CHAPTER 5

EVALUATION OF ANTIOXIDANT ACTIVITIES AND TOTAL POLYPHENOLS OF EDIBLE PARTS OF *CAPARIS SPINOSA* L.
COLLECTED FROM TRANS-HIMALAYA
ABSTRACT

The antioxidant activity and total polyphenols of the methanolic extract of leaves, flower buds, roots and fruits of *C. spinosa* were assessed in an effort to corroborate the medicinal and culinary potential of the edible parts of the plant. To estimate the mentioned antioxidant capacity, three different methods were performed: the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH), 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity and ferric reducing/antioxidant power assay (FRAP). Highest DPPH and ABTS radical scavenging activity was observed in leaves and least in dried fruit samples. FRAP assay illustrated that leaves samples possess maximum antioxidant contents and dried fruit sample restrain minimum as compared to the other edible parts and well-known antioxidant butylated hydroxytoluene (BHT). The IC$_{50}$ value of DPPH were highly correlated with IC$_{50}$ value of ABTS (R$^2$=0.9074) and FRAP assay (R$^2$=0.9771). However, IC$_{50}$ value of ABTS reasonably correlated with FRAP assay (R$^2$=0.5737). The highest phenolic and flavonoid content was found in leaves samples (24.77-5.69 mg GAE/g DW) and lowest in dried fruit samples (4.07-0.00 mg quercetin equivalent/g DW). The total phenolic contents were highly correlated with IC$_{50}$ value of ABTS (R$^2$=0.9074), DPPH (R$^2$=0.9377) and FRAP value (R$^2$=0.9617). But total flavonoid contents were reasonably correlated with ABTS (R$^2$=0.7449), DPPH (R$^2$=0.7791) and FRAP value (R$^2$=0.9577). This study, has to some extent, validated the medicinal potential of all the edible parts of the *C. spinosa*. 
5.1 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 to the increased consumer perception of this problem in recent years. Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the initiation or propagation of oxidizing chain reactions, and also involved in scavenging free radicals. A great number of edible medicinal plants contain chemical compounds that exhibit antioxidant properties. Edible parts of plant such as fruits, buds and roots are reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be correlated with antioxidant potential [235].

*Capparis spinosa* L. (Capparidaceae) also called ‘Caper’ and locally known as ‘Kabra’ is one of the oldest known medicinal plant in ‘Amchi system’ (local medicinal system) for the treatment of various ailments like gastrointestinal infection, diarrhoea and rheumatism and occasionally used by local people of Ladakh as a leafy vegetable and forage [36]. This plant has multiple uses in cuisine as salad, pickle and condiments. *Capparis* is known to contain a wide variety of antioxidant compounds including phenolic compounds. These compounds are found to be well correlated with antioxidant potential.

Previous chemical studies on *C. spinosa* have shown the presence of alkaloids, lipids, polyphenols, flavonoids, indole and aliphatic glucosinolates [210]. Ethanolic extract from the fruit of *Capparis spinosa* exhibits a notable activity in protecting against oxidative stress and suggesting its protective effect against skin sclerosis [163]. However, methanolic extract of *C. spinosa* buds, rich in flavonoids, including several quercetin and kaempferol glycosides, was demonstrated to possess strong antioxidant/free radical scavenging effectiveness, antiviral and immuno-modulatory effects in different *in vitro* tests [153]; whereas based on *in vivo* test this extract showed a noteworthy anti-allergic effectiveness against bronchoapasm in guinea pigs [152], and when topically applied it
afforded significant *in vivo* protection against UV-B light induced skin erythema in humans [23]. Natural antioxidants present in *Capparis 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 defenses or by supplementing with proven dietary antioxidants [211].

Until now, there has been no report determining the polyphenol contents and antioxidant activity of edible parts of *Capparis* from Ladakh region. In the present study we investigated and compared the above mentioned parameters of four different edible parts: leaves, buds, fruits and roots. In addition, correlations between all the analysed parameters were evaluated.

5.2 MATERIALS AND METHODS

5.2.1 Plant samples, estimation of total phenolic content and antioxidant activity

The edible parts such as leaves, flower buds, roots and dried fruits were collected in triplicate from *Capparis* plants growing in wild from Ladakh during the month of July 2009. The total phenols and flavonoids are extracted from the plant samples as described previously (chapter 4). Similarly the antioxidant activities of plant samples were studied based on DPPH, ABTS and FRAP assays as described previously (Chapter 4).

