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Studies on phytochemicals, antibacterial efficacy and antioxidant potency of *Capparis sepiaria on enteric pathogens*

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Abstract

In this investigation, *Capparis sepiaria* was analysed for its phytochemical constituents qualitatively and quantitatively. The antibacterial property of aqueous, ethanolic and hexane extracts of *Capparis sepiaria* was studied against different bacteria include *Escherichia coli, Proteus mirabilis, Salmonella typhi, Enterobacter aerogenes, and Shigella flexineri*. Hexane extract of *Capparis sepiaria* showed the maximum growth inhibition zone of 20.4±0.2 mm at concentration of 500 mg against *Salmonella typhi*. Please mention the highest inhibition result including extract name and concentration. The antioxidant effect of those extracts was also studied against α-tocopherol as a control. From the results, Alkaloids, flavonoids, saponins, and tannins were revealed to be present in *Capparis sepiaria*. Ethanol extract at the concentration of 500 µg/ml showed 57.81% antioxidant activity against 500 µg/ml of α-tocopherol which showed 66.76% as a standard reference.

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Introduction

Plants are effective in the treatment of infectious diseases and many plant extracts have been shown to possess antimicrobial properties *in vitro* (Sofowora, 1983). The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents inefficient to control some bacterial diseases (Tanaka et al., 2006). Herbal medicine is frequently a part of a larger therapeutic system such as traditional and folk medicine. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. The search and use of drugs and dietary supplements derived from plants have been accelerated in recent years. Ethnopharmacologist, botanist, microbiologist and natural product chemist are combing the medicinal flora for biological substances that could be developed for the treatment of infectious diseases. Capparis is a genus of flowering plants in the Capparidaceae family and found in most of the parts of south India. Capparis Sepiaria (Fam; Capparidaceae) is a profusely branched hedge plant with slender prickly shrubs, zigzag stems (Mathew KM, 1983 and Anonymous, 1992) Traditionally it is used as a blood purifier, stomachic, tonic and appetizer. Its flowers, leaves and roots are used in the treatment of cough and toxemia and root powder is also used as cure for snakebite (Kiritkar KR, 1993). It also possesses febrifuge properties and used to treat skin diseases, inflammation and diseases of the muscle (Trivedi PC, 2002). Some previous findings related to Capparis genus or Capparidaceae family should be mentioned with reference.

The aim of the present investigation is to analyze *Capparis sepiaria* qualitatively to study the phytochemicals and antibacterial effect of aqueous, ethanolic and hexane extract of *Capparis sepiaria* against enteric pathogens with the assessment of its antioxidant potency.

Materials and methods

Preparation of plant extract

The leaf of *Capparis sepiaria* used in this study were collected from Thirukkalukundram village, Kanchipuram District, TamilNadu, South India in the month of June, 2010. The plant was identified by the experts of Centre for Advanced Studies in Botany, University of Madras, Guindy campus, Chennai and a voucher specimen was deposited in our departmental laboratory. The collected plant sample was refluxed in running tape water for 1-2 h and shade dried at room temperature for 15 – 20 days. Aqueous, ethanolic and hexane extract of *Capparis sepiaria* was prepared using soxhlet apparatus (Hoffman et al., 2004) for about 24h. The extract was distilled and concentrated *in vacuo* with addition of CaCl2. Lyophilized aqueous fractions were further used to test for the antifungal, antibacterial and antioxidant properties.

Chemicals and microorganisms

Escherichia coli, Proteus mirabilis, Salmonella typhi, Enterobacter aerogenes and Shigella flexineri were purchased from IMTECH, Chandigar, India. Solvent and other chemicals which were used during this study were from Himedia, Merck and s.d. Fine-Chemicals, Mumbai.

Methods

Phytochemical screening procedures carried out were adopted from (Oloyed, 2005). This analysis determines the biologically active compounds that contribute to the flavour, colour and other characteristics of leaves.

