Chapter III

Evaluation of in-vitro antioxidant potential of *Fagonia cretica*, *Carum carvi* and *Parmelia perlata*
1. Introduction

Natural physiological and biological processes occurring in the living cells are associated with the generation of free radicals and other reactive oxygen species (ROS) (Pourmorad et al., 2006). In living cells there is a well established homeostasis of the free radical generation and antioxidant defence mechanism. Any toxic insult to the cell can lead to the misbalance between the oxidant and antioxidant levels in the cells. This results in the generation of more free radicals, which cause oxidative damage to the cellular macromolecules such as, lipids, proteins and DNA and eventually leading to many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases (Harman, 1998). The cellular damage caused by the free radicals could be peroxidative damage to membrane lipids leading to membrane instability and cell death due to necrosis, the adduct formation with cellular proteins and enzymes leading to their dysfunction or these free radicals may damage DNA directly leading to the cellular mutations.

Nature has endowed plants with free radical scavenging molecules such as, vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, and other metabolites, which possess rich antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). Numerous studies have shown that most of these antioxidant compounds from plants possess anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities (Rice-Evans et al., 1995; Sala et al., 2002). Moreover, it has been observed that the ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, (Yang et al., 2002; Ashok et al., 2008; Veerapur et al., 2009), and in recent years, there has been a worldwide trend towards the use of the natural phytochemical present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts et al., 2000; Wang and Jiao, 2000; Muselik et al., 2007), that are rich source of antioxidants. In the present chapter, an attempt has been made to evaluate the in-vitro antioxidant potential of the methanolic extract of *Fagonia cretica*, *Parmelia perlata* and *Carum carvi* in terms of total phenolic content, inhibition of lipid peroxidation, DNA sugar damage, DPPH scavenging activity and ferric reducing potential. *Fagonia cretica* L., (previously known as *Fagonia arabica*) a tropical herb belonging to family Zygophyllaceae, commonly known as “Dhamasa”. It is a small spiny under shrub 1 to 3 feet height, mostly found in dry calcareous rocks throughout the Mediterranean region of Africa, Afghanistan, India and Pakistan (Rizvi et al., 1996; Hooker 1881; Chopra et al., 1982). It is reputed medicinal plant in scientific and folkloric literature, and its medicinal
values are well documented (Saeed, 1969). Different parts of this herb have been used to
cure various ailments, including hematological, neurological, endocrinological and
inflammatory disorders (Chopra et al., 1982; Saeed and Wahid, 2003). It has also been
reported to contain wide variety of antioxidants and triterpenoids saponins (Miyase et al.,
1996; Khalik et al., 2000). The plant is bitter in taste and is used for the treatment of fever,
thirst, vomiting, dysentery, asthma, urinary discharges, liver trouble, typhoid, toothache,
stomach troubles and skin diseases (Baquar, 1989). Its infusion is effective as a cooling
agent in stomatitis. It is known to purify blood and also acts as a deobstruent (Said, 1996).
It is also used for skin diseases, small pox and for endothermic reaction in the body (Watt,
1972). The twigs of the plant are used as remedy for snake bite and also applied externally
as paste on tumours and for the swellings of neck (Rizvi et al., 1996).

*Carum carvi* Linn, commonly known as caraway belongs to the family Umbelliferae, is a
globally distributed spice with a history as a medicinal plant since ancient times (Hartmans
et al., 1995). The dried ripe fruits of the plant are used in folk medicine especially in the
treatment of digestive disorders in both adults and infants (Reynolds et al., 1993,
Thompson et al., 2002). The main constituents of *Carum carvi* are the volatile oils including
carvone (40–60%), limonene, carveol, dihydrocarveol and thymol in addition to glycosides
and flavanoids (Zheng et al., 1992; Matsumura et al., 2002). Experimental studies have
shown that *Carum carvi* possesses, antidyseptic (Holtmann et al., 2003), antispasmodic
(Eddouks et al., 2004), antiulcerogenic (Khayyal et al., 2001), antibacterial (Singh et al.,
2002), antitumor (Kamaleeswari et al., 2006), antiproliferative (Nakano et al., 1998),
antioxidant (Kamaleeswari et al.,2006), antihyperglycemic (Eddouks et al., 2004),
antihyperlipidaemic (Lemhadri et al.,2006) and diuretic (Lahlou et al.,2007) activities.

