

Pharmacognostic and Pharmacological Studies on Flower Buds of *Capparis spinosa* L.

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ABSTRACT

Capparis spinosa L. (Capparaceae) is growing wild on walls or in rocky coastal areas all over India and normally known as *Maratimokku* in Tamil. The current study was aimed to reveal the botanical and chemical pharmacognostic standards, and pharmacological and toxicity properties of aqueous extract of *C. spinosa* flower buds. Powder microscopy revealed the characteristic features of the sample, which are useful in fixing the pharmacognostic standards. Preliminary phytochemical screening revealed the presence of alkaloids, reducing sugars, carbohydrates, saponins, phenolic compounds, tannins, anthraquinones and lignins in aqueous extract. LC-MS/MS analysis revealed the presence of quercetin as the major constituent in the aqueous extract. Strong antioxidant activity was observed in aqueous extract centered on the outcomes of DPPH radical scavenging (IC-50 1.74 µg/ml) and inhibition of lipid peroxidation (IC-50 4.01 µg/ml) assays. The aqueous extract exhibited anti-inflammatory activity in terms of inhibition of protein denaturation (IC-50 8.01 µg/ml), inhibition of protease activity (14.24%) and RBC membrane stabilization (IC-50 226.69 µg/ml) and also possess antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Salmonella enteritidis* strains. Acute oral toxicity study revealed that it is safe up to the dose level of 2000 mg/kg bw in the experimental animals.

Keywords: *Capparis spinosa*, Powder microscopy, Chemical composition, Medicinal value.

INTRODUCTION

Plants have played a significant role in the maintenance of human health and in improving the quality of human life since thousands of years¹. Increased cost and serious side effects of existing synthetic drugs and increased number of incidences with chronic diseases lead the society to search for alternative source to meet their requirements². This prompted us to focus our search towards the identification of safe and efficacious herbals which are used in indigenous system of medicine in Southern Peninsular India.

Capparis spinosa L. (Capparaceae) is a perennial spiny bush that bears rounded, fleshy leaves and big white to pinkish-white flowers³. In English it is commonly known as Caper and locally called as *Maratimokku* in Tamil and being used as a spice in Indian cuisine. It is native to the Mediterranean region and growing wild on walls or in rocky coastal areas throughout India⁴. The phytochemicals identified in this plant were lipids, flavonoids, alkaloids, saponins, tannins, lignins, glucocapperin, (6S)-hydroxy-3-oxo- α -ionolglucosides and polyphenols⁵⁻⁷. It has been used in traditional Indian system of medicines for various human diseases⁸ and the summation of its medicinal properties was reviewed⁴. *C. spinosa* L. has anthelmintic, cytotoxic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-microbial, cardiovascular, chondroprotective, anti-

diabetic, hypolipidemic, anti-allergic, anti-histaminic, immunomodulatory, anti-carcinogenic and anti-hepatotoxic activities^{9, 10}.

Even though some traditional medicinal uses of this plant material are well known, there is no scientific approach to reveal its pharmacognostic and pharmaceutical properties. Hence, in the present study an attempt is made to explore the botanical and chemical characteristics of *C. spinosa* flower bud and its pharmacological properties.

MATERIALS AND METHODS

Sample collection

Flower buds of *C. spinosa* L. was procured from herbal market of Thanjavur. The selected plant drug was identified with the help of Flora of Presidency of Madras and authenticated by comparing with voucher specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's College, Tiruchirappalli. After proper identification and authentication, the collected material was cleaned, shade dried and finely (1 mm) powdered.

Powder microscopy

The powder microscopic characters of *C. spinosa* flower bud were studied according to the method of WHO¹¹. Presence of calcium carbonate crystals was observed by taking a pinch powdered drug and treated with acetic acid (60 g/L) and the preparation was mounted and observed

Table 1: Chemical composition and nutritional value of *Capparis spinosa* flower bud (Values are mean \pm S.D, n = 3).