Test for alkaloids

About 2 g of the ground sample were pounded separately on a mortar. 0.2 g was boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 min. The mixture was allowed to cool and filtered and the filtrate

was shared in equal proportion into 3 test tubes and labeled A, B, C. One (1) ml portion of the filtrate was treated with 2 drops of the following reagents respectively. With Dragendroff's reagent a red precipitate was shown. With Mayer's reagent a creamy white coloured precipitate indicated the presence of alkaloid (Harborne, 1973; Trease and Evans, 1989).

Test for flavonoids

About 0.5 g of the macerated sample of *Capparis sepiaria* was introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixture was then filtered and the filtrate used for the following test. 4 ml of the filtrate was shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of a yellow colour in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids (Harborne, 1973).

Test for saponins

About 0.1 g of the sample was boiled with 5 ml of distilled water for 5 min. Mixture was filtered while still hot and the filtrate was then used for the following tests (Trease and Evans, 1989). To 1 ml of the filtrates, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion. 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth (Trease and Evans, 1989).

Test for tannins

Into 2 g of the ground sample, 5 ml of 45% ethanol was added and boiled for 5 min. The mixture was cooled and filtered. 1 ml of the filtrate was added 3 drops of lead sub acetate solution. A gelatinous precipitates were observed which indicates the presence of Tannins. Another 1 ml of the filtrate was added 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins (Trease and Evans, 1989).

Test for glycosides

About 2g of the sample was mixed with 30 ml of distilled water and it was heated for 5 min on a water bath, filtered and used as follows: five ml of the filtrate was added to 0.2 ml of fehling solution A and fehling solution B until it turns alkaline and heated in a water bath for 2 min. A lightish blue colouration was observed (instead of brick red precipitate) which indicates the absence of glycosides (Oloyed, 2005).

Quantitative analysis of phytochemical constituents

Estimation of alkaloids: 0.5 g of the sample was dissolved in 96% ethanol-20% H_2SO_4 (1:1) mixture. 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI), and allowed to stand for 5 min. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 565 nm (Harborne, 1976).

Estimation of flavonoids: Flavonoid in the test sample was determined by the acid hydrolysis of spectrophotometric method. 0.5 g of processed plant sample was mixed with 5 ml of dilute HCl and boiled for 30 min. The boiled extract was allowed to cool and filtered. 1 ml of the filtrate was added to 5 ml of ethyl acetate and 5 ml of 1% NH_3. This was then scanned 3 from 420nm-520nm for the absorbance (Harborne, 1976).

Estimation of saponins: 0.5 g of the sample was added to 20 ml of 1N HCl and was boiled for 4 h. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes. 6 ml of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 min and the absorbance was taken at 490 nm (Oloyed, 2005).

Estimation of tannins: 5 g of the ground sample was shaken constantly for 1 min with 3 ml of methanol in a

test tube and then poured into a Buchner funnel with the suction already turned on. The tube was quickly rinsed with an additional 3 ml of methanol and the content poured at once into the funnel. The filtrate was mixed with 50 ml of water and analyzed within an hour. For aqueous extractions, 5 ml of water was used for the extraction and for the rinse and the filtrate was added to 50 ml of water. 3 ml of 0.1 ml $FeCl_3$ in 0.1 NH_4Cl was added to 5 ml of the extract and followed immediately by timed addition of 3 ml of 0.008 ml $K_2Fe(CN)_6$. The absorbance was taken at 720 nm spectrophotometrically (Onwuka, 2005).

