ANTIOXIDANT ACTIVITY IN SEEDS OF Avena fatua AND Chenopodium album  
WEEDS ASSOCIATED WITH WHEAT CROP

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https://doi.org/10.28941/24-3(2018)-2

ABSTRACT

Evaluation of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity through various assays of weed plants, Avena fatua (wild oat) and Chenopodium album (common lambsquarters), has never been reported before. In the present study, methanolic seed extracts of A. fatua and C. album were used. Total phenolic and flavonoid contents were determined by Folin-Ciocalteu and aluminum chloride methods, respectively. Moreover, antioxidant activity was determined through DPPH scavenging method, phosphomolybdenum assay and Ferric ion (Fe\textsuperscript{+3}) reducing assay. The TPC values of methanolic extracts were 26.1 and 28.9 mg GAE/g dw while TFC values were 1.11 and 2.23 mg CE/g dw for A. fatua and C. album, respectively. The antioxidant activity of both extracts had shown 62% and 74% inhibition at 0.1 mg/mL extract in DPPH free radical scavenging assay. In addition to that the total antioxidant activity through phosphomolybdenum assay, the values of 75 and 120 mg AAE/g dw of both extracts were obtained. Ferric ion (Fe\textsuperscript{+3}) reducing antioxidant power assay exhibited high antioxidant activity in both extracts by using ascorbic acid as a positive control. These results showed that phenolic and flavonoids were found in high concentration in two weeds which had strong antioxidant activity. However, C. album had higher antioxidant activity as compared to A. fatua.

Keywords: Antioxidant activity, Avena fatua, Chenopodium album, phenolic content (TPC), total flavonoid content (TFC).


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INTRODUCTION

A large number of weeds are associated with wheat crop. Some of these are used as traditional herbal medicines. Phytochemical analysis of the seeds of fifteen wheat weeds showed that these contained very active natural products like phenols and flavonoids etc. (Abbas et al., 2012) and possess medicinal and physiological or biochemical actions (Sofowara, 1993; Ajayi, et al., 2011).

*Avena fatua* (wild oats vern. jangli jai) is the most competitive weed in grain crops such as barley and wheat. It has been reported that extract of *A. fatua* in water exhibited pronounced allelopathic and phytotoxic effects on the wheat seedlings and its subsequent growth. The harmful effects on wheat are due to release of phytochemicals from various parts of *A. fatua* by different physiological processes, for example leaching and volatilization etc. thus, inhibiting the growth of wheat crop production (Ahmad et al., 2014). Nielsen et al. (1960) found toxic effect of extract of *Avena sativa* on growth of wheat. The same finding was observed from experiment conducted by Guenzi and Mc Calla (1962). In this weed plant, five phenolic acids were reported which cause inhibition of wheat growth (Guenzi and McCalla, 1966). Schumacher et al. (1983) found the effect of two more phenolic acids obtained from the roots of *A. fatua* by exudation process and reduced the growth of shoots of wheat plants. Similar results were obtained from 23 more weeds when aqueous extracts were used (Schumacher et al., 1982). Bhatia et al. (1982) also found identical results on inhibition of growth through study of allelopathic effects of some weeds on wheat. *C. album* (common lambsquarters vern. Bathu), is another one of the most important weeds of wheat, exhibited same toxic effect on seedlings. Beside adverse effects of weeds on the yield of wheat crop, they give some advantages too. Seeds of *A. fatua* serve as a tonic, laxative and nerve stimulant (Hussain et al., 2006).