5.3 RESULT AND DISCUSSION

5.3.1 DPPH radical scavenging activity

DPPH is one of the compounds that possess a proton free radical and shows absorption band at 517nm in visible region. When DPPH encounters proton radical scavengers, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. Figure 5.1 shows the dose-response curve of DPPH radical scavenging activity of the methanol extracts from all the edible parts of the *C. spinosa*. Methanolic extract of leaves showed the highest free radical scavenging activity (70.76%) at a concentration of 0.1 mg/ml, followed by methanolic extract of flower buds, then roots and least activity was detected from dried fruits, though the
DPPH radical scavenging abilities of the extracts were less than those of BHT (72.09%). The scavenging activity of methanolic extract was in the order of leaves > buds (6-7 mm) > buds (9-12 mm) > roots > fruits. The variation in antioxidant activity of plant extract from edible parts was statistically significant ($p < 0.05$).

IC$_{50}$ value was determined from the plotted graph of scavenging ability against the concentration of methanolic extract of *Capparis*, higher the IC$_{50}$ value signifies less antioxidant activity and *vice-versa*. Table 1 revealed that highest IC$_{50}$ value for DPPH found in dried fruits (0.097 mg/ml) and lowest were found in leaves sample (0.050 mg/ml). The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [236]. 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 the all edible parts of *Capparis* plant possess antioxidant activity.

![Graph of DPPH radical scavenging activity of edible parts of *Capparis spinosa*](image)

**Figure 5.1** DPPH radical scavenging activity of methanolic extracts of all edible parts of *Capparis spinosa* collected from Ladakh region.
Table 5.1 Free radical scavenging activity (IC$_{50}$) value for methanolic extract of all edible parts of *C. spinosa* collected from Ladakh region.

<table>
<thead>
<tr>
<th>Edible parts</th>
<th>IC$_{50}$ mg/ml</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>0.050 ± 0.003$^a$</td>
<td>0.033 ± 0.003$^a$</td>
<td></td>
</tr>
<tr>
<td>F. Buds (6-7 mm)</td>
<td>0.067 ± 0.002$^b$</td>
<td>0.047 ± 0.002$^b$</td>
<td></td>
</tr>
<tr>
<td>F. Buds (9-12 mm)</td>
<td>0.091 ± 0.002$^c$</td>
<td>0.077 ± 0.002$^d$</td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>0.094 ± 0.003$^{cd}$</td>
<td>0.066 ± 0.003$^c$</td>
<td></td>
</tr>
<tr>
<td>Fruits (Dried)</td>
<td>0.097 ± 0.002$^d$</td>
<td>0.076 ± 0.002$^e$</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation (*n* = 3). BHT used as a standard. IC$_{50}$ means with different superscript were significantly different ($p < 0.05$, ANOVA).

5.3.2 ABTS radical scavenging activity

ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [237]. The methanol extracts of the leaves of *C. spinosa* were fast and effective scavengers of the ABTS radical (Fig. 2) and this activity was comparable to that of Ascorbic acid and BHT. At 0.02, 0.04, 0.06 and 0.07 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 was similar to that of ascorbic acid and BHT (100%). Lowest activity was found in dried fruit extract (61.15%) at a concentration of 0.1 mg/ml, whereas the flower buds and roots showed reasonably better antioxidant activity.

The scavenging of the ABTS$^+$ radical by the extracts was found to be higher 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 [220]. Wang et al. [221] found that some compounds which have ABTS$^+$ scavenging activity did not show DPPH scavenging activity, but this is not the case in this study.
Figure 5.2 ABTS radical scavenging activity of methanolic extracts of all edible parts of *C. spinosa* collected from Ladakh region.

5.3.3 Ferric reducing antioxidant power (FRAP) assay

Figure 3 showed that methanolic extract of *Capparis* leaves had highest total antioxidant content (73.54-77.14%) compared to BHT (67.51-76.97%). The lowest percent of antioxidant content was found in dried fruit extract (64.70-71.71) based on the FRAP assay. However, other edible parts are not significantly different in their antioxidant contents.

Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom [238, 239]. In this study, phenolic compounds of all edible parts of *Capparis* exhibited high reducing power on Fe3+-TPTZ. Rice-Evans [240] reported that phenolic compounds have redox properties, which allow them to 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 [240].

89
**Figure 5.3** Antioxidant content (%) of methanolic extract of all edible parts of *C. spinosa* expressed as percent of antioxidant using FRAP method.

### 5.3.4 Total phenolic

Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds [241, 224]. 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 [225]. The content of phenolic compounds determined by the Folin-Ciocalteu method for the *C. spinosa* leaves analysed is shown in Figure 5.4. Total phenolic compounds ranged from 4.07 to 24.77 mg GAE/g dry wt. The highest total phenolic content was found in the leaves (24.77) followed by flower buds, roots and dried fruits in decreasing order. The lowest phenolic content detected in the dried fruits is 4.7 mg GAE/g dry wt.