*Parmelia perlata* (lichen) commonly known as Charila or Stone flower in India, belongs to
family Parmeliaceae. Parmelia perlata exhibited strong antioxidant, antibiotic and
antidiabetic activities (Ketchum, 1984; Patil et al., 2011). It is usually used as a spice to
enhance the taste and flavor of the foods. Because of its medicinal properties it is also
useful to treat sores, boils, inflammations, seminal weakness, and amenorrhoea (Warrier et
al., 2002). It is important to note that at present, various herbal preparations used to treat
seminal weakness and skin creams for wound healing contain *Parmelia perlata* as major
component.
2. Materials and Methods: as described in chapter II.

2.1. Preparation of methanolic extract of *Fagonia cretica*, *Carum carvi* and *Parmelia perlata*

Plant materials were shade dried and coarsely powdered in a grinder. The preparation was then repeatedly extracted in a soxhlet apparatus with a 4000ml round bottom flask with 2000 mL methanol. The reflux time for solvent was 4 hours. The extract was cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotatory evaporator (Buchi Rotavapor, Switzerland). The methanolic extract showed the efficacy and was suspended in water to prepare the required dilution at the time of dosing. The extract was stored at 4°C. The concentrations of the methanolic extracts used in this chapter were 20, 40, 60, 80 and 100µg/ml of the stock (1mg/ml).

3. Results

3.1. Total phenolic content

The total phenolic content of the methanolic extracts of *Fagonia cretica*, *Parmelia perlata* and *Carum carvi* (Table 1) in terms of mg gallic acid equivalent (the standard curve equation: \(y = 0.019x + 0.060, R^2 = 0.997\)) was 76, 66 and 71 GAE/g extract.

3.2. DPPH scavenging activity

The methanolic extracts of *Fagonia cretica*, *Parmelia perlata* and *Carum carvi* exhibits a significant concentration dependent DPPH scavenging activity of (38, 51, 88, 97, 98%), (9, 22, 34, 50, 69%) and (36, 40, 47, 61, 70%) respectively, at concentrations of 20, 40, 60, 80, 100µg/ml of extract. Increase in DPPH scavenging activity indicates better antioxidant property of the plant material (Table 2, Figure 1).

3.3. Inhibition of Lipid peroxidation

With the increase in concentration (20, 40, 60, 80 and 100µg/ml) of methanolic extract of *Fagonia cretica*, *Parmelia perlata* and *Carum carvi* extracts, there is significant increase in the inhibition of LPO by (30, 41, 55, 84, 84), (16, 36, 67, 83, 83) and (39, 41, 50, 59, 63%) respectively (Table 3, Figure 2).

3.4. Inhibition of DNA sugar damage

Increased concentration (20, 40, 60, 80 and 100µg/ml) of methanolic extracts of *Fagonia cretica*, *Parmelia perlata* and *Carum carvi* exhibit significant inhibition of in-vitro DNA sugar damage by (19, 28, 45, 49, 57%), (3, 26, 38, 42, 42%) and (2, 12, 28, 37, 50%) respectively (Table 4, Figure 3).
Table 1: Total phenolic content of *Fagonia cretica, Parmelia perlata and Carum carvi*.

<table>
<thead>
<tr>
<th>Total Phenolic content (GAE/g extract)</th>
<th>F. Cretica</th>
<th>P. Perlata</th>
<th>C. carvi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>76.35</td>
<td>66.38</td>
<td>71.87</td>
</tr>
</tbody>
</table>

Table 2: DPPH scavenging activity of *Fagonia cretica, Parmelia perlata and Carum carvi*.

<table>
<thead>
<tr>
<th></th>
<th>20µl/ml</th>
<th>40µl/ml</th>
<th>60µl/ml</th>
<th>80µl/ml</th>
<th>100µl/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fagonia cretica</td>
<td>37.95 ± 0.66</td>
<td>± 51.22</td>
<td>± 88.36</td>
<td>± 96.65</td>
<td>± 96.97 ± 0.09</td>
</tr>
<tr>
<td>Parmelia perlata</td>
<td>9.03 ± 0.17</td>
<td>± 21.76</td>
<td>± 34.09</td>
<td>± 49.7 ± 0.24</td>
<td>± 68.54 ± 0.14</td>
</tr>
<tr>
<td>Carum carvi</td>
<td>36.12 ± 0.04</td>
<td>± 40.24</td>
<td>± 47.18</td>
<td>± 61.29</td>
<td>± 69.51 ± 0.04</td>
</tr>
</tbody>
</table>

The Experiment were performed in triplicate and the results have been expressed in %Values ± SEM.

Table 3: Inhibition of Lipid Peroxidation by *Fagonia cretica, Parmelia perlata and Carum carvi*.