It is also used in chorea, epilepsy and nervous exhaustion. *C. album* grows very fast in wheat fields. It is a very nutritious and healthy addition to the diet. It is commonly used as herbal medicine (Yadav et al., 2007). *C. album* possesses some medicinal activity which include anti-inflammatory and laxative property etc. Its black seeds are rich in minerals, iron, phosphorus, and calcium. It also contains β- carotene, saponins and vitamins A, C and D. Dietary and medicinal plants are good sources of antioxidants (Schuler, 1990). Phenolics and flavonoids present in the plants are the important and more active antioxidants (Atoui et al., 2005) than the precursors of vitamin E, C and carotenoids (Dai and Mumper, 2010). These antioxidants present in medicinal plants were found more active than those in vegetables and fruits (Chodak et al., 2011). Therefore, potential of weed plants can be related to their antioxidant activity as flavonoids, phenols and tannins were found in high concentration in two weeds namely, *A. fatua* and *C. album* (Asma-Saeed et al., 2016).

During normal metabolic processes in the human body, large number of free radical species (with an odd, unpaired electron) and some non-radical species (the precursors of free radicals) are frequently formed (Keser, et al., 2014). Free radicals react with electron rich molecules like DNA, lipids and proteins in order to gain required electron for its stability. Due to this reaction, metabolism is disturbed, resulting in failure of cellular functions (Metin et al., 2013). Various diseases such as cancer, diabetes and aging are emerged out consequently (Wu and Ng, 2008). Vitamins C and E in the human body act as antioxidants and neutralize the free radicals so they act as scavengers. Antioxidants are the molecules which scavenge free radicals (oxidants) and inhibit oxidative damage to the tissue. The study on the antioxidant activity in weed plants has been not reported as yet. Many antioxidants in plants have different mode of actions and broad categories of assays are required to evaluate antioxidant activity of...
compounds. Therefore, we aimed to assess antioxidant activity through different assays and to estimate phenolic compounds in methanolic extracts of seeds of A. fatua and C. album.

**MATERIALS AND METHODS**

Seeds of the studied weeds were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan.

**Preparation of extracts**

20g of fine powder of seeds were added to 100 mL of 80% methanol and kept for at least seven days. From time to time, it was agitated vigorously for efficient extraction. Following normal filtration, it was evaporated to dryness until black-brownish color was obtained by using Rotavapor (vacuum distillation).

**Antioxidant activity by DPPH radical scavenging**

The DPPH is 2, 2-diphenyl-1-picrylhydrazyl, stable free radical. When it is scavenged by the extracts containing natural products, its red color is changed to yellow (Yen *et al.*, 2005). This scavenging capacity was determined by method of Hatano *et al.* (1988) which was performed as follows: The methanolic extract of different concentration ranging from 0.02-0.1mg/mL was prepared. To 2 mL of extract, 1 mL of 0.1mM DPPH methanolic solution was added. After mixing, it was incubated for half an hour and the absorbance was measured at 517 nm. Blank was prepared in which extract was replaced by methanol. Ascorbic acid solutions of same concentrations (0.02-0.1mg/mL) as standard were prepared and followed the same procedure of antioxidant activity as positive control. The DPPH scavenging activity was calculated as =\((\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}} / \text{Absorbance}_{\text{control}}) \times 100\)

**Determination of Total Phenolics Content**

TPC was determined according to a Singleton and Rossi (1965). Aliquot of 40 µL of various extracts (20 mg/5mL of methanol) was added to 3.16 mL of water and then added 200 µL of 10 time diluted Folin-Ciocalteu reagent. After incubation for 8 min, 600 µL of 20% \(\text{Na}_2\text{CO}_3\) was added. The whole content was again incubated for ½ h at 40°C. The absorbance was taken at 760 nm. In blank, extract was replaced by methanol. Gallic acid standard curve in range of 50 to 750 µg/mL was constructed following above procedure to develop straight line equation \((y=0.0009x-0.0837 \ (R^2=0.9992))\) where \(y\) is the absorbance and \(x\) is the µg/mL gallic acid. The results were expressed as mg of GAE per g of extract.

