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Study Antibacterial Activity of Crude *Capparis spinosa L.* Extracts Against *Helicobacter pylori* Infection and Determine Their Bioactive Compounds

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Abstract

The antibacterial activity of *Capparis spinosa* L. extracts obtained from Baghdad was evaluated against six clinical bacteria isolates of *Helicobacter pylori*. The results presented in this work demonstrated that the leaves extract showed a significant effect against bacteria as compared to the root and fruit extracts at 100 mg/ml concentration, with inhibition zone ranging from 12.0 to 30.7 mm in each bacteria strain. The end results of GC-MS analysis indicated that the ethanol extracts of caper have a lot of active chemical compounds, including twenty-five, eighty-two and sixty-eight phytoconstituent compounds, that are distinguished in the extracts of roots, leaves and fruits with *C. spinosa* L. respectively. In addition, the high area % revealed in each extracts were: 1-methyl-pyrrolidine-2-carboxylic acid 35.77%, prolin, N-methyl-, butyl ester 12, 63% and (23S)-ethylcholest-5-en-3.beta.-ol 19.12% respectively.

Keywords: *Capparis spinosa*, *Helicobacter pylori*, Ethanol extracts, Antibacterial, Gastric ulcer

دراسة النشاط المضاد للبكتيريا للمستخلصات الخام لنبات الشفلح *Capparis spinosa L* ضد عدوى الملوية البوابية *Helicobacter pylori* وتحديد مركباته النشطة بيولوجيًا

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الخلاصة :

تم تقييم النشاط المضاد للبكتيريا لمستخلصات نبات *Capparis spinosa* L. والتي تم الحصول عليها من بغداد ضد ستة عزلات من البكتيريا السريرية *Helicobacter pylori*. أظهرت النتائج المقدمة لهذا البحث, أن مستخلص الأوراق له تأثيراً معنوياً ضد سلالات البكتيريا بالمقارنة مع مستخلص الجذور والثمار عند تركيز 100 مجم / مل بقطر تثبيط يتراوح من 12.0 إلى 30.7 مم. في كل سلاله بكتيريا . أشارت النتائج النهائية لتحليل GC–MS إلى أن مستخلصات الإيثانول في نبات الكبر تحتوي على الكثير من المركبات الكيميائية النشطة المتضمنة خمسة وعشرون ، اثنان وثمانين وثمانية وستون مركبًا نباتيًا ، والتي

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يمكن تمييزها في مستخلصات الجذوروالأوراق والثمار *C. spinosa* على التوالي. بالإضافة إلى ذلك ، فأن اكبر مساحة مئوية للمركبات الفعالة تم الكشف عنها في كل مستخلص وهي:حمض 1- ميثيل-بيروليدين-2-كربوكسيليك 35.77٪ ، برولين ، N- ميثيل- ، بوتيل إستر 12,63 %و (S23) -إيثيل كوليست-5-إن. -3 بيتا اوول. 19,12٪ على التوالي.

1. Introduction

Over the past few decades, there has been a clear interest in medicinal plants due to them being an easily available source of traditional medicine as well as being useful in the treatment of numerous diseases with fewer side effects [1]. Several diseases of the digestive system, that are caused by *Helicobacter pylori*, are considered to be a serious reason of infection globally such as gastritis, peptic ulcer disease and gastric carcinoma [2]. Based on the results of epidemiological studies demonstrating its ability to induce carcinogenesis without the referee of co-carcinogens, the World Health Organization (WHO) considers this pathogen as a class I carcinogen for gastric cancer [3]. Several approaches have been depended on to prevent the growth of *H. pylori* with less side effects [4, 5]. Perennial plant *Capparis spinosa* (Capparidaceae) is a xerophytes deciduous plant that is able to grow in a broad range of conditions, varying from frozen to dry deserts. *C. spinosa* is used in folk medicine to treat different liver diseases, diabetes, urinary incontinence and rheumatic infection [5, 6, 7]. Various medical and health benefit effects of the chemical and bioactive components of *C. spinosa* against numerous diseases, have been extensively investigated and mentioned in various analytical studies as being antioxidant and anti-inflammatory [7, 8, 9]. The current study aims to evaluate crude ethanolic extracts activity from various parts of medical plant *C. spinosa* (root, leaf and the fruit), against *H. pylori*.

