ANTIOXIDANT AND PHYTOBIOLOGICAL STUDIES ON TWO ALLIUM CEPA L. EXTRACTS

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Antioxidant and Phytochemical Studies on Two Allium cepa L. Extracts (Abstract). Aim: To investigate the antioxidant potential of two Allium cepa L. extracts.

Material and methods: The antioxidant activity of the two extracts (encoded EC1 and EC2) was assessed using two methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity and determination of their reducing power; phytochemical studies were performed using grains of Triticum aestivum L., Falmura variety. Results and discussions: EC1 and EC2 extracts had a particular affinity for binding DPPH radicals. At equivalent concentrations, the reducing power of EC1 extract was about 2 times higher compared to EC2, increasing with the concentration of the analyzed sample. The phytochemical study demonstrated that the investigated extracts had no cytotoxic and genotoxic potential. Keywords: Antioxidant activity, Allium cepa, Phytochemical.

Onion (Allium cepa L.) is widely used around the world as a food product, but also for its medicinal applications. Onion contains many active sulfur compounds, mainly cysteine derivatives, as S-alkyl cysteine sulfoxides, which decompose into a variety of thiosulfimates and polysulfides under the action of the enzyme alliinase (1). They possess antidiabetic, antibiotic, hypcholesterolemic, fibrinolytic and other various biological actions. Approximately 90% of the soluble organic bound sulfur is present as γ-glutamylcysteine peptides, which are not substrates for alliinase (2, 3).

Flavonols are present in onion tissues in the form of free quercetin and kaempferol and as the glycosides of those compounds. The main sapogenins from onion are: sitosterol, oleanolic acid, diosgenin, β-chlorogenin and cepaginen (4).

Over time, onion has received a special attention from researchers, who observed its traditional therapeutic uses. Numerous studies have revealed a multitude of biological actions: antibiotic activity (antibacterial and antifungal), action on the cardiovascular system (antiplatelet, lipid lowering), on the respiratory system, antidiabetic, anticancer, antiinflammatory activities (5, 6).

Material and methods

Extract preparation: Onion extracts
were prepared using fresh bulbs of *Allium cepa* L. (*Wolska* variety) purchased from a local market. The onion bulbs were peeled, cut into small pieces and allowed to stand for 18 h. Then, the product was soaked in absolute ethanol for 10 days at room temperature, with intermittent shaking. The extractive solution was concentrated using a rotary evaporator (under reduced pressure) at 40 °C. The extract encoded EC1 was obtained with a DER (drug extract ratio) of 5:2.

In order to obtain the extract encoded EC2, the onion bulbs were peeled and homogenized in cold water in a blender. The homogenized mixture was filtered through cheesecloth, the filtrate was centrifuged and the clear supernatant was diluted to 100 ml with cold distilled water. The EC2 extract had a DER of 1:2. The aqueous extract was stored at -20°C until use.

**DPPH scavenging activity:** The scavenging activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. When a solution of DPPH is mixed with an extract that can donate a hydrogen atom, the reduced form of the DPPH radical is generated, leading to the reduction of the violet color (7). Briefly, 50 μL of sample (EC1 and EC2) was mixed with 2.95 mL DPPH solution (4 mg %). After 5 minutes, the absorbance was recorded at 517 nm using a V-550 Jasco spectrophotometer. Quercetine was used as positive control. The radical scavenging activity was expressed as percentage of inhibition and was calculated as follows:

\[
\text{Scavenging effect (％) = (Absorbance of control - Absorbance of test)/Absorbance of control) \times 100}
\]

**Reducing power:** The reduction potential of EC1 and EC2 extracts was determined taking into account the fact that substances which act like reducing agents can react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which then reacts with ferric chloride to form ferric ferrous complex, that has a maximum of absorption at 700 nm (8). Ascorbic acid was used as positive control.

**Phytobiological studies:** During our research on EC1 and EC2 extracts, phytobiological studies using grains of *Triticum aestivum* L., *Falmura* variety were performed (9). Three experimental samples (encoded V1, V2 and V3) were used for each set of tests, these representing different concentrations of extract (tab. I).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC1 (mL%)</th>
<th>EC2 (mL%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample (M)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>V2</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>V3</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

The studied aspects were the influence of EC1 and EC2 extracts on plantlet germination, elongation and biomass accumulation and the impact of the extracts on mitosis in root tissues.

Groups of 200 wheat grains were used for both the control and each dilution; these were soaked for 24 hours in 50 mL of each experimental sample and in the same volume of distilled water for the control. 150 wheat seeds were washed and placed uniformly upon a system made of sieves, with plastic vessels filled with water placed below. The cultivation was performed in an air-conditioned chamber at an average air temperature of 22±2°C and constant light of 2.500 lux. After 7 days, the germination was stopped, the seed germination rate was
calculated and the shoot and root length was measured. Seedlings having less than 50 mm in length (both root and stem) were excluded from the experiment. Dry weight of roots and shoots for each treatment version was determined, after oven drying at 40 °C for 24 h.

On day 10 the roots and shoots of the wheat seedlings were detached and weighed. After the determination of their fresh weight (FW) the seedlings were maintained in an oven at 105 °C for 24 h and weighed again in order to obtain the dry weight (DW). Their average fresh and dry mass was calculated for each experimental sample and also for the control. But, in order to objectively assess to what extent the biostimulatory or bioinhibitory effect was real, the ratio DW/FW x 100 was calculated.

