Iris aitchisonii (Bakar) Boiss.: A potential source of Natural Antioxidants

*MUHAMMAD AJAIB¹, ZAHEER-UD-DIN KHAN¹, MUHAMMAD ATHAR ABBASI² & SABAHAT ZAHRA SIDDIQUI²

¹Department of Botany, GC University, Lahore, Pakistan
²Department of Chemistry, GC University, Lahore, Pakistan

ABSTRACT

The organic and aqueous plant extracts of Iris aitchisonii (Bakar) Boiss. were obtained in petroleum ether, chloroform, methanol and water and were tested for their antioxidant potential, using four techniques, i.e. 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity, total antioxidant activity, ferric reducing antioxidant power (FRAP) assay and ferric thiocyanate assay along with the determination of their total phenolic contents. The results revealed that among these fractions the chloroform soluble fraction showed highest DPPH radical scavenging activity, i.e. 92.17±1.25% inhibition of DPPH radical at a concentration of 130 μg/ml with IC₅₀ value 48.55±1.08 relative to butylated hydroxytoluene (BHT), having IC₅₀ of 12.52 ± 0.89 μg/ml. Methanol extract showed highest total antioxidant activity, i.e. 1.182±0.09 as well as highest FRAP value, i.e. 142.33±0.96 TE μM/ml. Chloroform and methanol extract showed considerable amounts of total phenolic contents, i.e. 122.33±0.12 and 121.83±0.85 GAE mg/g respectively. Chloroform extract showed good value of inhibition of lipid peroxidation, i.e. 51.61±0.64. 

Key words: Antioxidant activities, Iris aitchisonii (Bakar) Boiss., FRAP assay, Total phenolic contents, Total antioxidant activity, IC₅₀

INTRODUCTION

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in medicine (Aggarwal et al., 2003). Medicinal plants are in greater demand due to their increased popularity and it is being suggested by a large number of conservation groups, that wild medicinal plants should be brought into cultivation. Numerous medicinal plants as well as their purified components have shown beneficial therapeutic potentials. Various herbs and other plant species are reported to show antioxidant activity. Majority of the antioxidant potential is due to the presence of flavones, flavonoids, isoflavones, anthocyanin, lignans, coumarin, catechins and isocatechins in plants (Aqil et al., 2006). Antioxidant-based drug products are being used for the treatment and prevention of complicated diseases like atherosclerosis, diabetes, stroke, Alzheimer’s disease, and cancer (Devasagayam et al., 2004). In living organisms, free radicals are produced as a result of the normal metabolic process, and also free radical chain reactions normally occurring as respiratory chain reaction in the mitochondria, through xanthene oxidase activity, liver mixed function oxidases, atmospheric pollutants and from the transitional metal catalysts, xenobiotics, and drugs. In addition to this chemical mobilization of the body fat stores in different conditions such as lactation, fever, exercise, infection, and even fasting, may result in enhanced radical activity, and damage. Oxidative injury or free radicals now appears as the fundamental mechanism, causing a number of the human neurologic and many other disorders. Peroxidation of lipids can be initiated by the oxygen free radical, which in turn stimulates the glycation of protein, inactivation of some enzymes, and alteration in the function and structure of collagen basement and a few other membranes, and also play a role in chronic complication of diabetes (Ara & Nur, 2009). Iris aitchisonii (Bakar) Boiss. belongs to a monocotyledous family Iridaceae and is locally used to treat various diseases. It is a herb up to 35cm in height. The plant is common in grassy fields of Brooth near Khuiratta, flowering during March-April. Locally this plant is called Sanp Buti and is used as diuretic, cathartic and antidote for snakebite. It is a toxic plant and is used very carefully (Ajaib, 2012).

MATERIALS AND METHODS

Plant Material

The plant Iris aitchisonii (Bakar) Boiss. was collected from District Kotli, Azad Jammu & Kashmir during April 2011, identified and deposited in Department of Botany, GC University, Lahore as a voucher specimen no. GC.Bot.Herb.0701.
Test organisms:

Gram -ve, Gram +ve bacteria and fungi were obtained from PCSIR Laboratories Lahore, as test organisms.

Extraction and Fractionation of Antioxidants

About 250 gm shade-dried and well ground whole plant was extracted successively with non-polar and polar solvents, like petroleum ether, chloroform, methanol and water using maceration technique. The extracts, concentrated on rotary evaporator, were used to evaluate their *in vitro* antioxidant potential.