2. Materials and Methods:

2.1 Collection and Preparation of Plant:

Capparis spinosa specimens were manually collected from Baghdad University, Iraq and then stored in plastic bags before their transportation to the laboratory. Location position of the source was longitude 33°01'.94"E and latitude 44°20'.41"N during September 2020. *C. spinosa* samples were washed with tap water to remove the dust and then dried for three days in the shade, continuously flipping over from time to time. Preparation of hot ethanolic extracts was done according to [10]. In this process, full 20 g dry powder of each plant part, prepared by using Soxhlet apparatus, was put in thimble. All extracts were kept in serial tube at 4^{o}C until used.

2.2 Phytochemical Screening by GC-MS Analysis:

5 µl of the caper root, leaf and fruit ethanol extracts were employed for GC-MS analysis by Agilent Technologies (SHIMADZU Japan), by employing a high temperature column. Helium was the carrier gas at a flow rate of 1.0 mL.min-1 and a split ratio of 1:10, the temperature program. The injector and detector temperatures were set at 280°C while the initial column temperature was set at 70°C. A 5 µl sample volume was injected into the column and ran using split (1:10) mode. After 2 min, the oven temperature was raised to 110°C at a ramp rate of 5°C/min (hold time 9 min). The oven temperature was then raised to 280°C at a ramp rate of 7.5°C/min (hold time 9 min). The compounds were identified by comparing their mass authentic standards [11].

2.3 Preparation of Bacterial Suspensions:

Six pathogenic bacteria of *H. pylori* isolates were obtained from postgraduate laboratories in Department of Biology, College of Science, University of Baghdad. Each strain was

identified in the biology department laboratory. In order to hide the identity of the patients from whom the samples were obtained, clinical isolates were coded with the number of access: Hp7, HP8, HP9, Hp10, Hp11 and Hp12. Inoculum of each fresh colonies were transferred into tubes containing sterile physiological saline solution while adjusting the turbidity to the 2.0 McFarland standards [12]. This turbidity produced a suspension that corresponded to approximate turbidity of (1.5×10^8) CFU/mL of *H. pylori*. All strains were previously evaluated against commonly used antibiotics in a conventional therapy: ciprofloxacin (10 μg), imipenem (10 μg), amoxicillin (25 μg), levofloxacin (5 μg), tobramycin (10 μg), trimethoprim + sulfamethoxazole (25 μg) and cephalothin (30 μg).

2.4 Screening of Antibacterial Activity by Using Well Diffusion Methods:

Each plant extract was prepared by dissolving it in DMSO solution to obtain four sets of dilution (100, 50, 25 and 12.5 mg/ml). The surface inoculation of Mueller Hinton Agar to produce bacteria isolates was done by using sterile swab from a 24-hour-old culture containing (1.5×10^8 CFU). The plates were then left to dry at room temperature. Five holes were made in the culture medium by using a sterile cork-borer with 5 mm diameter, where each hole contained one of concentration of extract. The last hole used DMSO as control. The plates were incubated at 37°C for 24 hours in anaerobic condition. It was followed by the calculation of the zone of inhibition (mm). The extract was diffused in the agar medium which inhibited the growth of the microbial strain tested [13].

2.5 Statistical Analysis

To compare different groups with one another, the acquired data was subjected to one way analysis of variance (ANOVA) test. Findings were expressed as mean + standard DEV(SD) and values of $p>0.05$ were regarded as statically non-significant. Whilst $p<0.05$ and <0.01, 0.001, 0.0001 values were regarded as significantly different, highly significantly different successively.

