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Evaluation of Antioxidant Capacities and total Polyphenols in Various Edible Parts of *Capparis spinosa* L. Collected from trans-Himalayas

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ABSTRACT

The phytochemical screening, antioxidant capacity, and total polyphenols in the methanolic extract of leaves, flower buds, roots and fruits of *Capparis spinosa* collected from trans-Himalayan region of Ladakh were assessed in an effort to corroborate its medicinal and culinary potential. Highest DPPH and ABTS radical scavenging activity were observed in the leaves and least in dried fruit samples, even FRAP assay also illustrated the same trend. IC50 values of DPPH assay was highly correlated with that of ABTS (R^2 = 0.9084) and FRAP assay (R^2 = 0.9771). However, IC50 value of ABTS was reasonably correlated with FRAP assay (R^2 = 0.5838). The highest phenolic and flavonoid content was recorded in the leaf samples (24.78 and 5.69 mg GAE/g DW respectively), whereas it was lowest in the dried fruit samples (4.07 mg quercetin equivalent/g DW and nil, respectively). The total phenolic contents were highly correlated with IC50 value of ABTS (R^2 = 0.9084), DPPH (R^2 = 0.9388) and FRAP value (R^2 = 0.9618). But, total flavonoid contents were highly correlated with ABTS (R^2 = 0.7449), DPPH (R^2 = 0.8791) and FRAP values (R^2 = 0.9588). Thus, this study has validated the medicinal potential of all the edible parts of the *C. spinosa.*

Keywords: Capers; Phytochemical screening; Antioxidant activity; Polyphenols; Correlation

1. INTRODUCTION

There is a growing demand for natural products in the human diet, both due to the possible negative effects of synthetic food additives on human health and also to increase consumer perception of this problem in recent years. The edible parts of *Capparis* like flower buds, fruits, leaves, and root barks are known to contain a range of antioxidants, including polyphenolic compounds, which are significantly correlated with antioxidant potential[1,2].

Capparis spinosa L. (Capparidaceae) also called 'Caper' and locally known as '*Kabra'* is one of the oldest known medicinal plants in 'Amchi system' which is local medicinal system of Ladakh (India). It has been utilised in preparations of various herbal formulations since long time for the treatment of a range of ailments like gastrointestinal infection, diarrhea, diabetes, rheumatism and also used as a leafy vegetable and forage by local people residing in trans-Himalayan region of Ladakh[3,4]. In India, it is found in the inner valleys of trans–Himalayas between 3020 m – 3890 m above mean sea level (AMSL) which includes Indus, Nubra and Suru valleys of Ladakh region. It is an under-utilised plant which grows wild at roadside, on dry rocky slopes of stony soils and can withstand extreme temperatures (–30°C to +35°C) of Ladakh and is also highly drought tolerant. This plant has multiple uses in cuisine as salad, pickle, and condiments[5,6]. *Capparis* is known to contain a wide variety of antioxidant compounds including phenolic compounds which are found to be well correlated with antioxidant potential[7].

Earlier phytochemical studies have shown the presence of alkaloids, flavonoids, polyphenols, indolic and aliphatic glucosinolates in *C. spinosa*[8-10]. It has also been studied pharmacologically, the alcoholic and aqueous extract of plant has antimicrobial, hepatoprotective, antihyperglycemic, and protects against oxidative stress in systemic sclerosis dermal fibroblasts[11,12]. The methanolic extract of leaves shows very high antioxidant activity and polyphenolic content[7]. The flower buds showed antioxidant and antibacterial activity and have excellent photoprotection against UVB-induced skin damage[13]. Natural antioxidants present in *C. spinosa* can scavenge harmful free radicals from our body. It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defense or by supplementing with proven dietary antioxidants[14].

Until now, there has been no report on comparative study of the polyphenol contents and antioxidant activity in different edible parts of *C. spinosa* from trans-Himalayan region of Ladakh, India. Keeping in view to validate the medicinal potential of *C. spinosa* growing wild in trans-Himalayas, the present investigation was designed to study the major phytochemicals, polyphenols and antioxidant capacity among different edible parts viz. leaves, flower buds, fruits and roots of *C. spinosa* and to correlate the antioxidant capacity with that of polyphenol contents in different edible parts of *C. spinosa*.