Antioxidant Assays

The DPPH radical scavenging activity of various extracts was examined by comparison with that of a known antioxidant, butylated hydroxytoluene (BHT) using the reported method of Lee et al. (2001). The percent of DPPH decoloration of the samples was calculated according to the formula: 

\[ \text{Antiradical activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \]

The total antioxidant activity of various extracts was evaluated by phosphomolybdenum complex formation method following Prieto et al. (1999). The FRAP assay was according to Benzie and Strain (1996) while Total phenolics of various extracts were determined after Makkar et al. (1993). The antioxidant activity of various extracts on inhibition of linoleic acid peroxidation was investigated by thiocyanate method following Valentao et al. (2002). Each sample was assayed in triplicate and mean values were calculated for statistical analysis.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The various extracts of *Iris aitchisonii* tested for their percent of DPPH radical scavenging activity indicated that activity was increased by increasing the concentration of the fractions in the assay. The various concentrations of chloroform extract exhibited highest percent inhibition of DPPH radical as compared to other fractions. It showed 92.17±1.25% inhibition of DPPH radical at a concentration of 130µg/ml. The IC50 values were also calculated (Table 1). Lowest the IC50 value, greater was the DPPH radical scavenging activity. Chloroform extract showed lowest IC50 value, i.e. 48.55±1.08 relative to IC50 12.52 ± 0.89µg/ml of butylated hydroxytoluene (BHT). Methanol extract showed moderate activity (IC50 189.10±2.36) while petroleum ether and aqueous extracts showed no significant activities.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample?</th>
<th>Concentration in assay (µg/ml)</th>
<th>% scavenging of DPPH ± S.E.Mb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum ether extract</td>
<td>1000 500 250 130</td>
<td>72.59±1.39 60.03±1.28 50.0±1.19 42.46±0.91</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform extract</td>
<td>130 60 30</td>
<td>92.17±1.25 52.41±1.06 43.01±1.13</td>
</tr>
<tr>
<td>3.</td>
<td>Methanol extract</td>
<td>500 250 130 60</td>
<td>69.08±1.97 59.04±1.86 48.37±1.05 36.44±0.94</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous extract</td>
<td>1000 500 250 130</td>
<td>73.79±1.63 52.71±1.58 48.49±1.61 34.33±0.89</td>
</tr>
<tr>
<td>5.</td>
<td>BHTb)</td>
<td>60 30 15 8</td>
<td>92.46 ± 0.25 74.57 ± 0.39 49.61 ± 0.55 28.33 ± 0.83</td>
</tr>
</tbody>
</table>

b) All results are presented as mean ± standard error of three assays.

<table>
<thead>
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<th>Concentration in assay (µg/ml)</th>
<th>% scavenging of DPPH ± S.E.Mb)</th>
</tr>
</thead>
</table>

Total Antioxidant Activity by Phosphomolybdenum Complex Method

The total antioxidant activities of the extracts were measured and compared with the standard antioxidant BHT (Table 2). It was revealed that methanol extract showed highest total antioxidant activity, i.e. 1.182±0.09 as compared to other fractions. The chloroform fraction also showed reasonably good value, i.e. 0.951±0.05. Petroleum ether and aqueous extracts showed very less values (0.432±0.03 and 0.139±0.02 respectively). The results were compared with BHT, a reference standard having total antioxidant activity 1.293 ± 0.09.
**DISCUSSION**

The model of scavenging the stable DPPH radical is very widely used to evaluate antioxidant activities in a relatively short time. The addition of extracts to the DPPH solution caused a rapid decrease in the optimal density at 517 nm. The degrees of decoloration indicated the scavenging capacity of the extracts. Free radicals cause autoxidation of unsaturated lipids in food. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. Antioxidants cease the free radical chain of oxidation to donate hydrogen from the phenolic hydroxyl groups. Hence, 92.17 ± 1.25% inhibition of DPPH radical at a concentration of 130 µg/ml of chloroform extract exhibited significant percent inhibition of DPPH radical as compared to the inhibition by other fractions as recorded by Abbasi et al. (2012). The significant total antioxidant activity in phosphomolybdium method, i.e. 1.182±0.09 and 0.951±0.05 of methanol and chloroform extracts, respectively, confirms the presence of ascorbic acid, some phenolics, tocopherols and carotenoids. The presence of such compounds has already been indicated by Prieto et al. (1999). Ferric Reducing Antioxidant Power (FRAP) assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions and the reducing power of methanolic extract showed good FRAP value, i.e. 142.64 ± 0.96 TE µM/mL while chloroform extract also possessed good FRAP values. High reducing power of antioxidants against oxidative effects of reactive oxygen species. Electron donating anti-oxidants can be described as reductants and inactivation of oxidants by reductants can be described as redox reactions and the reducing power of methanolic extract showed good FRAP value, i.e. 142.64 ± 0.96 TE µM/mL while chloroform extract also possessed good FRAP value.