Antibacterial effect

The antibacterial activity of *Capparis sepiaria* was evaluated by agar well diffusion method (Chung *et al*., 1990). Muller Hinton agar medium was prepared and poured into the petridishes. Then it was inoculated with a swab of bacterial culture (mid log phase) and spread throughout the medium uniformly with a sterile cotton swab. Using a sterile cork borer (10mm diameter) wells were made in the agar medium. The test compound was introduced into the wells and all the plates were incubated at 37°C for 24 h. The experiment was performed five times under strict aseptic conditions. Sensitivity of the organism was determined by measuring the diameter of the zone of inhibition. Each assay was repeated for five times and the mean value was taken for analyses. The control experiment was carried out with the antibiotics such as streptomycin and chloramphenical (S.Shobana et al, 2009).

Antioxidant effect

The antioxidant activity of aqueous, ethanolic and hexane extracts of *Capparis sepiaria* were determined by ferric thiocynate method (Mistuda et al., 1996). 10 mg of each extract was dissolved separately in 99.5% of ethanol and various concentrations (100, 200, 300, 400 & 500µg/ml) were prepared. A mixture of a 2 ml of sample in 99.5% ethanol, 2.052 ml of 2.51% linoleic acid in 99.5% ethanol, 4 ml of 0.05 M phosphate buffer (pH 7.0) and 1.948 ml of water was placed in a vial with a screw cap and placed in an oven at 60°C in the dark. To 0.1 ml of this sample solution 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocynate was added. After the addition of 0.1 ml of 2 x 10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of the red color developed was measured in 3 min at 500 nm (Matook and Hashinaga, 2005). The control and standard were subjected to the same procedures as the sample, except that for the control, only solvent was added, and for the standard, sample was replaced with the same amount of α-tocopherol (reference compound) (Ali Yildirim et al., 2001). The inhibition of lipid peroxidation in percentage (Table 3.0) was calculated by following equation:

% Inhibition = 1 - (A1/A2) x100

Where,

A1 - absorbance of the test sample

A2 - absorbance control reaction

Results and discussion

Phytochemical analysis

Phytochemical analysis is very useful in the evaluation of some active biological components of medicinal plants. The qualitative and quantitative analyses were carried out in both dry and wet samples. Alkaloids, flavonoids, saponins, tannins, were revealed to be present in *Capparis sepiaria* (Table 1.1). This shows high level of its possible medicinal and dietary values (Oloyed, 2005). Although, some of these analyzed constituents of the vegetable species may be completely harmful to both man and farm animals and some are species specific as observed in the case of tannins (Odebiyi and Sofowora, 1979). Some of these active components have been demonstrated to possess anti nutritional effects, following their ability to reduce palatability and digestibility of feedstuff (Odebiyi and Sofowora, 1979).

In Table 1.2, the levels of these phytochemicals (bioactive compounds) were shown. Generally, the dry

sample showed higher levels of these bioactive compounds than the wet sample. The reason may be that the bioactive compounds are not volatile compounds and hence have a high dried weight. High levels of flavonoids (65.13 ± 3.14 and 52.38 ± 3.21) in

Table 1.1. Qualitative analysis of different extracts of *Capparis sepiaria* for phytochemicals

Name of the phytochemical	Different extracts of *Capparis sepiaria*		
	Aqueous extract	Ethanolic extract	Hexane extract
Alkaloids	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Tannins	+ve	+ve	+ve
Glycosides	-ve	-ve	-ve

+ve – presence; -ve - absence

Table 1.2. Quantitative analysis of *Capparis sepiaria* for phytochemicals

Name of the phytochemical	*Capparis sepiaria*	
	Dry sample	Wet sample
Alkaloids (mg/100g	41.28±2.41	19.89±2.17
Flavonoids(mg/100g)	65.13±3.14	52.38±3.21
Saponins(mg/100g)	02.97±0.54	01.43±0.76
Tannins(mg/100g)	01.17±0.69	01.73±0.29

Table 1.2 showed that the vegetable is good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biologic antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Burlon and Ingold, 1984). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases (Parkinson's and Alzheiner's) (Palozza, 1998). 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 are inversely related to mortality from coronary heart diseases and to the incidence of heart attacks (Donald and Cristobal, 2006).

