VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 730-737 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Antimicrobial Effect of *Capparis sepiaria* L. against Some Clinically Important Pathogenic Microorganisms

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Received: 25th June, 2023; **Accepted:** 22nd September, 2023; **Published online:** 29th September, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.082

Abstract: The plants of *Capparis sepiaria* L., which are being used in ayurvedic system of medicine for curing several ailments, have been studied for antimicrobial activities. *Capparis sepiaria* L. leaf and stem powder was extracted in different solvents (water, ethanol, diethyl ether and methanol) separately by using Soxhlet extractor. The antimicrobial activity was screened by well diffusion method at different concentrations (50, 75 and 100 µl) against some clinically important pathogenic microorganisms. All the leaf extracts, most effectively inhibited the gram negative bacteria such as *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas fluorescens* compared to other gram positive bacteria. The leaf water extracts with minimum inhibition were observed against fungal pathogen. The chemical constituents of *Capparis sepiaria L.* was extracted with methanol. The obtained methanol extract from the leaf and stem of *Capparis sepiaria* L. was investigated by a GC-MS technique. A count of about 25 compounds from leaf extract and 35 compounds from the stem of *Capparis sepiaria* L. were identified. It was finally concluded that *Capparis sepiaria* L. could be exploited for new potent antimicrobial agents.

Keywords: *Capparis sepiaria*, Antimicrobial, Well diffusion, Gas Chromatography – Mass Spectrometry, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas fluorescens*

Citation: Kumar M. and Prince L.: Antimicrobial effect of *Capparis sepiaria* L. against some clinically important pathogenic microorganisms. Intern. J. Zool. Invest. 9(2): 730-737, 2023.

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Introduction

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin. Infectious diseases are the world's leading cause of premature deaths, killing almost 50000 people every day. Drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh *et al.,* 1992; Mulligen *et al.,* 1993). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of

antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune-compromised, AIDS and cancer patients (Rinaldi, 1991; Diamond, 1993). Infectious diseases are usually characterized by clear symptoms, so it is likely that traditional healers have been able to recognize such diseases and have developed effective therapies. Moreover, as antibiotics mostly have clear effects, the chance of finding antimicrobially active traditional medicine is considered of more importance (Sofowora, 1984).

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.,* 2008).

Capparis (Capparaceae) is a common Indian angiosperm fern referred in Indian traditional medicine as sedative and anticonvulsant (Goyal *et al.,* 2009). Several species of *Capparaceae* such as *Capparis zeylanica* L. and *Capparis bervispina* have been studied for their cytotoxic, anticonvulsant, antimicrobial (Petkar *et al.,* 2018) and antioxidant activities (Selva Kumar and Ramani, 2020). However, the antimicrobial activity of the *Capparis sepiaria* L. plant has not been thoroughly studied. This study was therefore designed to investigate the antimicrobial activity and chemical constituents of this plant especially against human pathogens.

Materials and Methods

Collection and preparation of plant extract:

The fresh plants of *Capparis sepiaria* L. were collected from the natural habitats of villiage, Tiruvarur district, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water and separated into leaves and stems. The separated plants parts were shade dried and grounded into fine powder. The powdered materials were stored in air tight polythene bags until use.

The powdered materials of the plant (50 g) were extracted successively with 500 ml of different solvents (water, ethanol, diethyl ether and methanol) separately by using soxhlet extractor for 18-20 h. The extracts were concentrated and dried in a rotary evaporator and then placed in a desiccator and was allowed to dry completely. Once completely dry, the extracts were stored at 4°C for further tests.

Test Microorganisms:

Bacteria (*Bacillus subtilis* MTCC 0421, *Escherichia coli* MTCC 25921, *Klebsiella pneumoniae* MTCC 15380, *Pseudomonas fluorescens* MTCC 27853, *Streptococcus pyogenes* MTCC 29212) and fungi (*Aspergillus niger* MTCC 9652, *Aspergillus flavus* MTCC 1884, *Aspergillus terreus* MTCC 2580, *Trichoderma viride* MTCC 2047, *Fusarium solani* MTCC 2082) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC). Chandigarh, India. The bacterial cultures were maintained in Nutrient Agar (NA) slants at 4°C.

Antimicrobial activity:

The antimicrobial screening was carried out using well diffusion method. The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts loaded in different concentrations (50, 75 and 100 µl) were placed on the pre inoculated Mueller Hinton Agar (MHA) plates with respective cultures and incubated at 37°C for 24 h. Streptomycin (20 µg) was used as a positive control for bacteria and amphotericin-B (50 µg) was used as a positive control for fungi. DMSO was used as negative control. After incubation the zone inhibition around the well (diameter in mm) was measured using Hiantibiotic zone scale (HiMedia, PW096).