3. Results and Discussion:

Recent study was conducted to emulate the effects of crude alcoholic extract of medical plant *C. spinosa*. All 6 bacterial strains showed resistance to antibiotic trimethoprim +sulfamethoxazole, amoxicillin, levofloxacin and cephalothin as seen in Table 1. While antibiotic tobramycin and ciprofloxacin showed 66.6% and 83.3% resistance respectively. Unfortunately, frequent and inappropriate use of antibiotics can cause bacterial resistance [14, 15]. Interestingly, the antibiotic imipenem showed higher sensitivity in each bacterial isolate. Due to imipenem formula $C_{12}H_{17}N_3O_4S$ which is a beta-lactam, semisynthetic thienamycin of the carbapenems class with a broad spectrum of antibacterial activity. The mechanism of action reason cell wall analysis or interference with cell wall synthesis in gram-negative bacteria was attained by binding to penicillin-binding proteins (PBPs) [3, 16, 17].

Table 1: Antimicrobial susceptibility to antibiotic for *H. pylori*

NO.	Bacteria Strains	Antibiotics						
		(TN)	(CIP)	(IPE)	(STX)	(AX)	(LEV)	(KF)
1.	Hp. 7	S	S	S	I	R	R	R
2.	Hp. 8	S	R	S	R	R	R	R
3.	Hp. 9	R	R	S	R	R	R	R
4.	Hp. 10	R	R	S	R	R	R	R
5.	Hp. 11	R	R	S	R	R	R	R
6.	Hp.12	R	R	S	R	R	R	R
%Resistant Bacteria		66.6	83.3	0.00	100	100	100	100

The diameters of the inhibition zones were interpreted according to CLSI (2016). The examined isolates were reported as *R* resistant, *I* intermediate, *S* sensitive, (TN) Tobramycin (10), (CIP) Ciprofloxacin (10), (IPE) Imipenem (10), (STX) Trimethoprim +sulfamethoxazole (25), (AX) Amoxicillin (25), (LEV) Levofloxacin (5), (KF) Cephalothin (30).

Clearly, increasing the concentration of the extracts of roots, leaves and fruits increased the efficiency of the inhibition which could be due to an increase in the concentration of the active compounds found in it. The statistical analysis showed significant differences at the P <0.01 in inhibiting the growth of bacterial isolates that were used in this study. When using ethanol extraction of root, leaves and fruits at 100 mg/ml, it showed extreme efficiency in inhibiting the growth of bacterial strain Hp. 8. The inhibition zones were: 28.3, 30.7 and 29.3 respectively. Generally, Hp.11 is considered to be extremely high virulence bacterial strain as seen in Table 2 when compared to the other strains, especially when using leaves and fruits extracts at 50 mg/ml. Numerous certain conditions make *H. pylori* transform from spiral-shaped into coccid form including: nutrient deprivation, exposure to anti-ulcer drugs and antibiotics and old culture. Due to the diversity and complexity of the natural mixtures of bioactive compounds in crude plants extract, it is rather hard to check and characterize all compounds present and elucidate their structure in a single study. As a result, it became clear that the roots, fruits and leaves of caper have a great significance and have a major number of available chemical compounds that might be valuable and numerous sources of starting materials for the synthesis of new antibacterial agents against *H. pylori* [18, 19, 21]**.**

Numerous studies mention that various flavonoids have been identified in the caper shrub such as: rutin (quercetin 3-rutinside), quercetin 7-rutinside, quercetin 3-glucoside-7-raminoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-3-rutinoside. Rutin is a powerful bioflavonoid antioxidant in the body that is used as a dietary supplement for capillary fragility. Rutin has no known toxicity [20, 21, 22]. Capers have more quercetin per weight than any other plant. It is extensively found in the bark of the trees and leaves and is considered as anticancer agent. Various studies confirm that the caper extracts exhibit a rich source of antioxidants such as flavonoids and phenolic acids, with the presence of rutin and quercetin in considerably high amounts [23, 24, 25, 26].