<table>
<thead>
<tr>
<th></th>
<th>Negative Control</th>
<th>20µl/ml</th>
<th>40µl/ml</th>
<th>60µl/ml</th>
<th>80µl/ml</th>
<th>100µl/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fagonia cretica</td>
<td>12.07 ± 0.28</td>
<td>8.42 ± 0.036</td>
<td>7.07 ± 0.039</td>
<td>3.83 ± 0.057</td>
<td>1.97 ± 0.04</td>
<td>1.97 ± 0.04</td>
</tr>
<tr>
<td>%Inhibition</td>
<td>30.2</td>
<td>41.4</td>
<td>54.5</td>
<td>83.7</td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>Parmelia perlata</td>
<td>12.07 ± 0.28</td>
<td>10.13 ± 0.43</td>
<td>7.77 ± 0.07</td>
<td>4.04 ± 0.035</td>
<td>2.09 ± 0.024</td>
<td>2.01 ± 0.011</td>
</tr>
<tr>
<td>%Inhibition</td>
<td>30.2</td>
<td>41.4</td>
<td>54.5</td>
<td>83.7</td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>Carum carvi</td>
<td>12.07 ± 0.28</td>
<td>7.34 ± 0.12</td>
<td>7.06 ± 0.08</td>
<td>6.04 ± 0.12</td>
<td>4.96 ± 0.08</td>
<td>4.48 ± 0.14</td>
</tr>
<tr>
<td>%Inhibition</td>
<td>30.2</td>
<td>41.4</td>
<td>54.5</td>
<td>83.7</td>
<td>83.7</td>
<td></td>
</tr>
</tbody>
</table>

The Values were expressed in mean ± SEM. (Units for LPO were nmol MDA formed/hr/g tissue)
Table 4: Table showing the inhibition of DNA sugar damage by *Fagonia cretica*, *Permelia perlata* and *Carum carvi*

<table>
<thead>
<tr>
<th></th>
<th>Negative Control</th>
<th>20µl/ml</th>
<th>40µl/ml</th>
<th>60µl/ml</th>
<th>80µl/ml</th>
<th>100µl/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. cretica</em></td>
<td>3.05 ± 0.03</td>
<td>2.47 ± 0.03</td>
<td>2.19 ± 0.036</td>
<td>1.69 ± 0.038</td>
<td>1.55 ± 0.028</td>
<td>1.32 ± 0.02</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>19.0</td>
<td>28.2</td>
<td>44.6</td>
<td>49.2</td>
<td>56.7</td>
<td></td>
</tr>
<tr>
<td><em>P. perlata</em></td>
<td>3.05 ± 0.03</td>
<td>2.96 ± 0.08</td>
<td>2.26 ± 0.05</td>
<td>1.9 ± 0.12</td>
<td>1.78 ± 0.13</td>
<td>1.76 ± 0.04</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>2.95</td>
<td>25.90</td>
<td>37.70</td>
<td>41.64</td>
<td>42.30</td>
<td></td>
</tr>
<tr>
<td><em>C. carvi</em></td>
<td>3.05 ± 0.03</td>
<td>3.01 ± 0.06</td>
<td>2.69 ± 0.01</td>
<td>2.21 ± 0.04</td>
<td>1.91 ± 0.03</td>
<td>1.54 ± 0.09</td>
</tr>
<tr>
<td>% Inhibition</td>
<td>1.6</td>
<td>11.8</td>
<td>27.5</td>
<td>37.4</td>
<td>49.5</td>
<td></td>
</tr>
</tbody>
</table>

The Values were expressed in mean ± SEM. (Units for DNA sugar damage were nmol MDA formed/hr/g tissue)
Figure 1: In-vitro DPPH scavenging activity, inhibition of LPO and DNA sugar damage by Fagonia cretica

Figure 2: In-vitro DPPH scavenging activity, inhibition of LPO and DNA sugar damage by Parmelia perlata

Figure 3: In-vitro DPPH scavenging activity, inhibition of LPO and DNA sugar damage by C. carvi
4. Discussion

In humans, more than hundred disorders are contributed by free radicals generated by environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods or by physical stress (Su et al., 2007). These free radicals cast their effect through the depletion of the immune system antioxidants, the altered gene expression and induce abnormal protein synthesis (Dillard and German, 2000; Pourmorad et al., 2006; Turkoglu et al., 2007).

From centuries plants have been used for coping with various illnesses. Till today phytotherapy has been integrated into all systems of traditional medicines, often as the main source of health care in low and middle income countries (Kosalec et al., 2009). Due to the widespread assumption that “Natural” means “harmless”, the use of medicinal plants against various disorders has been also increased in the developed countries. However, with the advancement in the use of medicinal plants against various ailments, the safety of these plant products has become a major concern in public healthcare (WHO, 2007).