**Table 2: IC₅₀, total phenolics, total antioxidant activity, FRAP values and lipid peroxidation inhibition values of different extracts of Iris aitchisonii (Bakar) Boiss.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>IC₅₀ (µg/mL) ± S.E.M²</th>
<th>Total antioxidant activity ± S.E.M²</th>
<th>FRAP value (TE µM/mL) ± S.E.M²</th>
<th>Total phenolics (GAE mg/g) ± S.E.M²</th>
<th>Inhibition of lipid peroxidation (%) ± S.E.M²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether extract</td>
<td>284.2±0.33</td>
<td>0.432±0.03</td>
<td>11.33±1.33</td>
<td>27.33±4.7</td>
<td>15.31±1.56</td>
</tr>
<tr>
<td>2</td>
<td>Chloroform extract</td>
<td>48.55±1.08</td>
<td>0.951±0.05</td>
<td>127.33±0.69</td>
<td>122.33±0.12</td>
<td>51.61±0.64</td>
</tr>
<tr>
<td>3</td>
<td>Methanol extract</td>
<td>189±2.36</td>
<td>1.182±0.09</td>
<td>142.64±0.96</td>
<td>121.83±0.85</td>
<td>27.99±0.69</td>
</tr>
<tr>
<td>4</td>
<td>Aqueous extract</td>
<td>415.12±3.05</td>
<td>0.139±0.02</td>
<td>42.33±0.96</td>
<td>14.83±0.64</td>
<td>9.22±0.87</td>
</tr>
<tr>
<td>6</td>
<td>BHT²</td>
<td>12.52 ± 0.89</td>
<td>1.293 ± 0.09</td>
<td>-</td>
<td>-</td>
<td>62.93 ± 0.78</td>
</tr>
</tbody>
</table>

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a) All results are presented as mean ± standard mean error of three assays.
b) Standard antioxidant.

**Ferric Reducing Antioxidant Power (FRAP) Assay**

The FRAP assay of the methanol extract showed highest value, i.e. 142.64±0.96 TE µM/mL. Chloroform extract also showed a good value of 127.33±0.69 TE µM/mL while petroleum ether and aqueous extracts showed poor FRAP values. High FRAP values may be ascribed partially to the presence of phenolic and flavonoid contents. The results were compared with the blank having value 10.73.

**Total Phenolic Contents**

Table-2 shows the phenolic contents in the studied fractions of plant extracts in milligrams of gallic acid equivalents (GAEs) per gram of fraction. Among them, chloroform and methanol extract showed good total phenolic contents, having values very near to each other, 122.33±0.12 and 121.83±0.85 GAE mg/g respectively. Petroleum ether and aqueous extracts showed poor values. The results were compared with the blank having a value, 12.68.

**Ferric Thiocyanate (FTC) Assay**

It was observed from the results (Table 2) that that chloroform extract showed highest value for % inhibition of lipid peroxidation, i.e. 51.61±0.64 %. Methanol extract showed moderate activity (27.99±0.69 %) while petroleum ether and aqueous extracts showed no significant activities in this assay. The results were compared with BHT, i.e. 62.93 ± 0.78 %.
value, i.e 127.33±0.69 TE µM/mL. These results were similar to those of Ajaib et al. (2013), but on different plant species, such as Echinochloa colona (Linn.) Link and Sporobolus comandelianus. Chloroform and methanol extract showed good total phenolic contents having values 122.33±0.12 and 121.83±0.85 GAE mg/g respectively. Similar results were also obtained by Abbasi et al. (2012) and Malik et al. (2012).

REFERENCES


Aqil, F., Ahmad,I., & Mehmood, Z., 2006 Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally used Iranian Medicinal Plants. Turkish J. Biology, 30: 177-183.