S. No.	Parameters	Content
1	Total carbohydrates (mg/g)	15.3 \pm 0.03
2	Total free amino acids (mg/g)	01.9 \pm 0.09
3	Total proteins (mg/g)	26.1 \pm 0.08
4	Total free fatty acids (mg/g)	79.1 \pm 0.04
5	Total fats (mg/g)	64.3 \pm 0.05
6	Total cholesterol (mg/g)	0.05 \pm 0.01
7	Energy value (Kcal)	744.3 \pm 0.06
8	Total crude fibre (mg/g)	07.2 \pm 0.02
9	Vitamin B1 (μ g/g)	0.40 \pm 0.30
10	Vitamin B2 (μ g/g)	0.20 \pm 0.06
11	Vitamin B3 (μ g/g)	0.80 \pm 0.10
12	Vitamin C (μ g/g)	1.50 \pm 0.04
13	Vitamin E (μ g/g)	0.02 \pm 0.01

Table 2: Phytochemical profile of aqueous extract of *Capparis spinosa* flower bud (Symbols + / - indicates the Presence / Absence of the compound, respectively).

S. No.	Phytochemical	Methods	Result
1	Alkaloids	Dragendroff's reagent	+
		Mayer's reagent	+
		Wagner's reagent	+
		Hager's reagent	+
2	Saponins	Foam with water	+
3	Glycosides	Anthrone reagent	-
4	Steroids	Liebermann burchard	-
5	Flavonoids	Shinado's reagent	-
6	Phenolic compounds	Ferric chloride	+
7	Tannins	Lead acetate	+
8	Quinones	Sulphuric acid	-
9	Anthraquinones	Aqueous ammonia	+
10	Lignins	Phloroglucinol	+
11	Carbohydrates	Molisch's reagent	+
12	Reducing sugars	Fehling's reagent	+
13	Proteins	Millon's reagent	-
14	Aminoacids	Ninhydrin reagent	-

under microscope. Presence of fats and fatty oils was analyzed by taking one pinch of powdered drug with 1-2 drops of sudan red solution, heated gently and the preparation was irrigated with ethanol (750 g/L) and the slides were mounted and observed under microscope. Mucilage was investigated by taking a pinch of powdered drug and treated with Chinese ink (1:10 with water) and the slides were mounted and observed under the microscope. For Starch test, a pinch of powdered drug was treated with Iodine (0.02 M) solution and the slides were mounted and observed under microscope. Presence of tannins was assessed by treating a pinch of powdered drug

with 1-2 drops of ferric chloride (50 g/L) and the slide was mounted and observed under microscope.

Extract preparation

Aqueous extract was prepared by soaking the powder (100 g) in distilled water (1 L) and kept on shaker for 48 h at room temperature. Then, the content was filtered over Whatman No. 42 filter paper and the filtrate was collected, frozen and lyophilized. Dry extract was re-dissolved at 10 mg/ml ratio in distilled water and used for further studies.

Chemical composition

Chemical profiles such as total carbohydrates¹², total proteins¹³, total free amino acids¹⁴, total fats¹⁵, total cholesterol¹⁶, total crude fibre¹⁷, thiamine¹⁸, riboflavin¹⁹, niacin²⁰, Vitamin E²¹, Vitamin C²² were estimated. Energy value was calculated using the formula 4 x (carbohydrates) + 4 x (proteins) + 9 (fats).

Phytochemical analysis

Different qualitative chemical tests were performed using standard procedures to identify the presence of major constituents²³⁻²⁵. The major phytochemical constituent of *C. spinosa* aqueous extract was investigated using LC-ESI-MS/MS (Make: Bruker, Model: MicroTOF-Q II). Solution (50 μ l) was injected for liquid chromatography separations in a C18 reverse phase column (120 \AA , 2.1 x 150 mm, 3.0 μ m, Dionex, USA) and UV detector was set arbitrarily at 330 nm. Gradient elution at a flow rate of 0.2 ml/min was performed using mobile phase (Solvent A: Acetonitrile and B: water with 1% acetic acid). The gradient started from 1% of A for 0.2 min and it was then brought to 75% A at 16th min and then reaching at 100% A at 19th min to 5% A at 21st min and was maintained at same condition till run ends at 30th min. Eluted compounds were then identified using MS and their respective MS/MS pattern. The result of molecular mass was compared with mass bank data and the major phytochemical was identified.

Bio-activity assays

Different concentrations of aqueous extract was analyzed for biological properties such as antioxidant, anti-inflammatory and antimicrobial activities. The antioxidant action was measured using DPPH free radical scavenging assay²⁶ and lipid peroxidation inhibition assay²⁷. Anti-inflammatory activity was examined based on *in vitro* methods such as inhibition of protein (Albumin) denaturation activity²⁸, protease inhibition activity²⁹ and RBC membrane stabilization assay³⁰. Four different concentrations of aqueous extract were investigated for antibacterial activity by the disc diffusion method against *Bacillus cereus*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Salmonella enteritidis* strains.