Table 2: Mean ± Std. Error of ethanolic *C. spinosa* extracts at various concentrations against *H. pylori* isolates.

Ethanol Extract of Root/Cons (mg/ml).									
Isolate no.	**12.5 Mean±SD**		**25 Mean±SD**		**50 Mean±SD**		**100 Mean±SD**		**P Value**
Hp7	22.0	5.2	19.3	4.9	21.0	1.7	23.3	2.4	**NS**
Hp8	C 20.3	2.9	B 23.3	2.9	B 24.7	2.5	A 28.3	2.4	**0.04**
Hp 9	C 11.7	1.2	C 12.3	1.2	B 14.7	1.5	A 16.0	0.8	**0.05**
Hp 10	11.0	0.0	10.5	0.7	11.0	1.7	12.0	0.8	**NS**
Hp 11	11.0	0.0	11.0	1.0	12.0	1.7	12.0	1.4	**NS**
Hp 12	21.0	3.5	15.7	3.1	13.7	2.3	14.3	2.5	**NS**
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**-----**
Ethanol Extraction of Leaves/Cons.									
Isolate no.	**12.5 Mean ± SD**		**25 Mean ± SD**		**50 Mean ± SD**		**100 Mean ± SD**		**P Value**
Hp7	D 10.7	0.6	C 15.0	4.4	B 20.0	3.6	*A 23.3	2.4	**0.01**
Hp8	B 24.3	1.2	B 25.7	1.2	A 29.0	1.7	A 30.7	0.9	**0.01**
Hp 9	D 11.0	0.0	C 18.0	3.5	B 21.7	5.8	A 25.0	2.8	**0.01**
Hp 10	10.0	0.0	11.0	0.0	0	0	0	0	**NS**

Hp 11	0	0	0	0	0	0	0	0	----
Hp 12	C 11.0	0.0	B 14.0	0.0	A 17.3	1.5	A 18.7	1.9	**0.01**
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-----

Ethanol Extraction of Fruit/Cons.

Isolate no.	**12.5 Mean±SD**		**25 Mean±SD**		**50 Mean±SD**		**100 Mean±SD**		**P Value**
Hp7	19.0	5.2	19.0	1.7	23.3	2.9	25.3	3.1	**NS**
Hp8	26.0	1.0	27.3	0.6	27.7	0.6	29.3	0.9	**NS**
Hp 9	15.0	0.0	18.0	1.0	19.3	0.6	21.0	0.8	**NS**
Hp 10	C 10.3	0.6	B 15.0	0.0	A 18.0	1.0	A 20.3	0.5	**0.03**
Hp 11	13.0	2.6	13.3	2.9	0	0	0.0	0.0	**NS**
Hp 12	19.0	2.6	0	0	0	0	19.7	3.3	**NS**
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-----

