



## Phytochemical analysis and antimicrobial activity of *Capparis sepiaria* L. against human clinical pathogens

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### Abstract

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. The present study is focused on exploring the phytochemical analysis and antimicrobial activity of *Capparis sepiaria* L. against human clinical pathogens. Analysis of phytochemical compounds was carried out on different extracts of the powdered specimens of the plant using standard procedures. In this study tannin, flavonoids, saponins and carbohydrates showed positive results for *Capparis sepiaria* L. leaf extract. Diffusion discs were prepared and immersed in various solvent extracts for antimicrobial activity study. Acetone and methanol extracts of leaves showed maximum antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus flavus*. The experiment confirmed the efficacy of *Capparis sepiaria* L. plant extracts as natural antimicrobials and suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms.

**Keywords:** phytochemical, antimicrobial activity, *Capparis sepiaria* L, human clinical pathogens

### Introduction

Traditional medicine has been defined as the sum of the knowledge, skills and practices, based on the theories, beliefs and experiences, indigenous to different culture, whether applicable or not, traditional medicines are used in the maintenance of health as well in the prevention, diagnosis, improvement or treatment of physical and mental illness [1]. In many developing countries, large parts of the population, especially in rural areas, depend mainly on traditional medicine for their primary healthcare. The indigenous knowledge of medicinal plants has been well documented in ancient Hindu literature.

According to an estimate, only 20 percent of the plant flora has been screened for drugs. Keeping in view the vast treasure of medicinal herbs, one can expect phytochemicals to play a significant role, as modern science has limited options for diseases like diabetes mellitus, rheumatoid arthritis, Alzheimer's disease and Parkinson's disease. Work on the identification and isolation of phytochemicals is an ongoing process and herbal medicine is expected to play critical role in the future healthcare system. Medicinal plant products could prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health [2]. The increasing global interest in the medicinal potential of plants during the last few decades is therefore quite logical. Similarly, a lot of concern has been generated world-wide over bacterial drug resistance, in view of the fact that cheap drugs have to be replaced with more effective and expensive ones. Sule and Olusanya, have reported the increasing prevalence of bacterial resistance to most of the commonly used antibiotics in this environment [3]. Harison Masih *et al* [4]. has studied the leaf extract of

*Pongamia pinnata*, *Calotropis procera*, *Nerium indicum* and *Curcuma longa* that were taken in aqueous solution and tested against plant pathogenic organisms – *Aspergillus fumigatus*, *Alternaria solani*, *Helminthosporium spp.* and *Fusarium solani*. *Capparis* species has been reported for their medicinal activity such as hepato-protective activity, analgesic activity [5], antibacterial activity, anti-diabetic activity [6], anti-hyperlipidemic [7], anti-inflammatory [8], treatment of stomach problems, cough, cold, asthma, ulcers, vomiting, diabetes, fever, gout, jaundice, dysentery, smallpox, cholera and diarrhea [9]. In the present study the phytochemical analysis and antimicrobial activity of *Capparis sepiaria* L. against human clinical pathogens was carried out.

### Materials and Methods

Plants were selected for this study based on their medicinal use. Fresh leaves of *Capparis sepiaria* L. was collected from Thanjavur Dt. of Tamil Nadu, India in Jan, 2018. The ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through questionnaire, interviews and discussions among the local practitioners in their language (Tamil). The plant parts were air-dried. After drying at 37°C for 7 days the plant materials were ground in a grinding machine (Thomas Wiley laboratory mill, model # 4, screen size-1mm). Exposure to direct sunlight was avoided to prevent the loss of active components.

### Phytochemical screening of *Capparis sepiaria* L. leaf extract

Chemical tests were carried out with the powdered specimens on aqueous, acetone and methanol extracts and

using standard procedures to identify the constituents, as described by Sofowara <sup>[10]</sup> and Trease and Evans <sup>[11]</sup>.

### Tannins

About 2ml of the leaf extract was taken in a test tube. Then 2ml of distilled water was added along with 2-3 drops of Ferric chloride. The test tube was observed for green coloration, which indicates the presence of tannins.

### Flavonoids

About 1 ml of leaf extract was taken and a few drops of 1% Ammonia Solution were added to it. A yellow coloration was observed in the extracts, thereby indicating the presence of flavonoids.

### Terpenoids

2ml of leaf and stem extracts were mixed in 2ml of acetic anhydride and 2-3 drops of Sulphuric acid was carefully added to form a layer. Deep red coloration shows positive test results for the presence of terpenoids.