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2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Crude Extract

The edible parts used in the present investigation were collected from *C. spinosa* plants growing wild in Ladakh. Ladakh is a part of Indian trans Himalaya situated at an altitude of 2200 m - 6100 m amsl, is characterised by diverse and complex land formations. It is located at the latitude of 31º44'57" to 32º59'57"N and longitude of 76º46'29" to 78º41'34" E[15]. The plant was identified by its vernacular name by the local people and later authenticated by the Herbarium of Defence Institute of High Altitude Research, Leh-Ladakh, India. The edible parts were first decontaminated by washing under tap water followed by sterilised distilled water, shade dried at room temperature (26 °C) to constant weights. The dried samples (bulk of five samples) were then pulverised individually and 10 g each was separately shaken in methanol for 72 h in an orbital shaker at room temperature. Extracts were then filtered using a Buckner funnel and Whatman No. 1 filter paper and filtrate was concentrated to dryness under reduced pressures (337 mbar) at 40 ºC using rotary evaporator. Then, the extract was resuspended in methanol to make 50 mg/ml stock solution[16].

2.2 Chemicals

The chemicals of various brands used include 2,2-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,4,6-tripyridyl-*s*-triazine (TPTZ), potassium ferricyanide, potassium persulfate, trichloroacetic acid, gallic acid and quercetin from Sigma Chemical Co. (USA); $FeCl_3$, ascorbic acid, and butylated hydroxyl toluene (BHT) from HIMEDIA Laboratories Pvt. Ltd. (India); Folin-Ciocalteu phenol reagent, anhydrous sodium carbonate (Na_2CO_3), hydrochloric acid (HCl), glacial acetic acid, potassium chloride, sodium acetate trihydrate and solvent methanol were from Merck Chemicals (Darmstadt, Germany). All the chemicals used were of analytical grade.

2.3 Phytochemical Screening

Phytochemical analysis of the crude extract of *C. spinosa* was performed qualitatively for the presence of alkaloids, tannins, saponins and anthraquinones as plant constituents according to a standard method[17]. The alkaloids were detected by treating the samples with Dragendorff's reagent, which resulted in the formation of a precipitate at the base of the test tube. Those samples, in which green or black color appeared when mixed with aqueous $FeCl_3$, were considered positive for tannins. Further, the presence of saponins was considered on the basis of frothing after vigorous shaking of diluted samples.

2.4 Determination of Antioxidant Activity

2.4.1 DPPH Radical Scavenging Assay

The effect of the extracts on DPPH radical ($DPPH^+$) was estimated using the standard method[18]. For this, the absorbance of the solution was measured spectrophotometrically at 517 nm using BHT as reference. The ability to scavenge $DPPH^+$ was calculated by the following equation: $DPPH^+$ scavenging activity (percent) = $[(Abs_{Control} - Abs_{Sample})]/(Abs_{Control})]x100$ where $Abs_{Control}$ is the absorbance of $DPPH^+$+ methanol; Abs_{Sample} is the absorbance of $DPPH^+$ + sample extract /standard.

2.4.2 ABTS Radical Scavenging Assay

ABTS radical ($ABTS^+$) scavenging assay was determined as per Re[19], *et al*. and the samples extract (1 ml) was mixed with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and per centage inhibition was calculated as ABTS radical scavenging activity (percent) = $[(Abs_{Control} - Abs_{Sample})]/(Abs_{Control})]$ x100 where $Abs_{Control}$ is the absorbance of ABTS radical+methanol; Abs_{Sample} is the absorbance of ABTS radical+sample extract/standard. IC_{50} (Inhibition coefficient) value was determined from the plotted graph of scavenging activity against concentration of all edible parts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH/ABTS radical concentration by 50 per cent.

2.4.3 Ferric Reducing Antioxidant Power Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was conducted using method of Wong[20], *et al*. The increase in absorbance was measured using spectrophotometer at 593 nm. The per cent of antioxidant was calculated using the formula, per cent of antioxidant (percent) = $[(Abs_{Sample} - Abs_{Control})/Abs_{Sample}]x100$.

2.4.4 Determination of Total Phenols

Total phenol contents in the extracts were determined by using modified Folin-Ciocalteau method[21]. The absorbance of the solution was measured at 765 nm. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$ (R^2=0.9365), where x was the absorbance and y was the gallic acid equivalent (mg/g).

2.4.5 Estimation of Total Flavonoids

Estimation of the total flavonoids in the caper extracts was carried out as per Ordon[22], *et al*. and absorbance was measured at 420 nm. The yellow color indicated the presence of flavonoids and total flavonoid was calculated as quercetin equivalent (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$ (R^2=0.9812), where x was the absorbance and y was the quercetin equivalent (mg/g).