Several investigations had reported that antioxidant property of plants could be therapeutically beneficial and this antioxidant property of these plants could be attributed to the phenolic components present in these plants (Cook and Samman, 1996). It is also well documented that plants containing higher phenolic content are potent scavengers of free radicals and possess better antioxidant activities (Deepa et al., 2009). Polyphenols have been used for the prevention and cure of various disorders which are mainly associated with free radicals (Havesteen, 1983). It has also been reported that compounds containing hydroxyl groups are responsible for the radical scavenging effect of most plants (Pereira, 1990). The chemopreventive mechanisms attributed to the plant polyphenols are through scavenging or chelating processes (Cook and Samman, 1996). The presence of these polyphenolic compounds in dry herbs is thus a significant finding of the present study. In the present study, the total phenolic content was found to be higher in Fagonia cretica followed by Carum carvi and Parmelia perlata. These results indicate that higher phenolic content in the methanolic extracts of Fagonia cretica, Carum carvi and Parmelia perlata may be extrapolated to their antioxidant properties. However, there might be any seasonal/batch-to-batch variations in the phenolic content of the methanolic extract of the herbal plants but this was not elucidated in the current study.

DPPH scavenging activity is a specific parameter to determine the antioxidant activity of plant extracts. DPPH, a stable free radical which when encounters antioxidants it gets convert it into a colorless α,α-diphenyl-β-picryl hydrazine and this conversion of DPPH
into α,α-diphenyl-β-picryl hydrazine results in the depletion in absorbance at 517nm. This depletion in absorbance is directly proportional to the antioxidant activity of plant extracts (Wu, et al., 2003). In this study, the methanolic extract of Fagonia cretica, Parmelia perlata and Carum carvi exhibits a significant concentration dependent DPPH scavenging activities. Fagonia cretica possess the highest DPPH scavenging activity followed by Carum carvi and Parmelia perlata. Increase in DPPH scavenging activity indicates better antioxidant property of these plant extracts.

Lipid peroxidation in biological membranes has been considered as one of the major mechanisms of cell injury in aerobic organisms subjected to oxidation stress (Poli et al., 1987). Since, free radical mediated peroxidation of lipids has received a great deal of attention in connection with oxidative stress and associated diseases. Oxidative degradation of polyunsaturated fatty acid in the cell membranes generate a number of degradation products, such as malondialdehyde (MDA), which is found to cause cell membrane destruction and cell damage, leading to liver injury, atherosclerosis, kidney damage, aging, and susceptibility to cancer (Rice-Evans and Burdon 1993). MDA, one of the major products of lipid peroxidation, has been extensively studied and measured as an index of lipid peroxidation and as a marker of oxidative stress (Janero, 1990). The addition of Fe^{2+} or Fe^{3+} is a common approach to experimentally induce in-vitro oxidative stress. In the present study, the methanolic extracts of Fagonia cretica, Carum carvi and Parmelia perlata exhibit concentration dependent inhibition of lipid peroxidation, indicating that these extracts could be used as potent modulators against free radical induced cell membrane damage and cell death.

In many diseases including cancer, DNA is also a major target of free radical induced damage. Under physiological conditions, there is the constant and endogenous rate of generation of free radicals that may lead to a minimal damage in DNA which is required to induce or activate the defensive systems and DNA-repair mechanisms. However, if this production increases than normal levels, oxygen radicals may attack DNA at either the sugar (deoxyribose) or the base, giving rise to a large number of products. Attack at a sugar ultimately leads to strand break with a terminal fragmented sugar residue (Imlay and Linn, 1988). Protection by methanolic extracts of Fagonia cretica, Carum carvi and Parmelia perlata against DNA damage was determined in terms of the damage to its deoxyribose sugar moiety. The presence of various concentrations (20-100µg/ml) of Fagonia cretica, Carum carvi and Parmelia perlata extracts prevented the free radical-mediated DNA-sugar damage in a dose-dependent manner.
It has also been reported that the reducing power of bioactive compounds present in the plants are associated with antioxidant activity (Sidduraju et al., 2002).

It is concluded that the methanolic extract of *Fagonia cretica*, *Carum carvi* and *Parmelia perlata* possessed the antioxidant activity such as free radical scavenging, lipid peroxidation and DNA sugar damage inhibitory activities. Furthermore, total phenolic content of these plant extracts showed a positive correlation with their antioxidant activity. In the present study only negative control was taken into the consideration as the main aim of the experiments was to find the dose dependent relationship between the concentration and in-vitro efficacy of the plant extracts. Further studies are needed to isolate and identify the individual compounds responsible for the antioxidant activity and to find the in-vitro efficacy of the plant extracts. The broad range of antioxidant activity of these extracts indicates their potential to be used as a source of natural antioxidants or nutraceuticals with potential application to reduce oxidative stress with consequent health benefits.