The results of the GC/MS analysis of the composition identified from root, leaves and fruits extract of *C. spinosa*, their retention indexes and percentage composition are summarized in Table 3. Twenty-five, eighty-two and sixty-eight peaks were found in the ethanolic extracts of root, leaf and fruit respectively. It constituted 95.61%, 72.02% and 67.5 % of the area percentage of the extracts respectively as shown in Table 3. Figure 1 shows that the root extract containing at least 6 peaks with percentage area more than 5% were: Pyrrolidine-5-one, 2-[3-hydroxypropyl] 5.34%, 1-Methyl-pyrrolidine-2-carboxylic acid 35.77%, Phytol5.63%, adipic acid, bis(2-ethylhexyl) ester 7.81% and (23S)-ethylcholest-5-en-3.beta.-ol 10.21%. While leaves extract containing at least 4 peaks more than 5% were: Prolin, N-methyl-, butyl ester12.63%, n-Hexadecanoic acid 9.01%, Heptadecane 5.70% and (23S)-ethylcholest-5-en-3.beta.-ol 6.95%. Finally, fruit extract containing at least 5 peaks more than 5% were: N-Methyl-L-prolinol 6.34%, 4,7-Methano-1H-inden-1-one, 2,3,3a,4,7,7a-hexahydro 9.93%, n-Hexadecanoic acid 5.67%, Disooctyl adipate 7.47% and (23S)-ethylcholest-5-en-3.beta.-ol 19.12%. Zhang and Ma [9] have recorded that caper possesses some therapeutic and antioxidant properties and is used as aromatic plant in Mediterranean cooking by investigation of the flavor profile [27]. It must be mentioned that the type of solvent used in this study played an important role in collecting the major amount of active compounds in each plant extract. Clinical and Laboratory Standards Institute [11] reports that major values of antioxidant activity have been obtained by using an ethanolic solvent to extract the bioactive compounds as compared with other types of solvents [7, 22, 23]. However, these findings are still in early stages and future experimental and clinical studies are subsequently needed in this kind of GC-MS investigation. It is an initial move towards recognizing the way of dynamic standards in this medicinal field for further point by point study. Tlili [28] showed that 1-Methyl-pyrrolidine-2-ca plant and this sort of project will be useful in producing rboxylic acid which has very promising antitumor properties of organizing (IV) compounds against a wide panel of tumor cell lines of human origin. It is a heterocyclic compound also called azaindoles

Among the identified bioactive components, two chemical compounds are common between all extracts, namely; Hexadecanoic acid that has antioxidant [8 ,9] and (23S)-ethylcholest-5-en-3.beta.-ol. While 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) have anti-inflammatory, cancer preventive, hepatoprotective, antioxidant, and hypocholesterolemic properties [23] and Cholestan-3-amine, N,N,4,4-tetramethyl-, (3.beta.,5.alpha.) are found both in leaves and root extracts. Finally, mitin and 4,7-Methano-1H-inden-1-one, 2,3,3a,4,7,7a-hexahydro can be found both in the leaves and fruit.

Ultimately, numerous phenolic compounds, esters, alkanes, aldehydes, alkenes, and ketones are the other major volatile compounds present that have antiulcer, anti-inflammatory, anti-arthritic, antidiabetic, hypolipidemic and cytotoxic properties [21, 22, 29]. Phytol was reported with antioxidant and neuroprotective, antimicrobial, anticancer, anti-inflammatory and anti-diuretic activities [29, 30]. 9,12- Octadecadienoic acid, methyl ester play an important role as having anti-inflammatory, anti-arthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, nematicide, 5-alpha-reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic and antiacne properties [27, 28]. Palmitate is considered as antioxidant, hypocholesterolemic, nematicide, flavoring agent, hemolytic and 5-alpha-reductase inhibitor [21, 23, 31].