In fact, many medicinal plants contain large amounts of antioxidants such as polyphenols. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases [242, 243]. The results strongly suggest that phenolics are important components of this plant, and some of its pharmacological effects could be attributed to the presence of these valuable constituents.
5.3.5 Total flavonoids

Figure 5.4 exhibits the flavonoid contents of all edible parts of *C. spinosa*. The total flavonoid contents in these samples ranged from 0.00 to 5.69 mg quercetin/g DW. The result reveals that leaves having maximum flavonoids contents (5.69 mg quercetin/g dry wt.) and dried fruits having no flavonoid contents (0.00 mg quercetin/g dry wt.) among all edible parts. This result strongly suggests that polyphenol 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.

Based on the antioxidant assays, it is thus suggested that phenolic compounds present in the leaves of *Capparis* have strong scavenging ability and ferric reducing power. This could be due to the antioxidant activity of phenolic compounds towards free radicals. Besides, phenolic compounds, the presence of flavonoids might also influence the antioxidant capacity. However, there are several methodological limitations for antioxidant determinations [244].

![Graph showing TFC and TPC levels in various parts of C. spinosa](image)

**Figure 5.4** 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* tender leaves.
5.3.6 Comparative study between total phenolic content and antioxidant activity

Several studies have reported correlations among the antioxidant activities measured by different methods, as well as the correlations between those methods and phytochemical concentrations in various food commodities [218]. However, this type of information is very limited for *C. spinosa*.

Fig. 5.5 shows the comparison among three different antioxidant assays- DPPH, ABTS and FRAP assay. The IC$_{50}$ value of DPPH were highly correlated with IC$_{50}$ value of ABTS ($R^2=0.908$) (Figure 5.6a) and FRAP assay ($R^2=0.977$) (Figure 5.6b). The result suggested that the three methods have similar predictive capacity for antioxidant activities of *C. spinosa*. However, IC$_{50}$ value of ABTS reasonably correlated with FRAP assay ($R^2=0.5737$) (Figure 5.6c). Leong and Shui [252] reported a high correlation ($R^2=0.90$) between ABTS and DPPH values for various fruit extracts and similarly Lachman et al. [231] reported high correlation ($R^2=0.937$).

![Figure 5.5](image)

**Figure 5.5** 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
Figure 5.6 Linear correlation between (a) DPPH IC50 and ABTS IC50 (b) DPPH 0.1 and FRAP 0.1 (c) ABTS 0.1 and FRAP 0.1
Figure 5.7 Linear correlation between (a) TPC and ABTS IC\textsubscript{50} (b) DPPH IC\textsubscript{50} and TPC (c) TPC and FRAP 0.1
Figure 5.8 Linear correlation between (a) TFC and ABTS IC$_{50}$ (b) TFC and DPPH IC$_{50}$ (c) TFC and FRAP 0.1
Several studies showed a correlation between antioxidant activity and total phenol contents [226]. The total phenolic contents were highly correlated with IC$_{50}$ value of ABTS ($R^2=0.908$), DPPH ($R^2=0.938$) and FRAP value ($R^2=0.961$). This result was in agreement with Benzie and Stezo [245], Othman et al. [232] who found a strong correlation between total phenolics and FRAP assay and Sun and Ho [255] who reported a significant correlation between total phenolics and scavenging ability of buckwheat extract on DPPH radicals. However, total flavonoid contents were reasonably correlated with ABTS ($R^2=0.745$), DPPH ($R^2=0.779$) but highly correlated with FRAP value ($R^2=0.958$) of measured value between ABTS and FRAP assay for Capparis samples.

5.3.7 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. Where Basgo, Nimmu, Phyang, Batalik samples had fallen in one cluster while samples from Thiksey, Skampuk, Phey, Skuru and Tirchey had fallen in another cluster (Fig. 5.9a and 5.9b). Whereas, in case of cluster based on total flavonoid content samples from Phey and Thiksey are falling in different cluster (Fig. 5.9c). It might be that in case of capers, total AOA activity is more contributed by total phenolics than total flavonoids.
Figure 5.9  Dendrogram of different sampling sites according to cluster analysis of similarity on the basis of (a) Total AOA determined by DPPH, ABTS and FRAP assays (b) Total Phenolic and Flavonoid contents using Ward method.
5.4 CONCLUSIONS

Antioxidant activities varied widely among all the edible parts of C. spinosa. The highest values of AOA showed unambiguously in the leaves and conversely the lowest values demonstrated in dried fruits. FRAP method revealed that leaves samples possess good antioxidant content comparable with that of the other edible parts. The highest total phenolic and total flavonoid content exhibits in the leaves and lowest in the dried fruits which is same as observed for AOA activity.

There were good correlations among the 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 and flavonoid contents indicated that the total phenolic contents can be used as indicator for methanolic antioxidant activities of edible parts of C. spinosa. This result again suggests that AOA of capers is caused mainly by phenolics and flavonoids.

Thus, the results revealed that the methanolic extracts of C. spinosa leaves possess a strong antioxidant/free radical scavenging effectiveness among all the edible parts, which is probably due to the presence of high amount 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.