The remaining 50 pre-imibed grains were placed in 10 cm diameter Petri dishes, on filter paper wet with distilled water. After 72 h, the primary roots were cut off and a squash preparation stained with Schiff reagent was made. Both normal ana- and telo-phases and those with different types of aberrations (bridges, fragments, fragments and bridges, tri- and tetrapolar anaphases, chromosomes in ring) were counted.

RESULTS AND DISCUSSION

Considering the mechanisms that express the antioxidant activity, the ability to give up a hydrogen atom (or an electron) of the investigated EC1 and EC2 extracts was lower compared to that of 5 mM quercetine, used as control.

Qualitative and quantitative differences concerning the active ingredients with antioxidant potential (flavones, polyphenolic acids, sulfur compounds) present in EC1/EC2 extracts (10) explained why the DPPH scavenger activity was higher for EC1 extract compared to EC2 (fig. 1).

The increase in absorbance was directly correlated with the reducing power, parameter that can serve as a significant indicator of the antioxidant potential. Data analysis suggested that at the same concentration the investigated extracts had different reducing abilities: at the concentration of 500 mg/mL EC1 extract had a reducing potential equal to that of 6.7 mmol/L ascorbic acid, while the EC2 reducing potential corresponded to that of 3.76 mmol/L ascorbic acid (fig.2).

The experimental data showed a low percentage of inhibition of Triticum seed germination for the investigated extracts. The influence of the treatment with onion extracts on the growth of Triticum aestivum plantlets was studied by length measurement of roots and shoots (tab. II).

As to the increase in root length, this parameter was generally positively influenced, except for sample V1 for EC1 and EC2 extracts.

The influence of EC1 and EC2 extracts on biomass accumulation is shown in table III.

In the case of EC1 extract, sample V2 caused a slight increase in root biomass accumulation, in both fresh and dried
plants. Fresh and dry shoot biomass accumulation was similar but lower than in the control (fig. 3, 4).

According to the results presented in figs. 3 and 4, it can be stated that the treatment of the caryopses with EC1 and EC2 extracts did not significantly influence the biomass accumulation in the wheat plantlets. The cytogenetic analysis showed that the frequency of chromosomal aberrations did not exceed 4% for all treatment samples, this value being considered normal for chromosomal aberrations during in vivo phytotoxicity testing (fig. 5).

![Graph showing reducing power of the investigated extracts](image)

**Fig. 2. Reducing power of the investigated extracts**

### TABLE II
**Effect of EC1 and EC2 extracts on germination rate and root and shoot length**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample of treatment</th>
<th>Germination rate %</th>
<th>Root length (mm) ± SD</th>
<th>Shoot length (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>M</td>
<td>93</td>
<td>119.97 ±12.40</td>
<td>149.34 ±12.17</td>
</tr>
<tr>
<td>EC1</td>
<td>V1</td>
<td>92</td>
<td>108.55 ±11.13</td>
<td>144.28 ±14.44</td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>91</td>
<td>134.07 ±13.66</td>
<td>146.93 ±15.28</td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>85</td>
<td>139.8 ±17.44</td>
<td>148.8 ±15.08</td>
</tr>
<tr>
<td>EC2</td>
<td>V1</td>
<td>87</td>
<td>114.76 ±13.19</td>
<td>146.5 ±14.06</td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>90</td>
<td>138.8 ±15.19</td>
<td>143.80 ±16.86</td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>68</td>
<td>128.2 ±13.82</td>
<td>144.36 ±12.35</td>
</tr>
</tbody>
</table>

FW=fresh weight; DW=dried weight

### TABLE III
**Influence of EC1 and EC2 extracts on biomass accumulation**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample of treatment</th>
<th>Root</th>
<th>Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FW (g)</td>
<td>DW (g)</td>
</tr>
<tr>
<td>water</td>
<td>M</td>
<td>7.74</td>
<td>0.74</td>
</tr>
<tr>
<td>EC1</td>
<td>V1</td>
<td>7.29</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>8.05</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>8.76</td>
<td>0.77</td>
</tr>
<tr>
<td>EC2</td>
<td>V1</td>
<td>6.05</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>V2</td>
<td>7.69</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>V3</td>
<td>6.55</td>
<td>0.60</td>
</tr>
</tbody>
</table>

FW=fresh weight; DW=dried weight
Antioxidant and phytobiological studies on two Allium cepa L. extracts

**Fig. 3.** Fresh and dry biomass accumulation in *Triticum* roots (a) and shoots (b) under EC1 treatment

**Fig. 4.** Fresh and dry biomass accumulation in *Triticum* roots (a) and shoots (b) under EC2 treatment

**Fig. 5.** Frequency of chromosomal aberrations in *Triticum aestivum* root meristem induced by treatment with EC1 and EC2 extracts

**CONCLUSIONS**

The results revealed that EC1 and EC2 extracts possess an interesting antioxidant activity. According to the phytobiological studies, the biostimulatory or bioinhibitory
effects of the investigated extracts were not significant compared with the blank. The results indicated that the extracts were well tolerated by plant cells, revealing the lack of cytotoxicity and mutagenicity.

REFERENCES