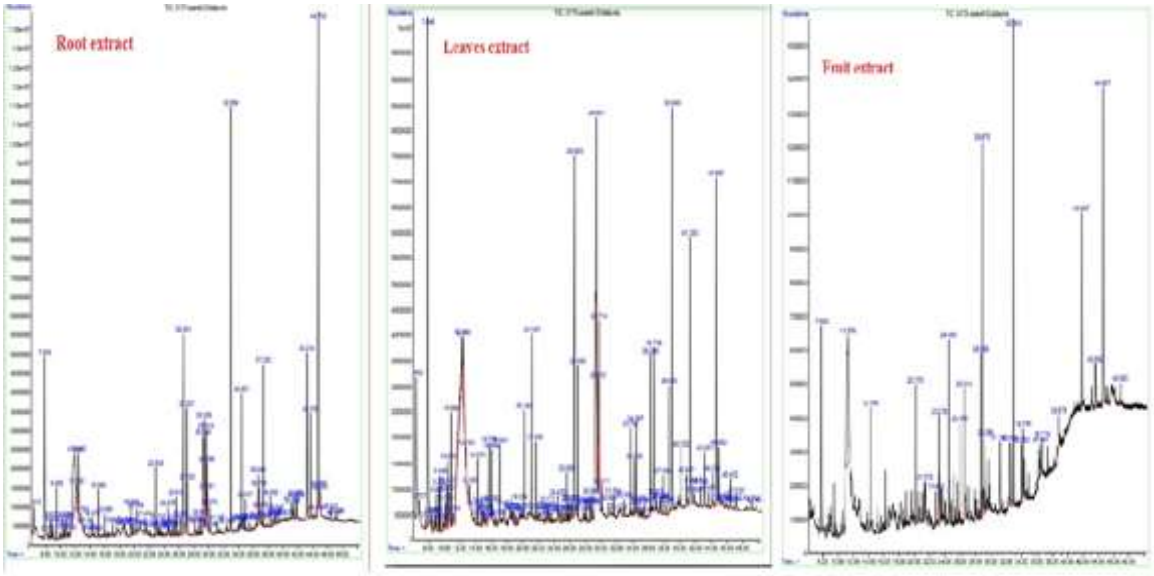

Figure 1: The main chemical compounds of hot ethanoic extract of *C. spanosa* by using GC-mass.

Table 3: The major compounds that are found in various parts of hot ethanoic extract of *C. spinosa* by using GC-mass.

Root Extract

Peak#	RT (min)	Area%	Name	M.W. g/mol	fFrmula
1	7.82	5.34	Pyrrolidine-5-one, 2-[3-hydroxypropyl]	143.18	$C_7H_{13}NO_2$
2	11.27	35.77	1-Methyl-pyrrolidine-2-carboxylic acid	129.16	$C_6H_{11}NO_2$
3	14.264	2.12	2-Methoxy-4-vinylphenol	150.17	$C_9H_{10}O_2$
4	20.169	2.43	Cholestan-3-amine, N,N,4,4-tetramethyl-, (3.beta.,5.alpha.)	443.8	$C_{31}H_{57}N$
7	23.277	1.16	Benzyl benzoate	212.24	$C_{14}H_{12}O_2$
8	24.47	2.35	Neophytadiene	278.5	$C_{20}H_{38}$
9	25.918	1.34	Hexadecanoic acid, methyl ester	270.5	$C_{17}H_{34}O_2$
10	26.515	2.13	Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$

11	28.694	2.59	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292.46	$C_{19}H_{32}O_2$
12	28.875	5.63	Phytol	296.5	$C_{20}H_{40}O$
13	29.291	1.94	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	264.4	$C_{18}H_{32}O$
14	31.143	2.13	4-BENZYL-3-OXAZOLIN-5-ONE	249.26	$C_{16}H_{11}NO_2$
15	32.508	1.91	cis-6-Phenyl-5-hexenal	174.24	$C_{12}H_{14}O$
16	32.943	7.81	Adipic acid, bis(2-ethylhexyl) ester	370.6	$C_{22}H_{42}O_4$
17	33.991	1.35	1H-Indole, 2-methyl-	131.17	C_9H_9N
18	34.215	1.49	Butane, 2-phenyl-3-(trimethylsilyloxy)-	222.40	$C_{13}H_{22}OSi$
20	36.736	1.09	1,1,1,3,5,5,5-Heptamethyltrisiloxane	222.50	$[(CH_3)_3SiO]_2SiHCH_3$
21	38.874	1.28	Cyclotrisiloxane, hexamethyl-	222.46	$C_6H_{18}O_3Si_3$
22	41.847	4.43	Vitamin E	430.71	$C_{29}H_{50}O_2$
24	44.649	10.21	(23S)-ethylcholest-5-en-3.beta.-ol	414.7	$C_{29}H_{50}O$
25	46.927	1.02 **95.6%**	Gibberellin A3	346.37	$C_{19}H_{22}O_6$

