، التشخيصية والنشاط الحيوي لمستخلص فاكهة كاباريس سبينوزا L

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الملخص

يعتبر الطب البديل والعلاج بالأعشاب من بين الأساليب الموروثة من قبل شعوب الشرق الأوسط. في الدراسة الحالية، قمنا بتقييم التركيب الكيميائي لثمار: كاباريس سبينوزا L، وكذلك التأثيرات المضادة للبكتيريا والتأثيرات المثبطة المحتملة لإنزيم نازعة الهيدروجين (LDH). تم تأكيد وجود المواد الكيميائية النشطة في المستخلص من خلال تقنيات الفحص والتوصيف الكيميائي النباتي مثل تحليل كروماتوجرافيا الغاز والكتلة الطيفية (GC-MS)، والتحليل الطيفي فوق البنفسجي (UV-Vis)، والتحليل الطيفي بالأشعة تحت الحمراء (FTIR). أظهرت النتائج وجود قلويدات، فلافونويد، بوليفينول، الاعفاص، وفيتامينات في المستخلص المائي. علاوة على ذلك، أظهر المستخلص نشاطًا مضادًا للبكتيريا خاصة ضد البكتيريا سالبة الجرام. في المقابل، امتلكت تركيزات المستخلص المائي نشاط تثبيط إنزيم ديهيدروجينيز بتركيز 0.13 ملي لتر/ لتر. خلصت الدراسة إلى أن المستخلص المائي لفاكهة C.spinose L. يحتوي على مركبات نشطة بيولوجيا يمكن استخدامها في عمليات تثبيط كل من مثبطات الإنزيم المضاد للبكتيريا ونازع هيدروجين اللاكتات (LDH).

الكلمات الدالة: كاباريس سبينوزا L، مادة كيميائية نباتية، مثبط للأنزيمات، ونازع هيدروجين اللاكتات.