2.5 Statistical Analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates ($n = 3$) and the data was subjected to one-way analysis of variance (ANOVA) using SPSS 11.5. Duncan's multiple range tests were used to assess difference between means. Regression test was used to assess correlation between means. The dendrogram was made using Ward method and distance is expressed as Euclid distance. *P-v*alues<0.05 were regarded as significant.

3. RESULT AND DISCUSSION

3.1 Phytochemical Screening

The phytochemical screening of *C. spinosa* extracts

showed the presence of different chemical classes, such as alkaloids and saponins. The literature has also shown the presence of various plants constituents and the presence of these phytochemicals in *C. spinosa* may be responsible for its usefulness in various diseases. Though, further studies are required to know the precise nature of chemical constituents mediating alleged biological activities.

3.2 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity provides information on their activity of the test compounds with a stable free radical. DPPH is one of the compounds that possess a proton free radical and shows absorption band at 517 nm in visible region. Methanolic extracts of leaves showed the highest scavenging effect (70.86 per cent) at a concentration of 0.1 mg/ml, whereas methanolic extract of dried fruits exhibited the lowest activity at the same concentration. Among all edible parts, though the DPPH radical scavenging abilities of the extracts were less than BHT (72.09 per cent).

IC_{50} value was deciphered from the graph of scavenging ability against the concentration of methanolic extract of *C. spinosa*. Higher IC_{50} value indicated lower antioxidant activity and *vice-versa*. Table 1 showed the highest IC_{50} value for DPPH in dried fruits (0.097 mg/ml) and lowest in leaf sample (0.050 mg/ml). Further, the degree of reduction in absorbance indicates the radical scavenging power of the extract. The effect of antioxidants on DPPH is probably due to its hydrogen donating ability[23]. Though the DPPH radical scavenging abilities of the extracts were less than BHT, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This study suggests that all edible parts of *Capparis* plant possess antioxidant activity (Fig. 1).

3.3 ABTS Radical Scavenging Activity

ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals[24]. The methanol extracts of the leaves of *C. spinosa* were fast and effective scavengers of the ABTS radical and this activity was comparable to that of ascorbic acid and BHT. At concentration of 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, and 0.08 mg/ml the ascorbic acid and BHT exhibited higher activity than the leaves extracts, but at 0.1 mg/ml the activity of the leaves extracts were similar to that of ascorbic acid and BHT (100 per cent). Lowest activity found in dried fruit extract (61.15 per cent) at a concentration of 0.1 mg/ml.

Table 1 revealed that highest IC_{50} value for ABTS found in dried fruits (0.086 mg/ml) and lowest were found in leaves sample (0.033 mg/ml). The scavenging of the $ABTS^{+}$ by the extracts was found more than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals[25]. Some compounds which have $ABTS^{+}$ scavenging activity did not show DPPH scavenging activity[26], but same was not fond true for this study (Fig. 1).

Table 1. Free radical scavenging activity (IC_{50}) value for methanolic extract of all edible parts of *C. spinosa* collected from Ladakh region. [Values are expressed as mean ± SD, n = 3]

Edible parts	IC_{50} mg/ml	
	DPPH	ABTS
Leaves	0.050 ± 0.003^{a}	0.033 ± 0.003^{a}
Flower Buds (6-8 mm)	0.068 ± 0.002^{b}	0.048 ± 0.002^{b}
Flower Buds (9-12 mm)	0.091 ± 0.002^{c}	0.077 ± 0.002^{d}
Roots	0.094 ± 0.003^{cd}	0.066 ± 0.003^{c}
Fruits (Dried)	0.097 ± 0.002^{d}	0.086 ± 0.002^{e}

Values with different superscripts in a column differ significantly ($p<0.05$)

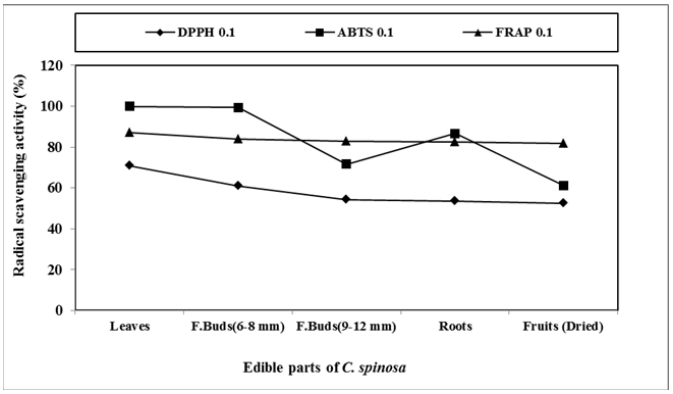

Figure 1. Free radicals scavenging activity determined with ABTS, DPPH and FRAP assays at 0.04 mg/ml concentration of methanolic extract of edible parts of *C. spinosa* samples.