**Determination of Total Flavonoids Content**

TFC was estimated by colorimetric assay as described by Kim *et al.* (2003). The incubation mixture consisting of 0.5 mL of extract (20 mg/5 mL), 4 mL of water, 0.3 mL of 5% \(\text{NaNO}_2\) and 0.3 mL of 10% \(\text{AlCl}_3\) was incubated for 5 min. Then 2 mL of 1M NaOH were added. The content was mixed and absorbance was measured at 510 nm against reagent blank in which extract was replaced by methanol. A standard curve was constructed with catechin (20-100 µg/mL) and catechin equivalents in the extract was determined from the equation \(y=0.003x-0.001 \ (R^2=0.999)\) where \(x\) is the catechin in extract as µg/mL. The results were expressed as mg catechin equivalents (CE)/g of dry sample.

**Total antioxidant assay**

A method of phosphor-molybdenenum assay was used (Prieto *et al*., 1999). 0.3mL of extract (20 mg/5 mL of methanol) was added to 3 mL of reagent mixture (composed of 1 mL of 0.6 M sulfuric acid, 1 mL of 28 mM sodium phosphate and 1 mL of 4 mM ammonium molybdate). The content was mixed thoroughly and incubated for 90 min at 95°C. After cooling, the absorbance at 695 nm was measured against a reagent blank in which methanol was used instead of extract. Standard curve was constructed with ascorbic acid (50 to 750 mg/L or µg/mL) and mg ascorbic acid equivalent (AAE) per g of extract was determined from equation \(y=0.0025x-0.0992 \ (R^2=0.9951)\).
The extract was determined as described by Saeed et al. (2012). The extract of different concentrations (0.02-0.1mg/mL) was prepared in methanol. To 1mL of each concentration of extract, 2.5 mL of 0.2M phosphate buffer pH 6.60 and 2.5 mL of 1% potassium ferricyanide were added. It was followed by incubation at 50°C for 30 min. and then 2.5 mL of 10% trichloroacetic were added. After mixing it was spun down at 2500 rpm for 5-10 minutes to get top layer. To 1 mL of top layer, 1 mL water and 0.2 mL of 0.1% FeCl₃ were added. The absorbance at 700 nm was recorded. Blank was prepared in which FeCl₃ was omitted from above reaction mixture. Ascorbic acid solutions of same concentrations (0.02- 0.1mg/mL) as standard were prepared and followed the same procedure for determining the ferric ion reducing antioxidant power.

**Statistical Analysis**

All Mean values were expressed as mean ± SD.

**RESULTS AND DISCUSSION**

While studying extraction of apple pomace by different organic solvents, the highest total phenolic contents were achieved when extraction was carried out with methanol (Zhang et al., 2016). Similar result was also found in the extraction of *Ranunculus arvensis* (Bhatti et al., 2015). Mohadjerani and Hosseinizadeh (2014) used hydro-methanolic extract for estimating antioxidant which offered good polarity and high yield of phenolic contents were achieved (Asghar et al., 2016). On the basis of this study methanol became a solvent of choice and hydro- methanolic extraction was used for evaluation of phenols, flavonoids & antioxidant activity.

For DPPH radical scavenging action, electron transfer (ET) as well as hydrogen transfer (HT) mechanisms are suggested but HT mechanism is likely to be dominant for some reasons (Marxen et al., 2007). Therefore, extract containing phenols and flavonoids has the ability to donate proton (H⁺) radical to DPPH radical to transform it into reduced form resulting in change of color. Table-1 shows that extracts from both plants had high DPPH radical scavenging activity which may due to protic flavonoids, facilitating HT mechanism (Ahmed et al., 2015). More than 60% DPPH inhibitions were displayed at concentration of 0.1mg/mL extract. However, antioxidant activity of *C. album* was greater than that of *A. fatua*.