GC-MS analysis:

The sample (20 g) was soaked and dissolved in 75 ml of methanol for 24 h separately. Then the filtrates were collected and evaporated under liquid nitrogen (Divya *et al.,* 2018). The GC-MS

analysis was carried out using a GC clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatography equipped and coupled to a Mass detector Turbo mass gold – perkin Elmer Turbomass 5.1 Spectometer with an Elite-1 (100% Dimethyl polysiloxane), 30 m x 0.25 mm id x 1 µl df Capillary column. The instrument was set to an initial temperature of 110 °C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 ev. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 mhz.

The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institution of Standards and Technology – Mass Spectral Database (NIST-MS). The percentage of each component was calculated from the relative peak area of each component in the chromatogram.

Statistical analysis:

Data are expressed as mean ± standard deviation. The data obtained was subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

Results and Discussion

In the present study the antibacterial activity of *Capparis sepiaria L.* leaf and stem different extracts such as water, methanol, ethanol and diethyl ether at different concentration (50, 75 and 100 µl) was analyzed against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas fluorescens and Streptococcus pyogenes*. The results clearly showed that leaf extracts were specific in action against the growth of pathogenic bacteria. Ethanol, methanol and water extracts were most effective followed by diethyl ether extracts. *Klebsiella pneumoniae* were more sensitive to all extracts. Diethyl ether had low inhibition against the tested organism compared to other extracts. The results showed inhibition diameters ranging from 7 mm to 27 mm (Table 1).

Due to the extensive use of antibiotics, the spread of multi resistant bacterial strains is one of the most worrying threats to public health (Nicolas Fotinos *et al.,* 2008). Compared to Gram-positive bacteria, Gram-negative bacteria are more resistant against the extracts, because of their impenetrable wall (Prescott *et al.,* 2005). In the present study all the leaf extracts of *Capparis sepiaria* L. effectively inhibited the gram negative bacteria such as *Klebsiella pneumonia, Escherichia coli,* and *Pseudomonas fluorescens* compared to gram positive bacteria. Besides marsiline *Capparis sepiaria* L. methanol extract contains various classes of saponins, alkaloids and polyphenols (Bhadra *et al.,* 2012). Marsiline (an ester of 1-triacontanol and hexacosanoic acid), isolated from *Marsilea minuta* Linn and *Marsilea rajasthansis* Gupta have been previously reported to be active against electroconvulsion. While the leaf extracts could not protect the rats against eletroconvulsions (Satyavati *et al.,* 1987).

Antibacterial potential of stem extract was assessed. The investigated results are presented in Table 2. *Bacillus subtilis* and *Escherichia coli* was more sensitive for ethanol and diethyl either extract of stem. Aqueous and methanol extracts had low inhibition against the tested organism compared to other extracts. The *Bacillus subtilis* exhibited relatively higher zone of inhibition followed by water, methanol and diethyl ether extracts compared to other test organisms. These results are supported by similar study done by Mathangi and Prabhakaran (2012) against ten bacteria (MRSA, *Acinetobacter spp., Enterobacter spp., Proteus mirabilis, MSSA, Proteus vulgaris, Enterococcus Spp.,* and *Pseudomonas aeruginosa*). This study clearly indicates that *Capparis sepiaria* L. has a profound antimicrobial activity and that it is used for medicinal uses as the plant has potent phytochemicals.

In the present study antifungal activity of

Table 1: Antibacterial activity of *Capparis sepiaria* L. - Leaf

S. No.	Bacteria	NC	PC	Zone of inhibition (mm in diameter) (Mean±SD)											
				H_2O			CH_4O			C_2H_6O			$(C_2H_5)_2O$		
				50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl
1	*Bacillus subtilis*	-	25±1.9	11±0.5	12±0.6	14±0.7	-	11±0.5	12±0.7	16±0.5	17±1.3	19±1.3	-	-	-
2	*Escherichia coli*	-	37±2.7	11±0.4	13±0.2	15±0.5	-	-	-	14±1.2	15±1.8	17±1.5	-	-	-
3	*Klebsiella pneumoniae*	-	36±1.6	11±0.2	13±0.7	15±0.4	11±0.3	13±0.4	15±1.1	16±1.6	20±1.9	21±1.8	10±0.2	12±0.7	13±0.8
4	*Pseudomonas fluorescens*	-	40±2.4	12±0.3	14±0.5	17±0.3	10±0.6	14±0.6	15±1.2	10±0.9	11±1.2	12±0.6	-	-	-
5	*Streptococcus pyogenes*	-	38±2.9	24±0.4	26±1.2	27±0.4	11±0.4	16±0.4	19±1.5	-	-	-	6±0.68	10±0.8	11±0.5