Toxicity study

Acute oral toxicity of the aqueous extract was investigated using the limit dose test of up and down procedure according to OECD Guidelines. The experiment was conducted at the Central Animal Facility, SASTRA University (Animal Ethical Clearance No. 358/SASTRA/IAEC/RPP). Wister type female rats (*Rattus norvegicus*) with 8 – 12 weeks age were acclimatized for one week and then administered with aqueous extract of *C. spinosa* in a single dose (2000 mg/kg bw) and observed for

Table 3: Antibacterial activity of aqueous extract of *Capparis spinosa* flower bud (Values are mean \pm SD, n = 3).

S. No.	Bacteria	Zone of inhibition (mm)				
		Standard (Gentamicin, 30 μ g / ml)	Extract (125 μ g/ml)	Extract (250 μ g/ml)	Extract (500 μ g/ml)	Extract (1000 μ g/ml)
1	<i>Bacillus cereus</i>	9 \pm 0.05	5 \pm 0.06	7 \pm 0.09	8 \pm 0.07	9 \pm 0.06
2	<i>Staphylococcus aureus</i>	9 \pm 0.06	4 \pm 0.05	5 \pm 0.07	6 \pm 0.06	8 \pm 0.08
3	<i>Campylobacter jejuni</i>	8 \pm 0.03	1 \pm 0.02	3 \pm 0.03	6 \pm 0.02	8 \pm 0.21
4	<i>Salmonella enteritidis</i>	8 \pm 0.04	5 \pm 0.04	6 \pm 0.05	8 \pm 0.09	8 \pm 0.10

Table 4: Acute oral toxicity results observed in female rats on the safety of aqueous extract of *Capparis spinosa* flower bud.

S. No.	Parameters	Observation
1	Feed intake on 1 st day (g)	4.29
2	Feed intake on 14 th day (g)	13.7
3	Body weight on 1 st day (g)	131.08
4	Body weight on 14 th day (g)	160.05
5	Appeared normal (1-14 days)	5/5
6	Found death (1-14 days)	0/5
7	Catalepsy (1-14 days)	0/5
8	Chromodacryorrhea (1-14 days)	0/5
9	Clonic (1-14 days)	0/5
10	Coma (1-14 days)	0/5
11	Convulsion (1-14 days)	0/5
12	Diarrhea (1-14 days)	0/5
13	Dullness (1-14 days)	0/5
14	Excessive grooming (1-14 days)	0/5
15	Change in Gait (1-14 days)	0/5
16	Hyperactivity (1-14 days)	0/5
17	Lacrimation (1-14 days)	0/5
18	Nasal discharge (1-14 days)	0/5
19	Nasal irritation (1-14 days)	0/5
20	Piloerection (1-14 days)	0/5
21	Polyuria (1-14 days)	0/5
22	Prostration (1-14 days)	0/5
23	Repetitive circling (1-14 days)	0/5
24	Respiratory distress (1-14 days)	0/5
25	Salivation (1-14 days)	0/5
26	Tonic (1-14 days)	0/5
27	Tremor (1-14 days)	0/5
28	Urogenital staining (1-14 days)	0/5

physical and clinical parameters for 14 days and the results were recorded.

RESULTS AND DISCUSSION

Powder microscopy

Powder microscopic studies were carried out in the selected plant and the results obtained were presented in Figure 1. Powder microscopy studies revealed the presence of lignified parenchyma cells, long curved uniseriate trichome, short trichome, sclerenchyma cells, striated sclereid, prismatic calcium oxalate crystal, xylem vessels with spiral thickening and simple starch grain. The noticed microscopic features of *C. spinosa* flower bud powder could be used as botanical standards of this herbal drug, which also might be useful in fixing its quality control standards.

