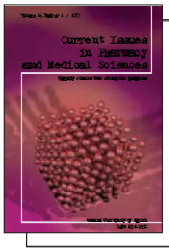

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Screening of the antioxidant potentials of polar extracts from fruits of *Eryngium planum* and *Eryngium amethystinum* using the β-carotene-linoleic acid assay

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ABSTRACT

The research shows the antioxidant potential of methanolic and methanolic-water extracts of two species *Eryngium amethystinum* and *Eryngium planum* using the β-carotene-linoleic acid assay. The fruits of both species were the source for preparing the samples. The antioxidant measurements were done by UV-VIS spectrophotometric using the phenomenon of bleaching of solution of β-carotene during its oxidation. The results are presented as AA_{50} value, which refers to the concentration of a substance needed to inhibit 50% oxidation of a sample and the standard activity coefficient (SAC), which corresponds to the amount of reference substance (trolox) to which it has the same potential like the tested substance.

Keywords: Eryngium planum, Eryngium amethystinum, antioxidant, β-carotene, trolox, β-carotene-linoleic acid assay

INTRODUCTION

Genus *Eryngium* belonging to *Apiaceae* family has been known in traditional medicine for a long time. The extracts from aerial parts of different species of *Eryngium* L. are used all over the world as anti-inflammatory agents and analgesics [13]. There are reports that some of *Eryngium* L. species have anti-microbial and antifungal effects [6,7]. Earlier reports show that the phenolic compounds are one of the major groups of active substances in the genus *Eryngium* [13]. It can be assumed, that they are responsible for great part of biological activity of these plants. They are well-known as good antioxidants, but there are only few papers indicating that plants form genus *Eryngium* have the antioxidant potential [12,13].

The results of many research studies indicate that the oxidative stress plays an important role in the development and the progression of neurodegenerative diseases like Alzheimer disease and Parkinson's disease [4]. There are many epidemiological studies, which show that a higher intake of antioxidant compounds is associated with a lower risk of mortality from cancer and coronary heart diseases. Because of that, there are thousands of publications appearing every year on the antioxidant properties of plant products [9]. Antioxidants are essential and important for plants and animal life. They protect cells from the damage caused by unstable molecules known as free radicals and reduce the oxidative stress. Still plants are the biggest source of new antioxidants. It is necessary to look for new ones because the use of some antioxidants that are well-known on the market is not entirely devoid of the side effects. For example, there are some reports that a high, long-term β-carotene supplementation increases lung tumor rates in smokers [5]. The discovery and examination of a new substance or plant extract with antioxidant properties, which will be completely free from side effects, when administered to a patient, is a challenge for medical science nowadays.

There are many methods that can be helpful in determining an antioxidant potential of plant extract. Majority of the methods are based on a comparison of the color change in the sample tested in the presence of an antioxidant and without it. It can be easily measured with spectrophotometer. Examples of this type of research are DPPH radical scavenging assay, β-carotene-linoleic acid, Ferric reducing antioxidant power assay (FRAP), Trolox equivalent capacity assay (TEAC) and many others [3].

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The results of this type of assays give information about the overall capacity of the tested substance. When the sample is complex, liquid chromatography comes to rescue. Combining the aforementioned methods with thin layer chromatography (TLC) allows constructing the antioxidant profile of complex samples. Commonly used method like this is TLC-DPPH assay [4,11]. In this kind of assays, the quantitative and semi-quantitative measurements are based on densitometry, or photographic analysis.

For this research, the β-carotene-linoleic acid method was chosen. This method is accurate and widely used in phytochemistry [1,2]. The aim of presented study was to investigate the antioxidant activity of two species: *Eryngium planum* and *Eryngium amethystinum.*

MATERIALS AND METHOD

Apparatus and reagents. The spectrophotometric measurements were carried out with the spectrophotometer Genesys 10S VIS (Thermo-Fischer Scientific). The solvents used in this study were of analytical purity grade purchased from POCH S.A. Polish Reagents (Gliwice, Poland). The β-carotene Trolox and linoleic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Plant material. Fruits of *Eryngium planum* and *Eryngium amethystinum* were collected in September 2011 in the Medicinal Plant Garden of the Department of Pharmacognosy, Medical University of Lublin, Poland. They were dried at the room temperature and then immediately powdered according to norms accepted for the fruits. The voucher specimen is deposited in the Department of Pharmacognosy, Medical University in Lublin, Poland.

Extraction. The extracts from the homogenized fruits of *Eryngium planum* and *Eryngium amethystinum* (20g) were obtained by an extraction on water bath under the reflux three times for 30 minutes at the temperature of 80°C, every time using the fresh portion of solvent (100ml). The pure methanol and 50% solution of methanol in water were used as solvents. Afterwards the solvents were evaporated in the vacuum at the temperature of 70°C. Two types of extracts were obtained for each plant (methanolic and methanolic-water). The yields of extraction are presented in Table 1.

β-*carotene-linoleic acid assay.* The antioxidant activity was evaluated using the modified β-carotene-linoleic acid model system created by Miller [10]. β-Carotene (0.5 mg) in 1ml of chloroform was added to 25 μg of linoleic acid, and 200 mg of Tween 80 emulsifier mixture. After evaporation of chloroform in a vacuum, 100 ml of distilled water saturated with oxygen was added by vigorous shaking. An amount of 3.8 ml of this mixture was transferred into different test tubes containing 0.2 ml different concentrations of the sample (from 1 mg/ml to 10 mg/ml). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50°C. A blank, devoid of β-carotene, was prepared for background subtraction. Trolox was used as the standard. The calibration curves were constructed based on dependence of two values, the percent of antioxidant activity (% AA) and concentration of examined sample. The % AA value was calculated from the equation [10]:

$$\%AA = 100\left[\frac{1-(A1_{(t=0)} - A1_{(t=1000)})}{A0_{(t=0)} - A1_{(t=100)}}\right]$$

where:
%AA – percent of antioxidant activity
$A1_{(t=0)}$ – absorbance of test sample/standard at zero time
$A1_{(t=120)}$ – absorbance of test sample/standard after 120 min.
$A0_{(t=0)}$ – absorbance of the aqueos control sample at zero time
$A0_{(t=120)}$ – absorbance of the aqueos control sample after 120 min.