Values are expressed as Mean ± Standard Deviation (n=3); NC (Negative control) – DMSO; PC (Positive control)- Streptomycin (20 µg); H_2O – Water; CH_4O – Methanol; C_2H_6O – Ethanol; $(C_2H_5)_2O$ - Diethyl ether

Table 2: Antibacterial activity of *Capparis sepiaria* L. - Stem

S. No.	Bacteria	NC	PC	Zone of inhibition (mm in diameter)											
				H_2O			CH_4O			C_2H_6O			$(C_2H_5)_2O$		
				50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl
1	*Bacillus subtilis*	-	25±2.10	11±0.58	13±0.65	15±1.20	-	15±0.78	17±0.57	18±1.24	20±1.58	21±1.39	19±1.42	21±1.80	25±2.10
2	*Escherichia coli*	-	34±2.45	11±0.45	14±0.38	15±1.49	-	-	-	17±1.27	20±1.93	21±1.45	17±1.28	19±1.32	22±1.99
3	*Klebsiella pneumoniae*	-	30±2.16	-	-	-	-	-	-	16±0.84	18±1.82	18±1.36	-	-	-
4	*Pseudomonas fluorescens*	-	36±1.98	-	-	-	12±0.34	14±0.94	16±0.92	12±0.43	13±0.72	14±0.91	-	-	16±1.36
5	*Streptococcus pyogenes*	-	22±2.20	10±0.33	13±0.56	13±0.95	11±0.56	13±0.49	14±0.88	-	-	10±0.83	-	-	27±1.64

Values are expressed at (Mean ± Standard Deviation) (n=3); NC (Negative control) – DMSO; PC (Positive control)- Streptomycin (20 µg); H_2O – Water; CH_4O – Methanol; C_2H_6O – Ethanol; $(C_2H_5)_2O$ - Diethyl ether

Table 3: Antifungal activity of *Capparis sepiaria* L. - leaf

S. No.	Bacteria	NC	PC	Zone of inhibition (mm in diameter)											
				H_2O			CH_4O			C_2H_6O			$(C_2H_5)_2O$		
				50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl
1	*Aspergillus niger*	-	19±1.10	-	-	-	-	-	-	-	-	-	-	10±0.52	12±1.10
2	*Aspergillus flavus*	-	10±0.42	-	-	-	-	-	-	-	-	-	07±0.22	10±0.33	13±1.22
3	*Aspergillus terreus*	-	10±0.96	-	-	-	-	-	-	-	-	-	10±0.42	12±1.02	13±1.52
4	*Trichoderma viride*	-	08±0.98	-	-	-	-	-	-	-	-	-	06±0.44	10±1.46	11±1.00
5	*Fusarium solani*	-	10±0.80	-	-	-	-		-	-	-	-	07±1.42	07±0.32	12±1.14

Values are expressed at (Mean ± Standard Deviation) (n=3); NC (Negative control) – DMSO; PC (Positive control) - Amphotericin-B (50 µg); H_2O – Water; CH_4O – Methanol; C_2H_6O – Ethanol; $(C_2H_5)_2O$ - Diethyl ether

Table 4: Antifungal activity of *Capparis sepiaria* L. - Stem

S. No.	Bacteria	NC	PC	Zone of inhibition (mm in diameter)											
				H_2O			CH_4O			C_2H_6O			$(C_2H_5)_2O$		
				50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl	50 µl	75 µl	100 µl
1	*Aspergillus niger*	-	14±1.10	10±1.10	12±1.10	13±1.10	-	-	-	-	-	-	-	-	-
2	*Aspergillus flavus*	-	12±0.42	7±1.10	12±1.10	13±1.10	-	-	-	-	-	-	-	-	-
3	*Aspergillus terreus*	-	12±0.96	10±1.10	11±1.10	12±1.10	-	-	-	-	10±1.10	11±1.10	10±0.42	11±1.02	12±1.52
4	*Trichoderma viride*	-	12±0.98	10±1.10	18±1.10	20±1.10	-	-	-	10±1.10	11±1.10	12±1.10	-	-	-
5	*Fusarium solani*	-	13±0.80	11±1.10	11±1.10	12±1.10	-		-	-	-	10±1.10	07±1.42	10±0.32	11±1.14

Values are expressed at (Mean ± Standard Deviation) (n=3)

NC (Negative control) – DMSO; PC (Positive control) - Amphotericin-B (50 µg); H_2O – Water; CH_4O – Methanol; C_2H_6O – Ethanol; $(C_2H_5)_2O$ - Diethyl ether

Table 5: Phytochemical constituents identified in the methanol leaf extract of *Capparis sepiaria* L using gas chromatography-mass spectrometry