In determining the total phenolic contents, a phenol donates H⁺ ion to Folin-Ciocalteu reagent and transform it into reduced form, producing blue color (Huang et al., 2005). The color change is measured. No reports were found regarding the phenolic contents in the extracts of weed plants by which the results of present work could be compared total phenolic contents were found 26.12 and 28.90 mg of gallic acid / g of extract of *A. fatua* and *C. album*, respectively as shown in Table-2. Our results are somewhat comparable with the findings of *Chalcas koeingii* leaves (Bokhari et al., 2013) who found total phenolic contents of 17.50 mg GAE/g extract. Song et al. (2010) reported wide range varying from 0.12 to 59.43 mg GAE/g in extracts among 56 selected Chinese medicinal plants. Similarly, Kahkonen et al. (1999) examined these contents in the extracts of 35 medicinal plants in range of 0.8-36 mg GAE/g dw. In the report of research work by Mahmood et al. (2012), strawberry and mulberry cultivars contained total phenolics (TPC) ranged from 4.91-18.84 mg GAE/g and 2.01-22.87 mg GAE/g dry weight extracts, respectively. These compounds were found good antioxidants (Rice-Evans et al., 1995) which were supported by Suchandra et al. (2007) who investigated phenolic compounds in *Myristica fragrans* had good DPPH scavenging activity and ability to inhibit lipid peroxidation.

All flavonoids have common structure (two six membered rings A and B connected by three carbons-oxygen ring C). Principle involved for flavonoids estimations that Al³⁺ produces pink color compounds by reacting with keto group at 4-positon of C ring and hydroxyl group at 3-positon of ring C or hydroxyl group at 5-position of ring A or with ortho hydroxyl groups in A or B ring (Amic et al., 2007).
The results for total flavonoid contents are presented in Table-3. The flavonoid contents were found 1.11µg CE / mg of extract of *A. fatua* and 2.23 µg CE /mg of extract of *C. album*. Marinova *et al.* (2005) found total flavonoid contents in fruits and vegetables in range of 0.15-1.9 mg CE/g and 0.025-0.76 mg CE/g, respectively while Mahmood *et al.* (2012) reported total flavonoid contents in strawberry and mulberry cultivars in range of 0.83-3.27 mg CE/g and 1.1-10.21 mg CE/g dry weight extracts, respectively. In fact, flavonoids act as antioxidants by donation electrons (Rice-Evan *et al.*, 1996). Antioxidant activity is due to hydroxyl groups in A and B rings but effective free radical scavenging activity is due to OH groups on B ring and double bond conjugated with keto group at 4-positon of ring C which are source of delocalization of electrons from B ring (Iriti and Varooni, 2015).

From above results(Tables-2 and 3), the TPC were found higher than the TFC which indicates that many of the flavonoids constitute a fraction of total phenolics. Mostly fruits have shown about flavonoids/phenolics ratio of 0.32 indicating that fruits had flavonoid contents around 32%. As compared to fruits, vegetables had smaller ratios of flavonoids/phenolics (0.11-0.2) means less amount of total flavonoid contents from 11 to 20% (Marinova *et al.*, 2005). Flavonoids/phenolics ratios were calculated for *A. fatua* and *C. album* also and found to be 0.036 and 0.037, respectively which are very less as compared to that of fruits and some vegetables but comparable to that of *Allium cepa* and *Capsicum anuum* which had ratios 0.07 and 0.08, respectively.

The total antioxidant assay involve an electron transfer mechanism in which H and electron from hydroxyl groups of flavonoids are transferred radical species to convert it into stable species and give rise stable flavonoid radicals (Dorman *et al.*, 2003) where molybdenum (VI) is reduced to molybdenum (V) and green phosphate-molybdate compounds are generated in acid medium. Table-4 shows the total antioxidant activity of both plants extract. The values of 75-120 mg AAE/g dry weight of both extracts were showing their reducing activity. However, the order of their reactivity was *C. album > A. fatua*.