Chemical composition

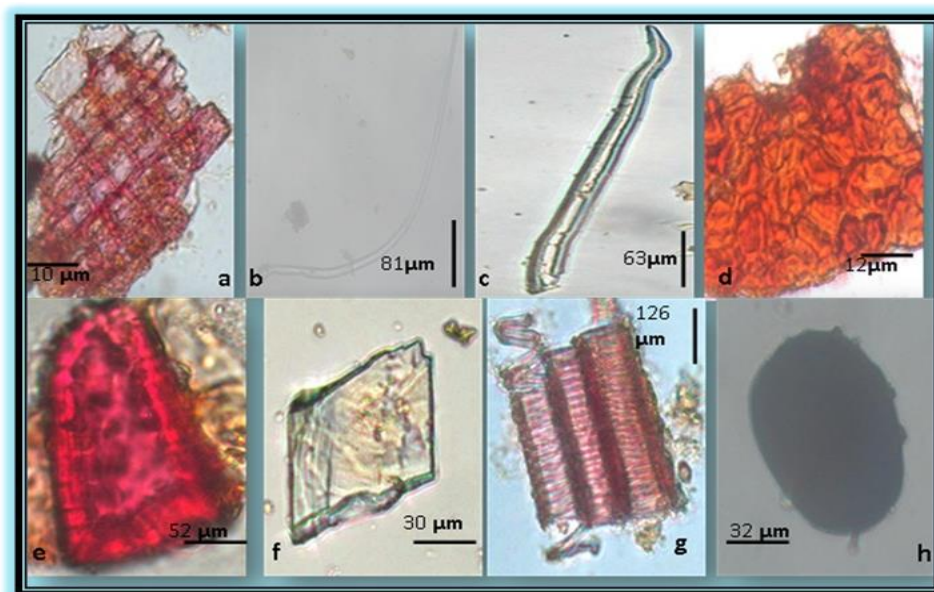
Chemical composition of aqueous extract of *C. spinosa* flower bud was given in the Table 1. From the nutritional point of view, flower buds of *C. spinosa* L. possess total carbohydrates (15.3 mg/g), total free amino acids (1.93 mg/g), total proteins (26.1 mg/g), total free fatty acids (79.1 mg/g), total fats (5.5 mg/g), cholesterol (0.05 mg/g), energy value (744.3 kcal) and crude fibre (7.2 mg/g). Chemical composition values revealed the presence of good amount of carbohydrates, proteins and fats in *C. spinosa* flower buds that play major roles in human nutrition. Deficiencies or excess of nutrients especially proteins, carbohydrates and vitamins lead to various complications and metabolic disorders in human beings. The presence of proteins will serve as building block of cells, muscles, cartilage, skin, hormones, enzymes, vitamins. Intake of crude fibers present in the selected plant material (7.2 mg/g) can lower the serum cholesterol level, risk of coronary heart disease, hypertension and constipation. Flower buds of *C. spinosa* L. also possess Vitamin B1 (0.40 μ g/g), Vitamin B2 (0.20 μ g/g), Vitamin B3 (0.80 μ g/g), Vitamin C (1.50 μ g/g) and Vitamin E (0.02 μ g/g) (Table 1). Ascorbic acid protects low density lipoproteins *ex vivo* against oxidation. The presence of high quantities of vitamin E confirms the capacity of providing protection from free radicals and products of oxygenation. Vitamin E works in conjunction with other antioxidant nutrients present in these plant materials to quench free radicals. Vitamin E also inhibits lipoxxygenase, an enzyme responsible for the formation of pro-inflammatory leukotrienes³¹.

Phytochemical profile

The data obtained in the preliminary phytochemical screening were given in Table 2. Preliminary phytochemical screening of flower buds of *C. spinosa* L. revealed the presence of alkaloids, reducing sugars, carbohydrates, saponins, phenolic compounds, tannins, anthraquinones and lignins in aqueous extract. LC-MS analysis of aqueous extracts confirms the presence of quercetin as the major phytoconstituent in the aqueous extract of *C. spinosa* flower bud (Figure 2) based on the detected mass/ion ratio of 302.236. Comparison of our results with mass bank data revealed the presence of Quercetin based on the fragmentation pattern of the daughter ions (285 and 251). In agreement with our findings, Behnaz et al.³² have also quantitatively analyzed the quercetin in the same plant.

Bio-activity assays

In the present study, the plant was screened for antioxidant activity using lipid peroxidation and DPPH free radical



(a). Lignified parenchyma cells, (b). Long curved uniseriate trichome, (c). Short trichome, (d). Sclerenchyma cells, (e). Striated sclereid, (f). Prismatic calcium oxalate crystal, (g). Xylem vessels with spiral thickening, (h). Simple starch grain.