Leaves Extract

Peak#	RT (min)	Area%	Name	M.W.	Formula
1	6.403	4.23	Ethanol, 2,2-diethoxy-	134.17	C6H14O3
4	7.851	12.63	Prolin, N-methyl-, butyl ester	185.26	C10H19NO2
15	10.886	1.46	1,3-Disilacyclobutane, 1,1,3,3-tetramethyl-	144.36	C6H16Si2
17	12.189	3.22	4,7-Methano-1H-inden-1-one, 2,3,3a,4,7,7a-hexahydro-	148.20	C10H12O
18	12.407	2.58	2-propyl cyclopentanone	126.20	C8H14O
28	15.753	2.07	DL-Proline, 5-oxo-, methyl ester	143.14	C6H9NO
40	20.184	1.01	Cholestan-3-amine, N,N,4,4-tetramethyl-, (3.beta.,5.alpha.)-	443.8	C31H57N
41	21.186	2.29	(4-Hydroxy-3-methoxyphenyl) ethyl methyl ketone	194.23	C11H14O3
52	26.624	9.01	n-Hexadecanoic acid	256.42	C16H32O2
53	27.023	1.42	Hexadecanoic acid, ethyl ester	284.5	C18H36O2
58	29.405	4.38	9,12,15-Octadecatrien-1-ol, (Z, Z, Z)-	264.4	C18H32O
60	29.716	2.12	9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)-	306.5	C20H34O2
67	34.396	1.10	Palmitin	330.5	C19H38O4

73	36.736	3.31	Pentacosane	352.7	C25H52
75	38.635	1.64	Hexacosane	366.7	C26H54
76	39.066	5.70	Heptadecane	240.5	C17H36
78	41.302	4.46	Eicosane	282.5	C20H42
79	43.201	1.27	(E)-5,10-secocholest-1(10)-en-3,5-dione	400.6	C27H44O2
81	44.68	6.95	(23S)-ethylcholest-5-en-3.beta.-ol	414.7	C29H50O
82	44.96	1.17	Cyclopenta [a] cyclopropa [2, 3] cyclopenta[1, 2-f] naphthalene, 3,5-cyclopregnan-6-one deriv. (CAS)	316.5	C21H32O2
		72.0%			

Fruit Extract					
Peak#	**RT (min)**	**Area%**	**Name**	**M.W.**	**Formula**
2	7.835	6.34	N-Methyl-L-prolinol	115.17	C6H13NO
12	11.898	9.93	4,7-Methano-1H-inden-1-one, 2,3,3a,4,7,7a-hexahydro-	148.2	C10H12O
28	22.836	1.54	2-Methoxy-3-isopropylpyrazine	152.19	C8H12N2O
36	26.592	5.67	n-Hexadecanoic acid	256.4	C16H32O2
37	27.028	1.76	Ethyl palmitate	284.5	C18H36O2
42	29.233	2.65	9,12-Octadecadienoic acid (Z, Z)-	280.4	C18H32O2
43	29.327	4.14	Octadec-9-enoic acid	282.5	C18H34O2
44	29.612	1.11	Linoleic acid ethyl ester	308.5	C20H36O2
50	32.959	7.47	Diisooctyl adipate	370.6	C22H42O4
51	34.401	2.54	Palmitin,	330.5	C19H38O4
66	43.217	4.96	Campesterol	400.7	C28H48O
68	44.732	19.12	(23S)-ethylcholest-5-en-3.beta.-ol	414.7	C29H50O
		67.5%			

Conclusion:

Data of the recent study expresses that the caper plant extracts are considered eco-friendly with great potential as antibacterial compounds against *H. plori*. Thus, they can be used in gastritis and peptic ulcer diseases treatment. In addition, they have numerous phytochemical compounds with higher biological activity which makes them promising drugs with low chance of bacteria in developing resistance and having less environmental hazards.

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