



Phytoconstituents and antioxidant activity in ethanolic extract of *Capparis sepiaria* L. leaf.

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ABSTRACT

India is known for its rich tradition in human wealth and medicine from decades. It is also home for many valuable medicinal plants which are being used in the different system of medicines. *Capparis sepiaria* known as himsra in Ayurveda, is used as an active ingredient in many oils. The present study aimed to evaluate phytoconstituents and antioxidant activity in ethanolic extract of *Capparis sepiaria* leaf by using standard methods. The phytoconstituents present in ethanolic extract of leaf, were flavonoids 570.0 ± 0.00 , phenols 480.0 ± 0.01 , tannins 476.0 ± 0.00 , alkaloids 381.1 ± 0.00 , terpenoids 0.321 ± 0.02 and glycosides 0.180 ± 0.00 mg/100gm respectively. *In vitro* antioxidant activity in ethanolic extract of *Capparis sepiaria* leaf revealed the DPPH and Nitric oxide radical scavenging activity of ethanolic extract of leaf showed near values to standard and control. Hydrogen peroxides radical scavenging activity and reducing power assay showed higher values than standard and control. Study concludes that ethanolic extract of *Capparis sepiaria* leaf was a natural source of phytoconstituents and antioxidants.

KEY WORDS: Antioxidants, *Capparis sepiaria*, ethanolic extract, DPPH, leaf, phenols, Phytoconstituents.

INTRODUCTION

Plants still constitute one of the major sources of drugs in modern and traditional medicine worldwide¹. Large sections of the Indian population still rely on plant-based medicines as they are abundantly available, economical, and have little or no side effects. In addition to their cultural acceptability of late medicinal plants have gained global importance in the alternative healthcare system, for their proven and effective curative properties². Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of the defense system against various diseases and stress conditions. The phytochemicals are grouped into two main categories³. namely primary constituents which includes amino acids, common sugars, proteins and chlorophyll etc., and secondary constituents consisting of alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds etc^{4,5}. Phytoconstituents are extracted by using

variety of solvents hexane, pet ether chloroform, methanol, acetone, ethyl acetate, ethanol and aqueous which is a universal solvent. The extracted of plant material can be used as crude drugs for treating diseases. Plants are rich sources of free radical scavenging molecules such as vitamins, phenolic acids, flavonoids and other metabolites which are rich in antioxidant activity, Flavonoids are a class of secondary plant metabolites with significant antioxidant and chelating properties^{2,6}. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid [Vitamin C] terminate these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes produced internally or the dietary antioxidants, vitamin A, vitamin C and vitamin E⁷. Polyphenols are a large and diverse class of compounds many of which occur naturally in a wide range of food and plants⁸. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity^{2,9}. *Capparis sepiaria* is hedge caper or wild bushy shrub belongs to the family Capparaceae, commonly known as kadu kattari or kanthari in Kannada and ahimsra in Sanskrit, native to India. **Botanical description:** It is a prickly, evergreen much branching shrub growing to 3 to 5 meters tall with velvet hairy stem. Leaves elliptic, oblong to ovate or nearly round, 1-5cm long, 1-2 cm broad often retuse, softly to hair less, leaf stalk 2-5 mm long with stipular spines. Flowers usually in corymbose at branch ends clusters of 10-30, small 5-10 mm across white, flowers February on words, fruits in April and seeds about 6 mm (Fig-6.). **Traditional uses:** It has febrifuge properties and is used to treat skin diseases, tumours, inflammation, jaundice, dysentery and diseases of the muscles¹⁰. It is used as blood purifier, stomachic, tonic and appetizer. It's flowers, leaves and roots are used in cough and toxemia and root powder is also used as a cure for the snakebite. In ayurveda, it is used as an active ingredient in many in oils and used for digestive disorders, anorexia, asthma, colds and abscess. Medicinal uses of *Capparis sepiaria* are well known but the supporting scientific data available is very scanty¹¹. Hence the ethanolic extract of *Capparis sepiaria* leaf is subjected for phytoconstituents and antioxidant activity.

MATERIALS AND METHOD

Collection and identification

The plant is identified by referring flora of Gulbarga and Gamble¹², and with the help of digital flora of Karnataka and the plant list (WFO), (Photograph of plant habit, leaf and flower etc. were represented in Fig 1 and 2).



Fig. 1. Habit



Fig. 2. Leafy twig with flower

Taxonomic classification

Kingdom: Plantae
 Division: Mangoliophyta
 Class: Magnoliopsida
 Order: Brassicales
 Family: Capparaceae
 Genus: *Capparis*
 Species: *Capparis sepiaria* L.

Preparation of plant extract

The plant material (leaf) was first washed with tap water thoroughly to remove dirt and soil deposits and dried under shade until the moisture content was completely removed; such dried leaves were powdered mechanically. Approximately 250 gm of this dried powder extracted with 100% ethanol by continuous hot percolation, using the Soxhlet apparatus. The resultant greenish-black extract was used to prepare different concentrations.