Figure 1: Powder microscopic studies on *Capparis spinosa* flower bud.

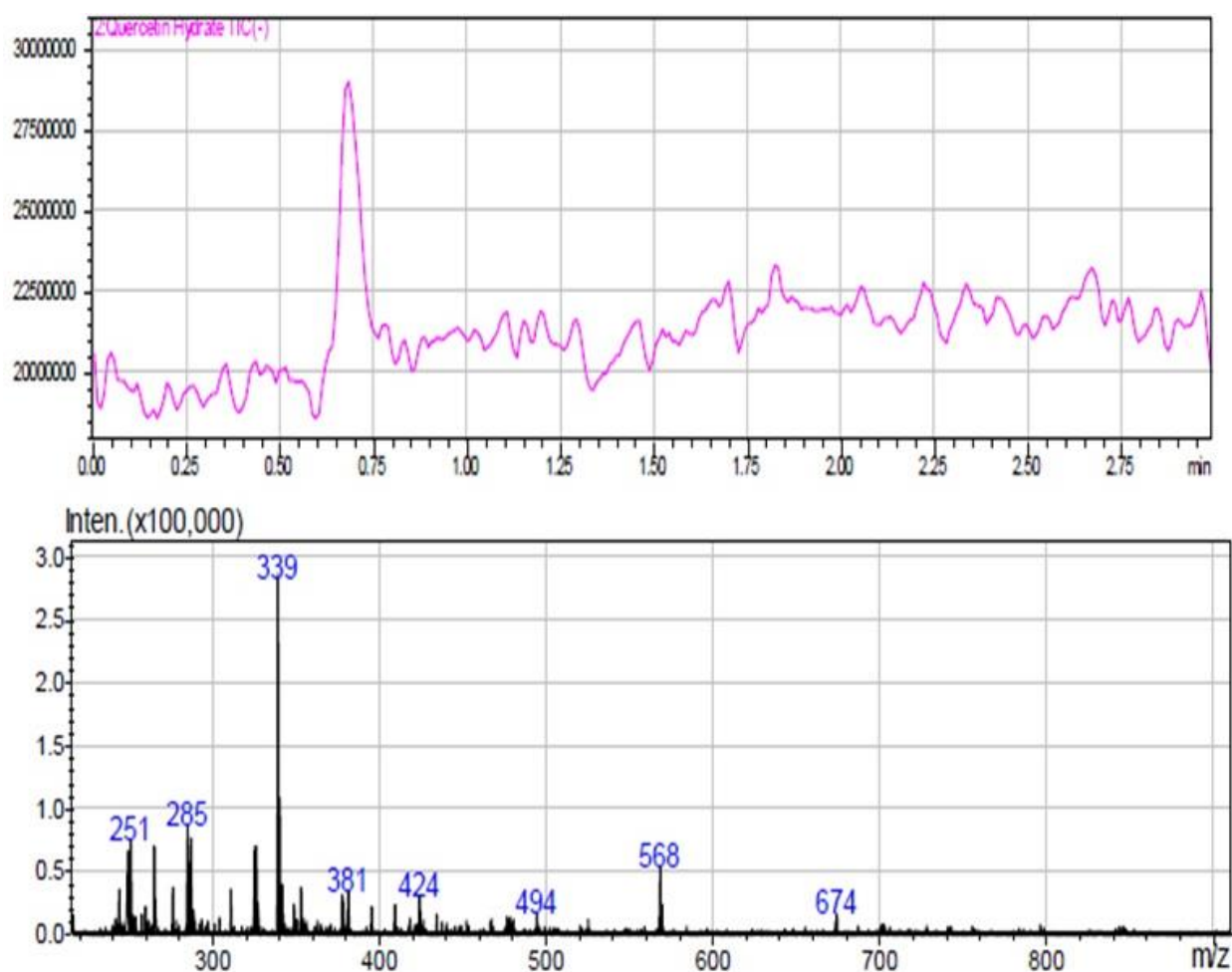


Figure 2: LC-ESI-MS/MS chromatogram of aqueous extract of *Capparis spinosa*.

scavenging methods and the data of the results obtained were shown in Figure 3. The flower buds of *C. spinosa* L.

extract exhibited maximum activity of 98.87% and 99.13% at 1000 μg/ml in lipid peroxidation and DPPH free radical