The results were presented as AA_{50} value and the standard activity coefficient (SAC). The AA_{50} value refers to the concentration of a substance needed to inhibit 50% oxidation of a sample. AA_{50} values were calculated from the calibration curves that are showed on Figures 1 and 2.

SAC was counted for the examined substances [11]. SAC refers to the amount of reference substance (Trolox) to act as antioxidant in the same way as the tested substance. It was calculated using the following formula:

$$SAC = \frac{m_s}{m_e}$$

where:
m_s – mass of reference compound
m_e – mass of tested substance

The m_s and m_e are in the linear range and both have the same antiradical activity (% of inhibition).

RESULTS AND DISCUSSION

The presented method is based on reaction between the linoleic acid free radical and the highly unsaturated β-carotene molecules. When there is no antioxidant in the solu-

Table 1. The results of antioxidant activity for all extracts of *E.planum* and *E.amethystinum* and trolox

Examined substance	Yields of extraction	EC_{50} [mg/ml]	SAC	Standard curve formula	R^2
Eryngium amethystinum ME	1.809%	8.19	0.1099	y = 5.5547x – 4.5066	0.9297
Eryngium planum ME	3.329%	8.64	0.1041	y = 5.0475x + 6.3737	0.9482
Eryngium amethystinum MWE	1.017%	2.37	0.3791	y = 4.0045x + 40.4	0.9648
Eryngium planum MWE	1.943%	4.51	0.1992	y = 4.0389x + 31.76	0.9817
Trolox	–	0.89	1	y = 14.708x + 36.765	0.9283

ME – Methanolic extracts
MWE – Methanolic-water extracts

tion, the orange color of β-carotene is rapidly bleached. The extent of discoloration is monitored spectrophotometrically at 470 nm [1]. The lowest discoloration of β–carotene refers to highest antioxidant activity. Table 1 shows the results of this investigation for the AA_{50} and SAC values. The calibration curves that are presented on Figures 1 and 2, confirm that the experiment was done in the linear concentration range with good correlation, the obtained R^2 value ranged from 0.9283 to 0.9817. It has been clearly proved that the methanolic-water extracts of both investigated plants are potentially stronger antioxidants than methanolic extracts. The highest antioxidant potential was obtained for methanolic-water extract of *E. amethystinum*. The obtained SAC value for this extract was 0.3791. It means that the 1g of extract acts as antioxidant like 0.3791 g of well-known antioxidant – Trolox. The SAC values for other extracts amounted to 0.1992 for methanolic-water extract of *E.planum,* and very similar to both methanolic extracts, 0.1099 for *E. amethystinum,* and 1.041 for *E.planum.*

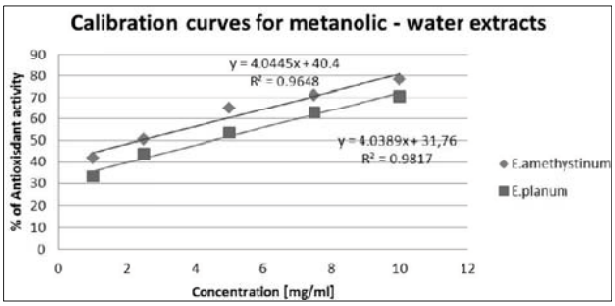

Fig. 1. Calibration curves of methanolic extracts of *Eryngium amethystinum* and *Eryngium planum*

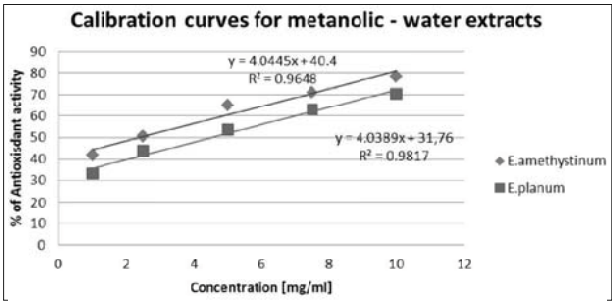

Fig. 2. Calibration curves of methanolic-water extracts of *Eryngium amethystinum* and *Eryngium planum*

It can be found in the literature that extracts of *E.planum* contain a lot of phenolic acids like rosmarinic, chlorogenic and caffeic [8,12]. Phenolic compounds are well-known as antioxidants with high potential because that they might be responsible for big part of antioxidant potential of *E.planum.* However, there is lack of information about the phytochemistry of *E. amethystinum*, but the close botanical relationship suggests that this plant also may contain many phenolic compounds. The extensive phytochemical analysis of this species should be done. The knowledge about phytochemistry of these plants will allow assessing this species as a source of antioxidant compounds. The β-carotene linoleic acid assay related with TLC chromatographic methods will be carried out in future.

CONCLUSIONS

The β-carotene-linoleic acid assay is a good and simple method to evaluate the overall antioxidant activity of plant extracts. The results show that the water-methanolic extracts of *E. amethystinum* and *E. planum* are more potent in antioxidant contents than the methanolic extracts. It was also found that *E.planum* extracts show stronger antioxidant activity than similar extracts for *E.amethystinum*. For more detailed information phytochemical examination should be done.

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