Antioxidative Effect of Extracts from *Erodium cicutarium* L.

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Erodium cicutarium (Storksbill, Hemlock, Storkshill) Extracts, Antioxidative Activity, *Oenothera paradoxa* (Evening Primrose) Oil

Extracts from *Erodium cicutarium* L. (EC) and some standard substances present in EC extracts were tested for their antioxidative properties during Fe^{2+}-induced triglyceride oxidation. Hydrophobic fractions such as petroleum ether (PEE), benzene (BE) and chloroform I (ChE I) extracts as well as hydrophilic fractions *i.e.* water (WE) and ethyl acetate (EAE) showed an antioxidative effect. Among the tested substances vitamin C and some polyphenolic compounds: tannin, (+)-catechin and gallic acid exhibited antioxidative activity, stronger than the one of the mentioned extracts.

Introduction

Recently, much attention has been paid to biological properties of various plant extracts and their application in therapy. They enhance antibody production (Kumazawa *et al.,* 1982; Kumazawa *et al.,* 1985), stimulate macrophages (Pace *et al.,* 1983), B lymphocytes or NK cells (Kumazawa *et al.,* 1984) and also increase interferon synthesis (Zielińska-Jenczylik *et al.,* 1988).

Erodium cicutarium (EC) is a one- or two-year plant found in the fields, roadsides of the lowlands and lower piedmonts in Poland. It is known for its antihemorrhagic activity, antiviral effect in relation to myxoviruses, *Herpes* virus type 1, vesicular stomatitis and vaccinia virus (Zielińska-Jenczylik *et al.,* 1987).

Reactions producing free radicals in the human body take place very often. Some of them such as unsaturated fatty acids oxidation are very important in natural physiological processes leading to synthesis of prostaglandins, thromboxanes and leukotrienes which are responsible for a number of physiological processes such as immunity, blood pressure and platelet aggregation (Halliwell and Gutteridge, 1988 a).

Another group of free radical reactions are processes caused among others by UV radiation, metal cations (Fe^{2+}, Cu^{2+}) or abnormal products of porphyrin metabolism. Disorders of the free radical control system lead to an concentration increase of very active free radicals such as $OH^{\cdot}$ (Halliwell and Gutteridge, 1988 b), which initiate processes leading, among others, to a modification of DNA or lipid peroxidation. Oxidation of lipids disrupts biological natural membranes which may result in pathological effects.

In this study the influence of extracts from *Erodium cicutarium* on oxidation of *Oenothera paradoxa* (OP) oil, in the presence of Fe^{2+} was investigated.

Materials and Methods

Preparation of extracts

The raw material (the dried *Erodium cicutarium* herb, collected from its natural habitat in the northern part of Poland in autumn) was subjected to fractionated extraction in the Soxhlet apparatus with the following solvents, supplied successively: petroleum ether (PEE), benzene (BE), chloroform (ChE I) and methanol (ME). From the obtained extracts the solvent was distilled off and the remaining residue was dried. After concentration of the ME extract the residue was mixed with 10 parts of boiling water, and heated of 100 °C (water bath) for 2 h. The cooled water solution was separated from the formed sticky precipitate. The clear filtrate (WE) was exhaustively extracted with chloroform, then with ethyl acetate and finally with ethyl ether. The obtained three fractions: chloroform (ChE II), ethyl acetate (EAE) and ethyl ether (EEE) were evaporated under reduced pressure and dried.

All the fractions: PEE, BE, ChE I, WE, ChE II, EAE, EEE and the water residue after extraction process (WR) were tested for their antioxidative activity simultaneously with the standard sub-

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stances identified previously in *Erodium cicutarium* herb.

Measurement of OP oil oxidation

Emulsion: 0.24% (v/v) OP oil in 3.5% (w/v) of arabic gum in 0.2 M Tris(2-amino-2-hydroxymethyl-1,3-propanediol)-HCl buffer, pH 7.4, was shaken vigorously for 10 min.

TBA reagent: 0.375 g of thiobarbituric acid (TBA) was dissolved (boiling bath) in 30 ml H_2O, then 15 g of trichloroacetic acid and 2.1 ml of 10 N HCl was added. The solution was adjusted to 100 ml with distilled water.

Methods: The antioxidant activities of extracts isolated from *Erodium cicutarium* were measured according to the method of Buege and Aust (1978) with some modifications.