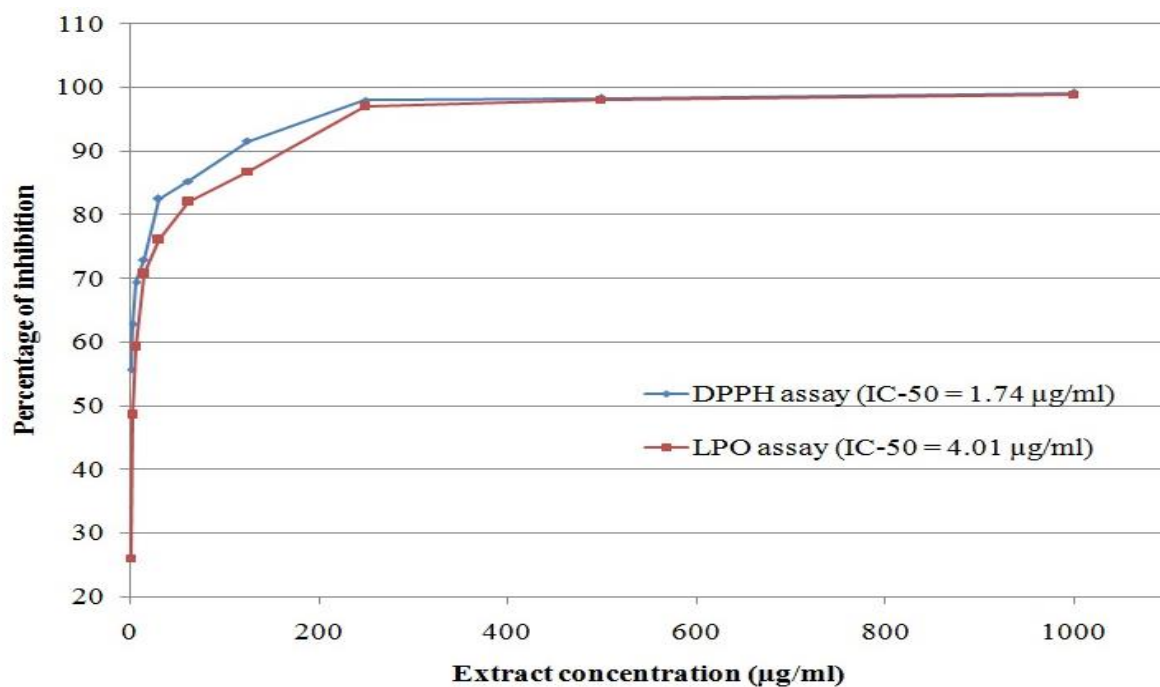


Figure 3: Antioxidant activity of aqueous extract of *Capparis spinosa* flower bud.