The reducing power was also determined by ferric ion (Fe$^{3+}$) reducing antioxidant power assay in which ferric ions were reduced to ferrous ions by the antioxidants in the extract. Potassium ferricyanide was reduced to potassium ferrocyanide which was converted ferri ferrocyanide in the presence of FeCl$_3$. The results showed that as the concentration of extract or ascorbic acid increased, the absorbance ($\Delta A_{\lambda700}$) was also increased (Table-5). The greater the absorbance ($\Delta A_{\lambda700}$), greater is the reducing antioxidant power. Thus extracts possessing greater phenolic contents and antioxidant activity also showed the good reducing power. Therefore reducing power assay may be considered as one of the most important methods for the estimation of plant antioxidant activity.

**CONCLUSION**

In conclusion, the results indicate that both extracts contained higher concentrations of total phenolic contents and possessed high antioxidant activity. The antioxidant activity of *C. album* was higher than *A. fatua*. However, the specific compounds responsible for antioxidant activity in *A. fatua* and *C. album* are not known as yet, hence further studies may be undertaken to identify phenolic acids, flavonoids and other natural products responsible for the antioxidant effects. These plants may be used as prevention against anti-inflammation and for the treatment of free radical related diseases such as coronary heart diseases, cancer and mutagenesis.

**AKNOWLEDGEMENTS**

The work was executed by the student by a PhD scholar of this department as a part of his Ph.D Dissertation.
Table-1. Comparison of % DPPH radical scavenging activity between different concentrations of extracts and ascorbic acid.

<table>
<thead>
<tr>
<th>Weeds/ Conc. of extracts/Ascorbic acid</th>
<th>0.02mg/mL</th>
<th>0.04mg/mL</th>
<th>0.06mg/mL</th>
<th>0.08mg/mL</th>
<th>0.1mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>21%</td>
<td>34%</td>
<td>43%</td>
<td>54%</td>
<td>62%</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>24%</td>
<td>41%</td>
<td>53%</td>
<td>63%</td>
<td>74%</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>77%</td>
<td>79%</td>
<td>78%</td>
<td>79%</td>
<td>80%</td>
</tr>
</tbody>
</table>

The absorbance against each concentration of ascorbic acid was taken as positive control.

Table-2. Quantitative estimation of total phenolics.

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
<th>St.dev</th>
<th>µg GAE /mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>0.008</td>
<td>0.013</td>
<td>0.010</td>
<td>0.010</td>
<td>0.010</td>
<td>104.48</td>
</tr>
<tr>
<td></td>
<td>0.010</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>104.48</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>0.015</td>
<td>0.021</td>
<td>0.022</td>
<td>0.022</td>
<td>0.022</td>
<td>115.59</td>
</tr>
<tr>
<td></td>
<td>0.021</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>115.59</td>
</tr>
</tbody>
</table>

Y= 0.0009x-0.0837 (R²=0.9992)

<table>
<thead>
<tr>
<th></th>
<th>µg GAE /mL</th>
<th>µg GAE /5mL</th>
<th>µg GAE /20mg dw</th>
<th>mg GAE /20mg dw</th>
<th>mg GAE /g dw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>104.48</td>
<td>522.41</td>
<td>522.41</td>
<td>0.52</td>
<td>26.12</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>115.59</td>
<td>577.96</td>
<td>577.96</td>
<td>0.58</td>
<td>28.90</td>
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</tbody>
</table>

The results are expressed as means ± SD of three replicate analyses.

Table-3. Quantitative estimation of total flavonoids.

<table>
<thead>
<tr>
<th></th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Mean</th>
<th>St.dev</th>
<th>µg CE /mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>0.012</td>
<td>0.013</td>
<td>0.012</td>
<td>0.012</td>
<td>0.012</td>
<td>4.44</td>
</tr>
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<td>0.012</td>
<td>0.012</td>
<td>0.012</td>
<td>4.44</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>0.0142</td>
<td>0.0141</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td>4.92</td>
</tr>
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<td></td>
<td>0.0141</td>
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<td>0.013</td>
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<td>4.92</td>
</tr>
</tbody>
</table>