3.4 FRAP

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+}–TPTZ)[27]. (Fig. 2) showed that methanolic extract of *Capparis* leaves had highest total antioxidant potential (73.54 percent to 87.14 percent) compared to BHT (67.51 percent to 86.97 percent). As per the FRAP assay, the minimum antioxidant activity was recorded in dried fruit extract (64.70 percent to 81.81 percent), however, other edible parts are not significantly different in its antioxidant contents. Generally, the reducing properties of any sample are associated with the presence of those compounds, which can break the free radical chain by donating a hydrogen atom[28]. In this study, phenolic compounds of all edible parts of *Capparis* exhibited high reducing power on Fe^{3+}–TPTZ (Fig 1). Due to its redox properties, phenolic compounds act as reducing agents, hydrogen donators and singlet oxygen quenchers. The redox potential of phenolic compounds plays an important role in determining the antioxidant capacity[29].

3.5 Total Phenols

Polyphenolic compounds are known to have antioxidant activity[30]. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[31]. The content of phenol compounds determined by the Folin-Ciocalteu method for the *C. spinosa* leaves analysed is shown in (Fig. 3). Total phenolic

compounds ranged from 4.07 to 24.78 mg GAE/g dry wt. The highest total phenolic content was found in the leaves (24.78) whereas the lowest phenolic content was found in the dried fruits (4.7 mg GAE/g dry wt).

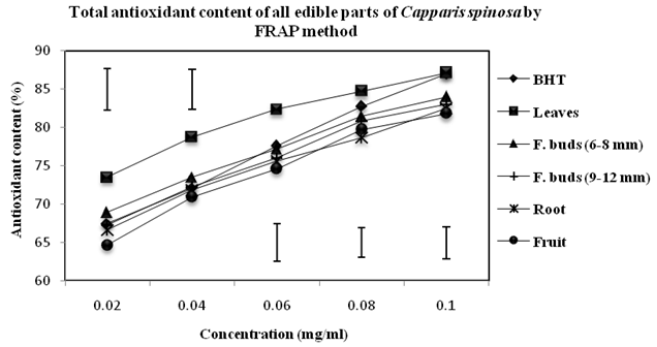

Figure 2. Antioxidant content (%) of methanolic extract of all edible parts of *Capparis spinosa* expressed as per cent of antioxidant using FRAP method.

In fact, many medicinal plants contain large amounts of antioxidants and many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases[32].

3.6 Total Flavonoids

Fig. 3 exhibits the flavonoid contents of all edible parts of *C. spinosa***.** The result revealed that leaves having maximum flavonoids contents (5.69 mg quercetin/g dry wt.) and dried fruits having no flavonoid contents among all edible parts. This result strongly suggests that polyphenols are important components of *Capparis*, which are responsible for not only its antioxidant activities but some of its pharmacological effects, could be attributed to the presence of these valuable constituents. The presence of flavonoids might also influence the antioxidant capacity[33].

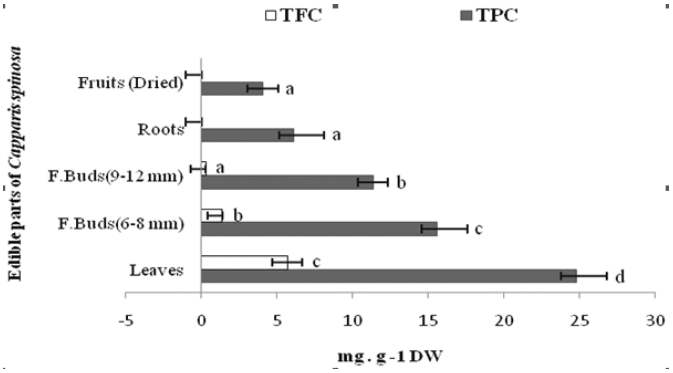

Figure 3. Total phenolic content (gray bars, mg of GAE g $^{-1}$ of DW) and total flavonoid content (white bars, mg of quercetin g $^{-1}$ of DW) of *C. spinosa* edible parts.