DETERMINATION OF PHYTOCONSTITUENTS

Estimation of Flavonoids

The estimation of alkaloids was carried out according to the procedure of²

Reagents: Vanillin reagent-1gm of crystalline Vanillin is dissolved in 100ml of 70% con H₂SO₄.

Procedure: 0.1 and 0.2ml extract is taken in the test tube diluted to 2ml with distilled water and to this 4ml Vanillin reagent was added rapidly after 15 minutes the brick red color is read at 599nm using spectrophotometer against reagent blank. The standard curve is plotted using different concentrations of Phloroglucinol as the standard flavonoid. The amount of flavonoids present in each sample was calculated with the help of a standard graph.

$$\text{Flavonoids in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}} \times 100$$

Estimation of phenols

The estimation of phenols was carried out according to the procedure of².

Reagents: 80% ethanol, Folin-Ciocalteu's reagent and 20% Na₂CO₃ Soln. Catechol std. 100mg of Catechol is dissolved in 100ml distilled water in a volumetric flask and further diluted to ten times to obtain a working standard.

Procedure: Take 0.1 and 0.5 ml aliquots and their final volume is made up to 3ml with distilled water. To these tubes, 0.5 ml Folin-Ciocalteu's reagent (FCR) and after 3 min, 2 ml of 20% (w/v) Na₂CO₃ were added and mixed thoroughly. All these tubes were incubated in a boiling water bath for a minute and cooled. The blue color appeared (due to molybdenum complex) absorbance is measured at 650 nm in a spectrophotometer against the blank reagent. A standard curve is plotted using 1% Catechol as phenol.

$$\text{Phenols in mg/100gm} = \frac{\text{Graphical value}}{\text{Wt. of the plant material}} \times \frac{\text{volume of total extract}}{\text{volume taken for reading}} \times 100$$

Estimation of alkaloids

The estimation of alkaloids was carried out according to the procedure of²

Reagents: 20% Acetic acid in ethanol (200ml), Concentrated NH₄OH.

Procedure: 5gm of the sample was weighed in to a 250ml beaker and 200ml of 20% Acetic acid in ethanol was added and stored for 4 hours. This content was filtered, and the extract was concentrated by using a water bath to one-quarter of the original volume. Then concentrated NH₄OH was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitation was collected and weighed.

Amount of alkaloid = W₂ – W₁,

W₁- Weight of the empty filter paper and W₂- Weight of the filter paper along with ppt.

Estimation of tannins

The estimation of tannins was carried out according to the procedure of² with little modification. Procedure: The working standard solution was pipette out in a series of test tubes ranging from 0.1, 0.2, 0.3, 0.4 and 0.5ml. A volume of 0.2ml of the leaf extract is added into another test tube and 0.8ml of distilled water is added to make up the volume to 1ml in all the tubes following which 1ml of ferric chloride and potassium ferricyanide each is added to all the test tubes and mixed well. The absorbance was read at 700nm spectrophotometrically.

Estimation of glycosides

The estimation of glycosides was carried out according to the procedure of¹³.

Reagents: Ethanol (70%), (Na₂HPO₄), (12.5%) Lead acetate and Methanol.

Procedure: 25g of powder plant material was mixed with 200ml of 70% ethanol and kept on rotator shake at 3000 rpm for 6 h at room temperature. Filter it and add 500ml of distilled water followed by 100ml of 12.5% lead acetate (to precipitate tannins, resins, and pigments). Later the volume made up to 800ml with distilled water and kept on the shaker for 10min at 3000rpm. To this, add 200ml of (4.77%) Disodium hydrogen phosphate (Na₂HPO₄) solution is added to ppt excess of Pb ions. The above solution is then filtered and evaporated to dryness. Calculate the percentage of glycoside using the following formula.

$$\text{Percentage of glycosides} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Determination of terpenoids:

The estimation of terpenoids were carried out according to the procedure of^{14,15}.

Procedure: About 10 gm of *Capparis sepiaria* leaf powder was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids.

DETERMINATION OF IN-VITRO ANTIOXIDANT STUDY**DPPH radical scavenging activity**

The DPPH (2, 2 diphenyl-1-picrylhydrazyl) radical scavenging activity was carried out following the procedure described by¹⁷.

Procedure: The 2ml of various concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg of (test sample) in ethanolic extract was prepare to which 1ml of 0.1 mM of DPPH in methanol solution was added. After 30 minutes of incubation period, the absorbance was measured at 517nm. The free radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution (Control). The Ascorbic acid and Butylated hydroxyl anisole were used as standards. The DPPH radical scavenging activity was calculated using the following equation. Percentage of inhibition = $(A_c - A_s) / A_c \times 100$. Where A_c is the absorbance of the control and A_s is the absorbance of test the sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging activity was carried out following the procedure described by¹⁶.