Y= 0.003x-0.001 (R²=0.999)

<table>
<thead>
<tr>
<th></th>
<th>µg CE /mL</th>
<th>µg CE /5mL</th>
<th>µg CE /20mg dw</th>
<th>mg CE /20mg dw</th>
<th>mg CE /g dw</th>
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</thead>
<tbody>
<tr>
<td>Avena fatua</td>
<td>4.44</td>
<td>22.2</td>
<td>22.2</td>
<td>0.0222</td>
<td>1.11</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td>4.92</td>
<td>24.6</td>
<td>24.6</td>
<td>0.0246</td>
<td>1.23</td>
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### Table-4. Quantitative estimation of antioxidant potential.

<table>
<thead>
<tr>
<th></th>
<th>R1 µg AAE/mL</th>
<th>R2 µg AAE/mL</th>
<th>R3 µg AAE/mL</th>
<th>Mean µg AAE/mL</th>
<th>Std. dev</th>
<th>µg AAE /mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena fatua</em></td>
<td>0.67±8</td>
<td>310.8±8</td>
<td>0.6±4</td>
<td>295.6±8</td>
<td>0.7±0</td>
<td>319.6±8</td>
</tr>
<tr>
<td></td>
<td>0.6±4</td>
<td>295.6±8</td>
<td>0.7±0</td>
<td>295.6±8</td>
<td>0.7±0</td>
<td>295.6±8</td>
</tr>
<tr>
<td></td>
<td>0.7±0</td>
<td>319.6±8</td>
<td>0.7±0</td>
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<td>0.7±0</td>
<td>319.6±8</td>
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<td></td>
<td>319.6±8</td>
<td>0.7±0</td>
<td>319.6±8</td>
<td>0.7±0</td>
<td>319.6±8</td>
<td>319.6±8</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>1.06±8</td>
<td>466.8±8</td>
<td>0.9±9</td>
<td>435.6±8</td>
<td>1.0±5</td>
<td>459.6±8</td>
</tr>
<tr>
<td></td>
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<td>459.6±8</td>
<td>1.0±5</td>
<td>459.6±8</td>
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<tr>
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<td>1.0±5</td>
<td>459.6±8</td>
<td>1.0±5</td>
<td>459.6±8</td>
<td>459.6±8</td>
</tr>
</tbody>
</table>

\[ Y = 0.0025x - 0.0992 \] (R²=0.9951)

<table>
<thead>
<tr>
<th></th>
<th>µg AAE /mL</th>
<th>µg AAE /5mL</th>
<th>µg AAE /20mg dw</th>
<th>mg AAE /20mg dw</th>
<th>mg AAE /g dw</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena fatua</em></td>
<td>308.75</td>
<td>1543.75</td>
<td>1543.75</td>
<td>1.5</td>
<td>75</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>487.41</td>
<td>2437.05</td>
<td>2437.05</td>
<td>2.4</td>
<td>120</td>
</tr>
</tbody>
</table>

### Table-5. Ferric Ion (Fe³⁺) reducing antioxidant power between different concentrations of extracts and ascorbic acid.

<table>
<thead>
<tr>
<th>Weeds/ Conc. of extracts/Ascorbic acid</th>
<th>0.02mg/mL (ΔA₇₀₀)</th>
<th>0.04mg/mL (ΔA₇₀₀)</th>
<th>0.06mg/mL (ΔA₇₀₀)</th>
<th>0.08mg/mL (ΔA₇₀₀)</th>
<th>0.1mg/mL (ΔA₇₀₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Avena fatua</em></td>
<td>0.4</td>
<td>0.9</td>
<td>1.3</td>
<td>2.15</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>0.51</td>
<td>1.2</td>
<td>1.7</td>
<td>2.45</td>
<td>2.9</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>2.0</td>
<td>2.75</td>
<td>3.75</td>
<td>3.8</td>
<td>4.0</td>
</tr>
</tbody>
</table>
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