THE INVESTIGATION OF ANTIOXIDANT ACTIVITY OF HEDERA HELIX L. COMPONENTS USING HPLC POST COLUMN ABTS METHOD

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Antioxidants are vital substances which possess the ability to protect the body from damages caused by free radical induced oxidative stress. Free radicals may disrupt normal or lead to the pathological cell metabolism causing diseases such as cancer, cirrhosis, and arteriosclerosis [5]. Natural antioxidants might play an important role in the free radical metabolism. Some research studies have demonstrated that *Hedera helix L.* serves as strong antioxidants [3]. Previously for determination of *Hedera helix L.* antioxidant activity spectrophotometric method was used [1]. There is a wide variety in nature of naturally occurring antioxidants that differ in their composition, physical and chemical properties and action mechanisms. That's why quite necessary to investigate the antioxidant activity of individual components, for finding main markers in ivy leaves. The ABTS-HPLC method is employed in rapid identification of antioxidants and determination of their activity. The objective of this studies was to evaluate components that show antioxidant in *Hedera helix L.* leaf obtained from different countries such as Ukraine, Lithuania, Poland, Czech Republic, Austria, Slovakia, Hungaria and Greece.

Prior to preparation of extract hedera leaves was ground in a cross beater mill. Powdered material (500 mg) was extracted with 10 ml of methanol on the UV-sound bath for 20 min. Prepared extracts were filtered through 0.45 µm membrane filter. HPLC analysis has been carried out using Waters 2695 chromatography system equipped with Waters 996 PDA Detector. For separation of substances ACE 5 C_{18} (250*4.6 mm) was used. Another HPLc conditions for determination of *Hedera Helix L.* antioxidant activity was as follows: the solvent A (0.1% acetic acid) and solvent B (acetonitrile), The following eluation profile was used: 95% A/5% B-0min, 85% A/15 % B – 8 min, 80% A/ 20% B – 30 min, 60% A/40% B – 48 min, 50% A/50% B – 58 min, 50% A/50% B – 65 min, 5% A/95% B -66 min. The column temperature was ambient. The flow rate was 1 ml/min and injection was 10 µL. After applying the HPLC-PDA detection system, the mobile phase was entered into a reaction coil through a mixing tee where the reagent (ABTS) was supplied at the same time by a Gilson pump 305. Reaction coils made of TFE (Teflon) of 3 m length, 0.25mm i.d. was used. The system with ABTS solution was monitored as follows: temperature set at 50˚ C and the flow rate of the reagent was set 0.5 ml/min. The ABTS solution was prepared following the methods described by Renolds et al [4].

The reaction of the antioxidant compounds with the ABTS reagent resulted in a color change that was detected at 650 nm wavelength. The post column antioxidant activity of the extract compounds was assessed by comparing their activity to standard, Trolox. Calibration curves was prepared from a Trolox ethanol solution at eight dilutions in the range of 0.625-80µg/ml [2].

The HPLC-ABTS assays have been developed and applied for rapid screening and identification of components that have antioxidant activity from the extracts of herbs. The results of ABTS postcolumn assay in terms of the TEAC values (mg/g) for the measured compounds showed statistically significant differences in antiradical response.

According to obtained data, the following components showed the highest antiradical response in the ivy leaves: chlorogenic acid (Rt is 12.4min), hyperoside (23.6min), apigenin 7-glucoside (32.06 min), the antioxidant activity was calculated in terms of TEAC for each of mentioned substances and for all substances in plant, the results are shown in the Table1.

Table 1

Antioxidant activity of components in the ivy leaves expressed as TEAC values

Country (region)	Chlorogenic acid (mg/g)	Hyperoside (mg/g)	Apigenin 7-glucoside (mg/g)	Total amount (mg/g)
Lithuania (Naujoji Akmene)	1.01	0.45	6.03	8.40
Lithuania (Klaipeda)	2.10	0.34	2.90	6.90
Lithuania (Plunge)	2.69	0.39	2.58	7.04
Lithuania (Silute)	2.40	0.17	0.55	8.97
Lithuania (Kaunas)	2.33	0.44	4.24	6.48
Lithuania (Pumpenai)	2.08	0.35	0.47	3.13
Lithuania (Kaunas)	3.08	0.39	1.79	6.74
Ukraine (Kharkiv)	3.81	0.63	4.67	12.42
Ukraine (Kiev)	0.94	0.35	1.91	4.02
Czech Republic (Praha)	1.62	0.66	1.36	4.19
Czech Republic (Brno)	1.01	0.55	0.80	2.88
Austria (Vienna)	0.38	0.19	0.41	0.98
Poland (Warsaw)	1.80	0.44	1.34	4.10
Poland (Krakow)	0.70	0.22	0.78	4.41
Slovakia (Bratislava)	0.17	0.18	-	2.11
Hungary (Budapest)	2.91	1.49	1.14	6.23
Greece (Athens)	0.65	0.11	1.07	2.32
Greece (Delphi)	1.24	0.24	2.35	5.13
Greece (Meteora)	1.89	0.37	2.06	5.99

The present study proved the antioxidant activity of the identified compounds and total activity of *Hedera helix L.* leaves obtained from different countries. Results showed statistically significant differences in antioxidant activities of the compounds between 19 different places of ivy leaf collecting. The highest value for chlorogenic acid was obtained from Ukraine (Kharkiv) 3.81 mg/g in terms of TEAC values, for hyperoside – Hungary (Budapest) with amount 1.49 mg/g, apigenin 7-glucoside – Lithuania (Naujoji Akmene) 6.03 mg/g, also the highest total amount was measured in Ukraine (Kharkiv) 12.42 mg/g.

In our study, *Heera helix L.* leaves have been tested for the first time in order to determine active components that have antioxidant properties. Thus, our study shows importance on raising awareness about antioxidant activity of the components of ivy leaf. The results showed that the potency of antioxidant activity of *Heedra helix L.* depends on the place where it was collected due to the different quantities of active substances (chlorogenic acid, hyperoside and apigenin 7-lucoside).

As a final conclusion of this study, the selective HPLC-ABTS postcolumn methodlogy was used to identify components that have antioxidant activity in methanol extracts of ivy leaves collected from 8 different countries. The potency of antioxidant activity varies due to different quantities of main components.

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