Procedure: The 0.5ml of 0.1M PBS (pH7.4) is added to the 2ml of 10 mM Sodium nitroprusside and mixed well. To this mixture various concentrations of ethanolic leaf extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg were added and incubated for 160 min at 30°C. After the incubation period, its absorbance was measured at 546nm. The nitric oxide radical scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution. Ascorbic acid and Phloroglucinol were taken as standards. The percentage of inhibition of nitric oxide radicals by extracts was calculated by using the following formula. Percentage of inhibition = $(A_c - A_s) / A_c \times 100$. Where A_c is the absorbance of the control and A_s is the absorbance of the test sample.

Hydrogen peroxide assays

Hydrogen peroxides radical scavenging activity was carried out following the procedure described by¹ with little modification¹⁶.

Procedure: A solution of H_2O_2 (30 mM) is prepared in distilled water. The ethanolic leaf extract of different concentrations such as 0.2, 0.4, 0.6, 0.8, 1 and 2mg in 2ml phosphate buffer (0.1 M, pH 7.4) mixed well and 0.5 μ l of H_2O_2 (30 mM) solution is added. After 10 minutes the absorbance of the reaction mixture was recorded at 230 nm. The hydrogen peroxide scavenging activity of each sample was determined by comparing its absorbance with that of a blank solution. The percentage of inhibition of hydrogen peroxide radical was calculated by using the following formula, H_2O_2 scavenging activity (%) = $(A_c - A_s) / A_c \times 100$. where A_c is the absorbance of the control and A_s is the absorbance of the sample.

Reducing power assay

Reducing power assay was carried out according to the procedure described by².

Procedure: The 1ml of different concentrations of ethanolic leaf extract such as 0.2, 0.4, 0.6, 0.8, 1 and 2 mg were mixed with 0.1M phosphate buffer (pH 6.6) and potassium ferricyanide (1ml, 1%), and the mixture was incubated at 50°C for 20 min. Next 2ml of Trichloroacetic acid (10%) is added to the reaction mixture, and then centrifuged at 10000 RPM for 10 min. The upper layer of the solution (1ml) is mixed with distilled water (1ml) and ferric chloride (150 μ l, 0.1%), and the absorbance was measured at 700nm against the blank sample (Control).

STATISTICAL ANALYSIS

The results were expressed as mean $\pm$ Standard error mean [Significant value $P < 0.01^{***}$, $P < 0.01^{**}$ and $P < 0.05^{**}$] using one-way ANOVA (Graph Pad Instat3) and Microsoft Excel.

RESULTS

The ethanolic extract of *Capparis sepiaria* leaf was subjected to phytoconstituents and *in vitro* antioxidants activity. The quantitative estimation in ethanolic extract of *Capparis sepiaria* leaf showed the presence of flavonoids 570.0 ± 0.00 phenols 480.0 ± 0.01 , tannins 476.0 ± 0.00 , alkaloids 381.1 ± 0.00 , terpenoids 0.321 ± 0.02 and glycosides 0.180 ± 0.00 mg/100gm respectively (tabulated in (Table-1). Flavonoids were present in highest amounts followed by phenols, tannins, alkaloids, terpenoids and glycosides respectively.

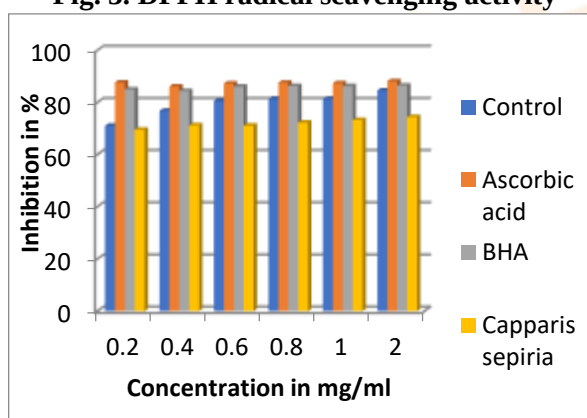
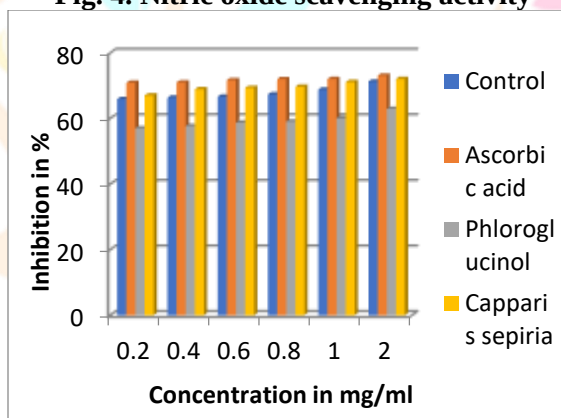
Table-1. Phytoconstituents activity