### Saponins

5 ml of leaf extract was taken and 5ml of water was added. The solution was then allowed to heat. Persistent frothing on warming the extract indicated the presence of saponins. The same extract with few drops of olive oil formed a soluble emulsion confirming the presence of saponins.

### Phlobatannins

2ml of leaf extracts was taken in a test tube and 2ml of 1% Hydrochloric acid was added and heated. The red precipitate formed in test tube indicates the presence of phlobatannins.

### Steroids

Leaf extracts were mixed with few drops of acetic anhydride, boiled and then cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids, respectively.

### Carbohydrates

Leaf extracts were mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrates.

### Glycosides

To about 2 ml of leaf extracts 2 ml of CHCl<sub>3</sub> solution and 2ml of CH<sub>3</sub>COOH solution was added. A color change from violet to blue-green color, confirmed the presence of glycosides.

### Courmarins

1 ml of 10% sodium hydroxide was added to 1ml of the extract. Formation of yellow colour indicates the presence of coumarins.

### Emodins

3 ml of leaf and stem extracts were evaporated. The dry residue was dissolved in 1 ml of concentrated NH<sub>4</sub>OH and the solution was treated with the Borntrager reagent. A

positive test result is revealed by the appearance of a bright orange or red.

### Protein

Leaf extracts were treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution was added and observed for the formation of violet and pink colors, respectively.

### Anthraquinones

3 ml of leaf extracts were treated with 3 ml of benzene solution and 5 ml of 10% of NH<sub>3</sub> solution separately and observed for the formation of Pink and Violet or Red coloration, respectively in the ammonical layer, indicates the presence of Anthraquinones.

### Anthocyanins

2 ml of leaf extracts were mixed with 2 ml of HCl (2N) solution and 3 drops of NH<sub>3</sub> solution separately and observed for the formation of Pinkish-red to bluish-violet coloration, which indicates the presence of Anthocyanins.

### Leucoantho cyaninsturns

5 ml of leaf extracts were mixed with 5 ml Isoamyl alcohol and observed for the formation of red colored organic layer indicates the presence of Leucoantho cyaninsturns.

### Quantitative analysis of *Capparis sepiaria* l. leaf extract Tannins

2 ml of plant extracts were added in a test tube. Then 5ml of Folin-Denis reagent, 10ml of sodium carbonate solution and, few drops of water were mixed and the solution was shaken well. The solution was read at 700nm in a spectrophotometer.

### Flavonoids

0.5g of sample was weighed and extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whattman filter paper NO: 4 (110 MM) size. The filtrate was evaporated over a water bath and weighed to a constant weight. The percentage of flavonoids was then calculated.

### Saponins

The extract was dissolved in 80% of methanol, 2ml of phenolic aldehyde in ethanol and 2ml of 72% sulphuric acid mixed well and heated on a water bath at 60°C for 10min. Absorbance was measured at 544nm against reagent blank. Diosgenins were used as a standard material and compared with Diosgenin equivalents.

### Carbohydrates

The 100mg of plant sample was hydrolyzed in a boiling tube with 5ml of 2.5N HCl in a boiling water bath for a period of 3 hours. It was cooled to room temperature and solid sodium carbonate was added until the effervescence ceases, the contents were then centrifuged and the supernatant was made up to 100ml using distilled water. From this 0.2ml of sample was pipetted out and made up to 1ml with distilled water, then 1.0ml of phenol reagent was added followed by 5.0ml of sulphuric acid. The tubes were kept at 25°-30°C for 20min. The absorbance was then read at 490nm.

### Assay of antimicrobial activity

A Soxhlet apparatus was used for extracting the antimicrobial active compounds from the plant leaves. 20g of the plant powder sample was ground and soaked with 100ml water, acetone and methanol (separately) in a 250ml conical flask. The flask was covered with cotton wool and aluminium foil, to prevent the solvent from escaping. The flask was placed in a shaker for 24hrs. The plant extracts prepared by using Soxhlet apparatus were collected and stored in a vial for further studies. The 6 mm (diameter) discs were prepared from Whatman NO: 1 filter paper. The discs were sterilized by autoclaving at 121°C. After the sterilization the moist discs were dried in hot air oven at 50°C. Then various solvents extract discs and control discs were prepared.

The pathogenic bacteria such as *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus terreus* pure cultures were inoculated in nutrient and PDA broth separately. The inoculated broths were incubated at 37°C for bacteria and for fungi at 28°C for 48 hrs. After incubation the strains were used for further studies.