Peak	Retention time	Area %	Height %	A/H	Mark	Name
1	22.01	2.77	4.41	2.2		Oxalic acid, cyclohexylmethyl tridecyl ester
2	24.54	11.39	15.95	2.5		Neophytadiene
3	24.64	1.96	2.35	2.92	V	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
4	24.96	2.28	3.37	2.37		3,7,11,15-Tetramethyl-2-hexadecen-1-ol
5	25.28	3.4	4.91	2.42		3,7,11,15-Tetramethyl-2-hexadecen-1-ol
6	26.69	1.37	2.14	2.24		4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone
7	27.44	3.32	4.96	2.34		Decane, 5,6-bis(2,2-dimethylpropylidene)-, (E,Z)-
8	28.99	10.25	14.29	2.51	V	Phytol
9	33.29	4.62	2.6	6.2	V	.alpha.-Amyrin
10	33.41	4.98	2.96	5.88	V	Olean-12-en-3-ol, acetate, (3.beta.)-
11	36.27	1.25	2.1	2.07	V	11-HEXADECENOIC ACID, METHYL ESTER
12	36.35	5.52	4.7	4.11	V	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl-, [1R-(1.alpha.,3a.beta.,4.alpha.,8a.beta.,9S*)]-
13	36.48	21.19	8.74	8.48	V	Lupeol
14	36.76	1.43	1.8	2.78	V	2-Hexanone, (2,4-dinitrophenyl)hydrazine
15	36.80	2.08	2.23	3.26	V	9,10-DIHYDRO-12'-HYDROXY-2',5'-BIS(1-METHYLETHYL)ERGOTAMAN-3',6',18-TRIONE (5'ALPHA,10ALPHA)-
16	36.89	5.08	3.25	5.47	V	D:B-FRIEDO-B':A'-NEOGAMMACER-5-EN-3-ONE
17	36.97	1.19	2.4	1.74	V	EISEN, TRICARBONYL-(HAPTO-4-2,4,5-TRIETHYL-2-ENDO,3-DIMETHYL-1,2,5-AZASILABOROLIN)
18	36.99	0.82	1.89	1.51	V	1,3-Diazabicyclo[4.4.0]decan-4-one, 3-[3-(1,3-dioxolan-2-yl)propyl]-
19	38.52	4.76	2.83	5.87	V	ERGOST-5-EN-3-OL, (3.BETA.,24R)-
20	38.95	0.88	2.14	1.44	V	Cyclopropanedecanoic acid, 2-hexyl-.alpha.-hydroxy-, methyl ester
21	39.17	2.26	1.8	4.39	V	3-(2-Benzyl-benzoimidazol-1-yl)-propane-1,2-diol
22	39.22	1.71	2.06	2.91	V	3-(2-BROMOETHYL)-8-CYCLOHEXYL-2,3,3A,4,5,6-HEXAHYDRO-1H-PYRAZINO[3,2,1-JK]CARBAZOLE
23	39.51	1.38	2.36	2.04	V	9,12-OCTADECADIENOIC ACID (Z,Z)-, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER
24	39.55	1.53	1.97	2.73	V	(5S,6aR,10aS)-5-Propyldecahydrodipyrrolo[1,2-a:1',2'-c]pyrimidine
25	39.61	2.56	1.8	4.99	V	9,12,15-OCTADECATRIENOIC ACID, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER, (Z,Z,Z)-

Capparis sepiaria L. (leaf and stem) was analyzed. The diethyl ether leaf extract had the maximum effect as it inhibited all the fungal strains. No antifungal activity was observed in other extracts of water, methanol and ethanol (Table 3). The stem extracts in water had most effective inhibition. Ethanol and diethyl ether extracts were observed to have intermediate inhibition. All the tested fungal organisms were resistant to the methanol plant extracts (Table 4).

Amongst the four plant extracts, methanol and diethyl ether leaf extract has high inhibitory effect against all pathogens. In the present study, the leaves were subjected to antimicrobial activity against selective microbes in which the results revealed that the methanolic extracts had good antimicrobial activity. Twenty five compounds were identified in the methanol extract of *Capparis sepiaria* L leaves. The peak report of the total ion current chromatogram obtained with details of peak number, retention time, area, area percentage, name of the identified phyto-component, its molecular formula and molecular weight, is presented in Table 5. Compounds predicted by comparing with the standard mass spectral databases. However, they varied in their respective retention time, area and area percentage. In the present study we applied GC-MS predicted Neophytadiene and Oxalic acid compounds.

Conclusion

This study has revealed the presence of many secondary metabolites in the leaves of *Capparis sepiaria* L. It has further confirmed that the plant extracts could be used for the treatment of various infections including skin transmitted infections. The results lend credence to the folkloric use if this plant in treating microbial infection and shows that *Capparis sepiaria* L. could be exploited for new potent antimicrobial agents.

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