Sl. No.	Phytoconstituents	Milligrams/100gm
1	Flavonoids	570.0±0.00, P<0.01.***
2	Phenols	480.0±0.01, P <0.01.*
3	Tannins	476.0±0.00, P <0.01.*
4	Alkaloids	381.1±0.00, P <0.01.**
5	Terpenoids	0.321±0.02, P <0.05.**
6	Glycosides	0.180±0.00, P <0.05.**

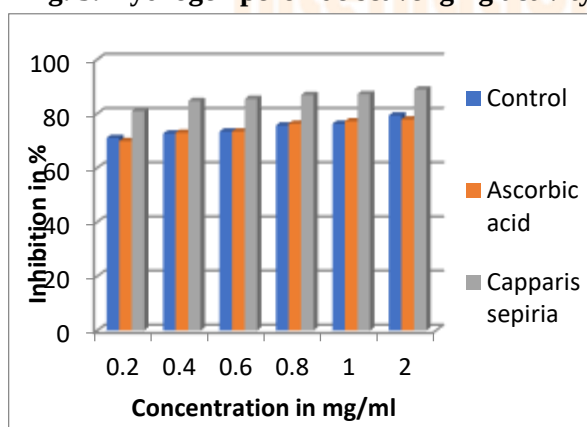
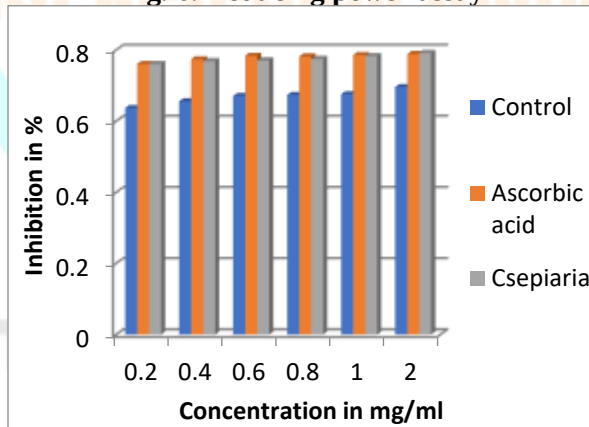
Mean ± Standard error mean

In vitro antioxidant activity in ethanolic extract of *Capparis sepiaria* leaf revealed that DPPH radical scavenging activity of ethanolic extract of leaf showed near values to standard and control [Fig.3], Nitric oxide radical scavenging activity was near to control lower than ascorbic acid and higher than std Phloroglucinol [Fig. 4], Hydrogen peroxide radical scavenging activity and reducing power assay showed higher values than standard as well as control at various concentrations [Fig. 5 and 6].

In vitro antioxidant activity

Fig. 3. DPPH radical scavenging activity**Fig. 4. Nitric oxide scavenging activity**

BHA: Butyl hydrated anisole

Fig. 5. Hydrogen peroxide scavenging activity**Fig. 6. Reducing power assay**

Discussion

Phytotherapy is gaining popularity as WHO encourages the appropriate ethnomedicinal use and signifies safety evaluation of herbal medicines^{17,18,19}. The earlier results of *Capparis sepiaria* indicated that the plant possess numerous biologically active compounds which could serve as potential source of drugs in herbal medicine²⁰. Present results showed the presence of flavonoids, phenols, tannins, alkaloids and glycosides which can act as effective antioxidants. The various solvents that are used in the extraction of plays important role for defining effectiveness of phytoconstituents. Various solvents like water (Aqueous) are universal solvent, used to extract plant products with antimicrobial activity. Though traditional healers use primarily water but plant extracts from organic solvents have been found to give more consistent antimicrobial activity compared to water extract^{21,2}. Ethanol: The higher activity of the ethanolic extract as compared to the aqueous extract can be attributed to the presence of higher amounts of polyphenols as compared to aqueous extracts. Since nearly all of the identified components from plants

active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol²². Phytoconstituents are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas. By boosting the human immune system plant-based antioxidants block free radicals produced through oxidation, thus inhibiting chain reactions that could lead to the degradation and death of cells²³. Traditional knowledge about the therapeutic potential of plants is necessary to isolate and identify biologically active products from plants. Therefore, isolation and identification of bioactive compounds present in a crude extract serve as the building block for the development of new type of therapeutics with new mechanisms of action²⁴.

Conclusion

The present study concludes that the ethanolic extract of *Capparis sepiaria* natural source of phytoconstituents and antioxidants which justify the use of this plant as crude drug in folklore medicine. Since *Capparis sepiaria* was a well-known plant in Ayurveda, it has to be explored more for its therapeutic uses.

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Conflict of interest

There is no conflict of interest

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