3.7 Correlation

Several studies have reported correlations between the antioxidant activities measured by different methods, as well as the correlations between those methods and phytochemical concentrations in various food commodities[34]. However, this type of information is very limited for *C. spinosa*. The IC_{50} value for DPPH was highly correlated with that of ABTS (R^2=0.9084) and FRAP assay (R^2 = 0.9771). The result suggested that the three methods have similar predictive capacity for antioxidant activities of *C. spinosa.* However, IC_{50} value of ABTS is reasonably correlated with FRAP assay (R^2 = 0.5838). There was a high correlation (R^2 = 0.90) between ABTS and DPPH values for various fruit extracts[35].

Several studies have shown correlation between antioxidant activity and total phenolic contents[7]. The total phenolic contents were highly correlated with IC_{50} value of ABTS (R^2 = 0.9084), DPPH (R^2 = 0.9388) and FRAP value (R^2 = 0.9618). However, total flavonoid contents were reasonably correlated with ABTS (R^2 = 0.7449) and DPPH (R^2 = 0.8791) but highly correlated with FRAP value (R^2 = 0.9588).

3.8 Cluster Analysis

Cluster analysis of *C. spinosa* edible parts collected from Ladakh region showed that cluster based on total antioxidant activity (DPPH, ABTS, and FRAP) was almost similar to cluster based on total phenolic contents (Figs. 4 and 5)[36]. It revealed that in case of capers, total Antioxidant capacity is equally contributed by total phenolics and total flavonoids. Antioxidant activities varied widely among all the edible parts of *C. spinosa.* There were good correlations between the

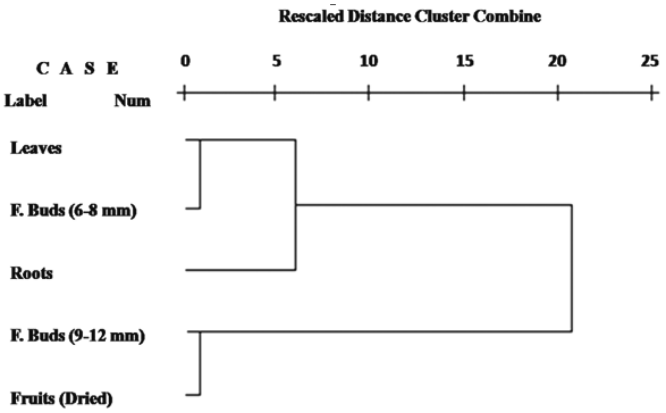

Figure 4. Dendrogram of different edible parts of *C. spinosa* according to cluster analysis of similarity on the basis of total AOA determined by DPPH, ABTS and FRAP assays using Ward method.

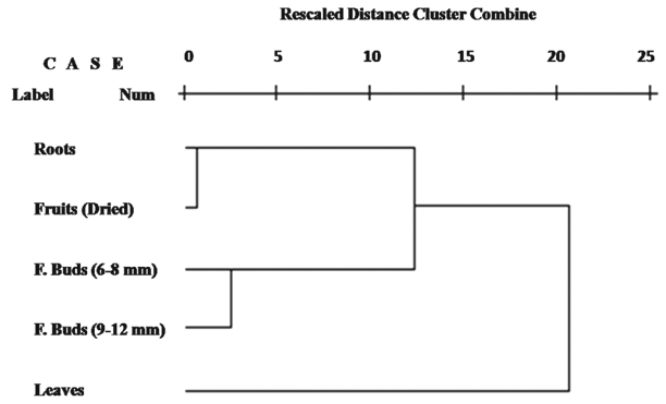

Figure 5. Dendrogram of different edible parts of *C. spinosa* according to cluster analysis of similarity on the basis of total phenolic and flavonoid contents using Ward method.

antioxidant activities measured by DPPH, ABTS, and FRAP as well as total phenolic contents, suggesting that these methods have similar predictive capacity for antioxidant activities of edible parts of *C. spinosa*. High correlation between the DPPH, ABTS, FRAP and phenol as well as flavonoid contents indicated that the total phenolic contents can be used as indicator for antioxidant activities of edible parts of *C. spinosa*. This result again suggests that Antioxidant capacity of capers is caused mainly by phenolics as well as flavonoids.

4. CONCLUSION

The results revealed that the methanolic extracts of *C. spinosa* leaves possess a strong antioxidant/free radical scavenging activities among all the edible parts, which is probably due to the presence of high concentration of polyphenolic compounds. The strong antioxidant activity of all edible parts of *C. spinosa* may, therefore, be a good candidate for functional foods as well as plant-based pharmaceutical products. Further studies are required to identify the active principle responsible for the significant antioxidant effect.

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