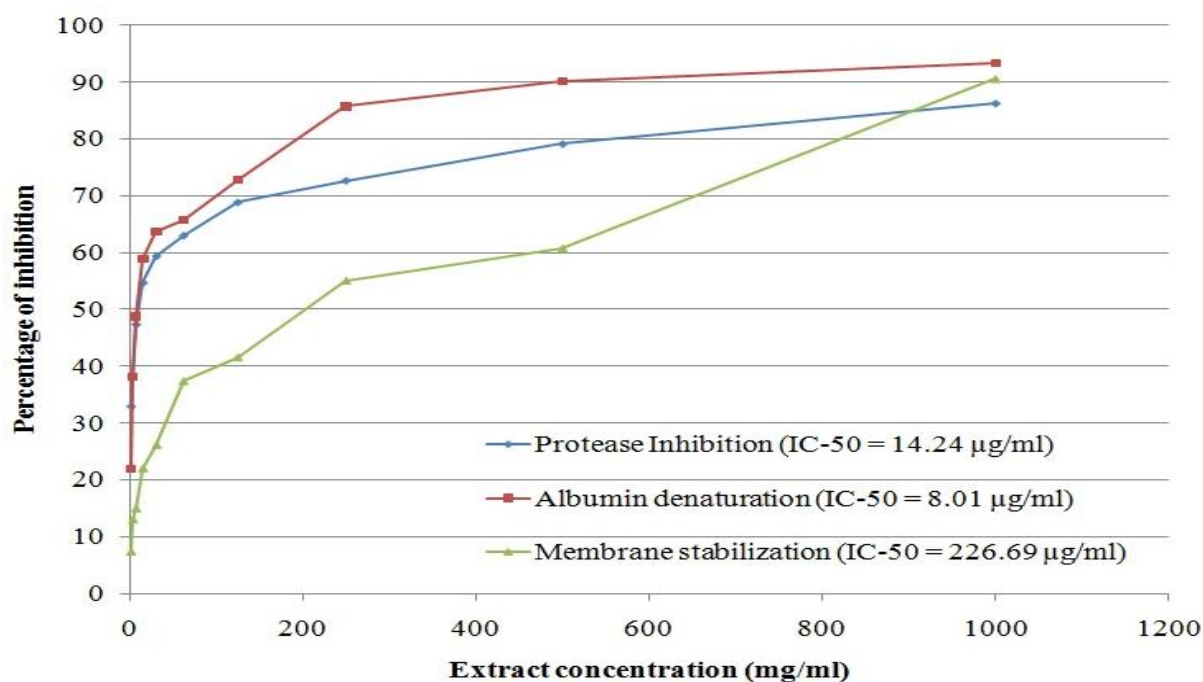


Figure 3: Anti-inflammatory activity of aqueous extract of *Capparis spinosa* flower bud.

scavenging assays respectively. Both DPPH radical scavenging (IC-50 = 1.74 µg/ml) and lipid peroxidation inhibition (IC-50 = 4.01 µg/ml) activities were dose-dependent, which has been found to be increased with increase in the concentration of the extract. Such radical scavengers may protect tissues from ROS and thereby prevent oxidative-damage related diseases.

Anti-inflammatory activity

In the present study, the aqueous extract was evaluated for anti-inflammatory activity employing *in vitro* methods like

inhibition of protease activity, inhibition of albumin denaturation and RBC membrane stabilization and the results were presented in Figure 4. High concentration (1000 µg/ml) of the extract was revealed anti-inflammatory efficacy up to 86.36%, 93.40% and 90.64% by inhibiting of protease activity (IC-50 = 14.24 µg/ml), albumin denaturation (IC-50 = 8.01 µg/ml) and membrane stabilization (IC-50 = 226.69 µg/ml). The extract exhibited anti-inflammatory efficacy in a dose-dependent manner. In

support of our findings, previously an anti-inflammatory compound was reported from this plant⁵.

Antibacterial activity

Flower buds of *C. spinosa* L. were screened for their antibacterial activity employing disc diffusion method and the data of the results obtained were given in Table 3. The extract showed different zones of inhibition at different concentrations against bacterial pathogens. The results obtained in mm represent the diameter of bacterial growth inhibitory zone. Extract exerted better activity at 1000 µg / disc concentration against the tested microorganisms. The results suggested that antibacterial activity of flower buds of *C. spinosa* L. against test organisms increased when used in high concentration. The result of this study indicated that quercetin present in the extract might have inhibited the bacterial growth. Previously, the inhibiting potential of aerial parts of *C. spinosa* plants has been reported against Gram-positive and negative bacteria³³. Already Ibrahim³⁴ reported the anti-bacterial activity of *C. spinosa* flower extracts against skin wound causing bacterial species. Likewise, extracts obtained from fermented caper fruits have been found to contain effective antimicrobial agents against bacterial strains that had become resistant to drugs like ciprofloxacin, vancomycin and teicoplanin³⁵⁻³⁷.

Toxicity

Toxicity of the aqueous extract of *C. spinosa* was investigated in animal model and the data obtained presented in Table 4. At a dose of 2000 mg/kg body weight of extract, animals did not show any signs of adverse reactions and no changes in animal's behavior during daily monitoring upto 14 days after the administration of the extract. No significant difference in body weight gain was observed after 1, 7 and 14 days. In addition, no mortality was recorded throughout the period of observation. As there was no mortality and clinical signs of toxicity in the tested dose, LD₅₀ value of flower buds of *C. spinosa* L. was found to be greater than 2000 mg/kg.

CONCLUSIONS

The present research work revealed the pharmacognostic and pharmacological properties of an Indian traditional medicinal plant, *C. spinosa* (flower buds). The botanical and chemical characteristics recorded from the present work could be set as quality control standards of this plant material for authentic use. This plant material is noted to possess significant biological activities such as antioxidant, anti-inflammatory and anti-bacterial activities without any toxicity.

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