Disc diffusion method [12] was adopted for evaluation of antibacterial activity of *Capparis sepiaria L.* plant parts. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. Discs of various solvents extracts that were prepared were individually placed on each petriplates along with control discs (Streptomycin for bacteria and Amphotericin for fungi). The plates were incubated at 37°C for 24 hrs for bacteria and 28°C for 72 hrs for fungi. After the incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

### Results and Discussion

The plant exhaustively showed medically useful phytoconstituents in the acetone and methanolic extract of *Capparis sepiaria L.* (Table. 1). The results of the determinations of the phytochemical tests indicated that the plant possesses numerous biologically active compounds which could serve as potential source of drugs in herbal medicine [13]. Tannins, flavonoids, saponins and carbohydrates showed positive results in the methanolic leaf extract of *Capparis sepiaria L.* The phytochemicals are reported to have many biological and therapeutic properties, so this plant species is expected to have many medicinal uses. These secondary metabolites are reported to have many biological and therapeutic properties [14], so this plant species is expected to have many medicinal uses. In previous studies it was reported that reducing sugar, flavonoids, steroids, tannins, glycosides, alkaloids, gums, resins, amino acids, proteins and anthraquinones were present in ethanolic extract of the *Capparis sepiaria L* while saponins and carbohydrates were found to be absent in it [14]. The previous literature reported that the *Capparis sepiaria L* fruit extract possesses hypolipidemic and anti-atherosclerotic effect was reported [15]. In the present study, saponins (50.76±2.45, 36.20±0.86 and 42.13±1.24 %) were present in maximum amount in *Capparis sepiaria L.* leaf extracts in aqueous, acetone and methanol extracts

compared to other compounds such as tannins, flavonoids and carbohydrates (Figure 1). Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxy radicals, hydroxyl radicals and peroxy nitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage [16]. Flavonoid may help provide protection against these diseases by contributing along with antioxidant vitamins and enzymes, to the total antioxidant defense system to the human body. Epidemiological studies have shown that flavonoids and carotenoids intake is inversely related to mortality from coronary heart diseases and to the incidence of heart attacks [17].

The antibacterial activity of aqueous, acetone and methanol crude extracts obtained from the leaves parts of *Capparis sepiaria L.* was examined by agar well diffusion method against the human pathogenic bacterial strains. The results clearly showed that plant extracts were specific in action against the growth of bacteria. Methanol and acetone extracts were the most effective followed by other aqueous extracts. *Escherichia coli* and *Pseudomonas aeruginosa* were more sensitive for methanol extract of leaves. Satyanarayana *et al* [18]. reported that ethanol soluble fractions of *Capparis sepiaria* stem showed potent activity against certain Gram positive (*Enterococcus faecalis*, *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) pathogenic bacteria of Gastrointestinal tract. Aqueous extracts had low inhibition against the tested organism compared to other extracts. *Escherichia coli* (08±1.45; 15±1.84; 17±1.55 mm in diameter) exhibited relatively higher zone of inhibition in aqueous, acetone and methanol extracts compared then other test organisms. The results showed inhibition diameters ranging from 08 mm to 17 mm (Table 2). Mahboubi and Mahboubi [19] revealed the scientific support to the traditional uses of leaves extracts as antimicrobial agents by studying the methanol, ethanol and ethyl acetate extracts from fruit and root of *Capparis spinosa*, another important medicinal plant species of Capparis, which exhibited good activity against microorganisms, especially fungi.

The results of present research highlights, the fact that the organic solvent extracts exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium [20]. Antifungal activities of different solvent extracts of *Capparis sepiaria L.* were analysed against human pathogenic fungi. Antifungal activities of plant extracts were also compared with standard antibiotic (Amphotericin). In this study maximum antifungal activity was recorded against *Aspergillus flavus* (9±0.61; 12±1.74; 22±0.98 aqueous, acetone, methanol, respectively). Moderate antifungal activity was noted against *Aspergillus terreus*. Lowest antifungal activity was recorded against *Aspergillus niger* (08±1.55; 10±0.81; 20±1.24, aqueous, acetone, methanol respectively). Amongst the three plant extracts, methanol 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.