Table 2.1. Antibacterial activity of aqueous extract of *Capparis sepiaria*

Aqueous extract	Conc. (µg)	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Capparis sepiaria	100	NS	NS	NI	NS
	200	NS	10.1	NI	NS
	300	10.5±0.3	11.2±0.3	NS	10.1±0.3
	400	13.1±0.2	13.2±0.4	11.7±0.3	13.2±0.4
	500	16.5±0.2	14.8±0.5	14.6±0.2	16.8±0.2
Streptomycin	100	20.8±0.5	19.7±0.4	18.8±0.3	20.1±0.5
Chloramphenical	100	22.1±0.4	20.5±0.6	19.4±0.4	21.1±0.2

NI – No Inhibition

We have considered <10mm as non-significant and >10mm as significant value to denote the efficacy of our plant of interest.

Table 2.2. Antibacterial activity of ethanolic extract of *Capparis sepiaria*.

Aqueous extract	Conc. (µg)	Mean diameter of the zone of inhibition (mm)			
		E. coli	*P. mirabilis*	*S. typhi*	*E. aerogenes*
Capparis sepiaria	100	NI	NI	NS	NS
	200	NS	NS	NS	NS
	300	11.6±0.4	10.2±0.6	10.8±0.3	11.1±0.4
	400	12.7±0.3	11.3±0.6	11.9±0.2	13.5±0.2
	500	14.8±0.2	13.2±0.5	14.7±0.4	16.1±0.3
Streptomycin	100	19.6±0.4	20.1±0.5	18.9±0.3	19.9±0.3
Chloramphenical	100	18.6±0.5	19.4±0.3	19.3±0.2	20.2±0.3

NI – No Inhibition

You must mention mean with standard deviation along with t-test or ANOVA (P value).

Antibacterial activity

From Table 2.1, 2.2 & 2.3 it is very clear that the aqueous, ethanolic and hexane extracts of *Capparis sepiaria* showed growth inhibition activity only at higher concentrations ranging of 300mg to 500mg. *E.aerogens* and *P.mirabilis* were sensitive to aqueous extracts of *Capparis sepiaria* rather than ethanolic and hexane extracts. *S.typi* showed moderate resistance to all the extracts when compared to others (Table 2.1, 2.2 & 2.3). It was observed that in both ethanolic and hexane extracts of *Capparis sepiaria*, bacterial strains are not highly susceptible even at high concentration than aqueous extract.

Table 3.0. Antioxidant activity of aqueous, ethanolic and hexane extracts of *Capparis sepiaria*.

Extract	% of inhibition of lipid peroxidation				
	100µg/ ml	200µg/ ml	300µg/ ml	400µg/ ml	500µg/ ml
Water	10.43	17.22	26.63	35.94	47.90
Ethanol	12.23	21.83	30.18	41.79	57.81
Hexane	11.46	20.21	31.13	40.34	49.15
α-Tocopherol	19.13	30.11	42.17	52.66	66.76

Antioxidant activity

The antioxidant activity of the aqueous, ethanolic and hexane extracts of *Capparis sepiaria* were determined by ferric thiocynate (FTC) and the values are presented in Table 3.0. FTC method was used to determine the amount of peroxide formed and that react with ferrous chloride (FeCl2) to form a reddish ferric chloride (FeCl3) pigment. In this method, the concentration of peroxide decreases as the antioxidant activity of extract increases. Aqueous, ethanolic and hexane extracts at various concentration (100,200, 300,400 and 500 in µg/ml), showed antioxidant activities in a concentration dependent manner. Ethanol extract at the concentration of 500 µg/ml showed 57.81%, an antioxidant activity at the concentration of 500 µg/ml of α-tocopherol 66.76%, the reference compound. The aqueous and hexane extracts of *Capparis sepiaria* also have showed some significant level of inhibition of lipid peroxidation. It has been observed that the extract exhibited moderate antioxidant activity with the increase in concentration.

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