**Table 1:** Phytochemical Screening of *Capparis sepiaria L.* Leaf extracts

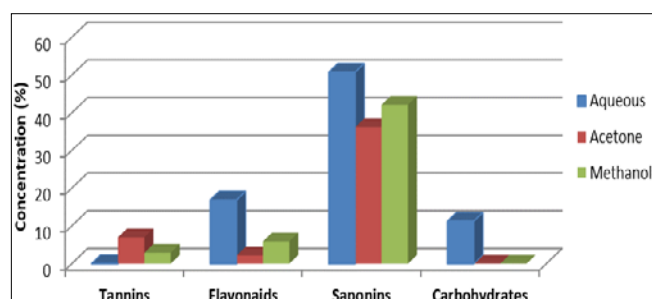
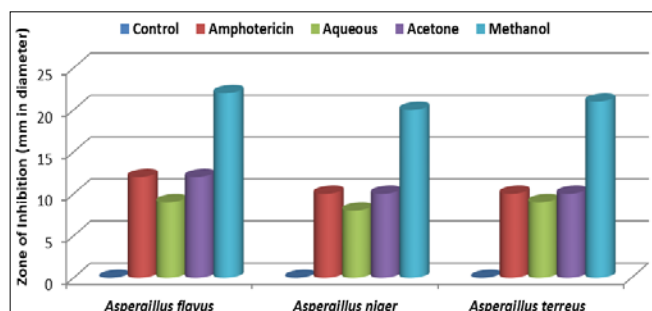
S. no.	Phytochemical compounds	Observation			
		Control	Aqueous	Acetone	Methanol
1	Tannins	-	-	+	+
2	Flavonoids	-	+	+	+
3	Terpenoids	-	-	-	+
4	Saponins	-	+	+	+
5	Phlobatannins	-	-	-	-
6	Steroids	-	-	-	+
7	Carbohydrates	-	+	-	-
8	Glycosides	-	-	-	-
9	Coumarins	-	-	-	-
10	Proteins	-	-	-	-
11	Emodins	-	-	-	-
12	Anthraquinones	-	-	-	-
13	Anthocyanins	-	-	-	-
14	Leucoanthocyanins	-	-	-	-

+ indicates presence; - indicates absence

**Table 2:** Antibacterial activity of *Capparis sepiaria L.* Leaf extracts

S. no.	Bacteria	Zone of Inhibition (mm in diameter)				
		C <sup>^</sup>	*S	Aqueous	Acetone	Methanol
1	<i>Bacillus cereus</i>	-	27±1.21	10±1.72	15±1.27	16±1.07
2	<i>Escherichia coli</i>	-	22±0.81	08±1.45	15±1.84	17±1.55
3	<i>Klebsiella pneumoniae</i>	-	22±1.51	11±1.23	10±1.04	10±1.09
4	<i>Staphylococcus aureus</i>	-	28±1.75	09±0.73	16±1.42	15±1.22
5	<i>Pseudomonas aeruginosa</i>	-	29±1.41	10±1.28	13±1.87	17±1.25

Values are expressed in Mean ± Standard deviation; n=3 C<sup>^</sup> - Control, \*S - Standard (Streptomycin (120 mg/disc)

**Fig 1:** Quantitative analysis of Phytochemical Compounds in *Capparis sepiaria L.* Leaf extracts**Fig 2:** Antifungal activity of *Capparis sepiaria L.* leaf extracts

## Conclusion

The present study showed that methanolic extract of *Capparis sepiaria L.* is rich in basic nutrients. Qualitative phytochemical screening showed that it is abundant in

phytochemicals such as tannins, flavonoids, saponins and carbohydrates especially it was found in high amount in methanolic extract than other extracts. Quantitative analysis showed that methanolic extract contains higher amounts than other two extracts (aqueous and ethanol extract). From the findings of the study it may be concluded that the methanolic extract of *Capparis sepiaria L.* acts as the potential source of phytochemical which may be used traditional medicine for prevention of several diseases. In case of the antimicrobial analysis, *Escherichia coli* and *Pseudomonas aeruginosa* were more sensitive for acetone and methanol extract of *Capparis sepiaria L.* leaves. In this investigation aqueous, acetone and methanol extracts from the leaves of *Capparis sepiaria L.* exhibited antifungal activity against *Aspergillus niger*, *Aspergillus terreus* and *Aspergillus flavus*. Thus the crude extracts as well as certain isolated compounds of *Capparis sepiaria L.* were found to exhibit its phytochemical activity and were found to be active against certain human pathogens and could be useful for the development of new antimicrobial drugs.

## Acknowledgement

The authors are thankful to the PG and Research Department of Microbiology, Marudupandiyar College and Specialty Lab and Research, Hi-Quality Microbiology Center, Thanjavur, Tamilnadu, India for providing the necessary facilities for this study.

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