When antioxidative effect of WE, ChE II, EAE, EEE, WR and standard substances were studied the following procedure was applied. 1, 2 or 5 mg of examined substances were added into 12 ml of emulsion. Control tests were prepared without effectors. After 5 min preincubation at 25 °C, 100 µl of 37.8 mM of freshly prepared Mohr salt ($(NH_4)_2SO_4 \times FeSO_4 \times 6H_2O$) solution in water was added into the mixture. 0.5 ml of emulsion was sampled and moved into 2 ml of TBA solution, heated (boiling bath) for 20 min, and centrifuged for 10 min at 1000×*g*. Sample absorption was measured at 535 nm in a 1 cm glass cuvette.

The amount of oxidation product was determined after 0, 30, 60, 120, 180 min of incubation with ferrous ions and expressed as µg of malonaldehyde formed in a 12 ml reaction mixture.

When the influence of hydrophobic fractions PEE, BE and ChE I on oxidation OP oil was determined the following changes in measurement procedure were introduced. 1,2 or 5 mg of the extract to be assayed were dissolved in 2 ml of chloroform. Then, 0.5 ml of 6% (v/v) solution of OP oil in chloroform was added. After stirring, the sample was evaporated under reduced pressure in the presence of nitrogen. 12 ml of 3.5% (w/v) of arabic gum solution in 0.2 M Tris-HCl buffer, pH 7.4, was added and the emulsion was prepared as previously described. Further steps in the measurement of the oxidation process were carried out according to the method described above. The inhibition of the reaction in the presence of the investigated compounds was calculated according to the equation shown below.

$$\frac{\mu gC - \mu gP}{\mu gC} 100 = I$$

µgC, µg of malonaldehyde formed in control test; µgP, µg of malonaldehyde measured in sample after tested compound addition; *I*, percentage of inhibition.

Table I. The influence of various extracts from *Erodium cicutarium* on the oxidation of OP oil. The rate of the reaction was expressed as µg of malonaldehyde formed in the sample after 30, 60, 120 and 180 min incubation.

Type of extract		Time of reaction [min] 30	60	120	180
		Malonaldehyde formed in the reaction mixture [µg]			
Petroleum ether extract (PEE)	control	0.90	1.12	1.85	2.41
	1 mg	0.22	0.56	0.90	1.57
	2 mg	0.34	0.34	0.78	1.01
	5 mg	0.17	0.06	0.22	0.50
Benzene extract (BE)	control	1.06	1.51	2.36	3.03
	1 mg	0.56	0.95	1.68	1.91
	2 mg	0.34	0.78	1.51	1.68
	5 mg	0.45	0.73	1.23	1.23
Chloroform extract I (ChE I)	control	0.95	1.51	2.24	2.86
	1 mg	0.00	0.56	0.95	1.12
	2 mg	0.00	0.56	0.90	1.01
	5 mg	0.00	0.11	0.22	0.50
Water extract (WE)	control	1.40	1.57	2.13	2.69
	2 mg	0.92	1.04	1.15	1.37
	5 mg	0.17	0.00	0.11	0.22
Chloroform extract II (ChE II)	control	1.35	1.79	2.58	3.42
	1 mg	1.23	1.40	2.24	2.92
	2 mg	1.12	1.35	2.13	2.52
	5 mg	1.06	1.18	1.46	1.63
Ethyl acetate extract (EAE)	control	1.40	1.57	2.47	3.20
	1 mg	1.12	1.29	1.85	2.13
	2 mg	1.12	1.12	1.68	1.57
	5 mg	0.22	0.11	0.22	0.00
Ethyl ether extract (EEE)	control	1.12	1.68	2.02	2.52
	1 mg	1.18	1.63	1.91	2.75
	2 mg	1.12	1.63	1.85	2.24
Water residue (WR)	control	0.90	1.29	1.68	2.36
	1 mg	0.90	1.12	2.13	2.24
	2 mg	0.78	1.23	1.68	2.02
	5 mg	0.73	1.12	1.63	2.36

ChE I, chloroform extract obtained from raw material (see preparation of extracts – section Materials and Methods). ChE II, chloroform extract obtained from hot water fraction (see preparation of extracts – section Materials and Methods).

Results and Discussion

The malonaldehyde formed during OP oil oxidation and the reaction inhibition in the presence of EC extracts is shown in Tables I and II.

PEE and ChE I exhibited similar effect on oxidation of OP oil. In the presence of 1 mg of PEE in the sample inhibition of malonaldehyde formation was equal to 76, 50, 51, 35% of the control (without extract) after 30, 60, 120 and 180 min of incubation. A somewhat slower effect took place when 1 mg of benzene extract was present in the emulsion and inhibition was respectively 47, 37, 29, 37%. The water (WE) and ethyl acetate extracts (EAE) had a similar influence on oxidation of OP oil. In the presence of 2 mg of EAE the inhibition of the reaction was lower than the one observed in the presence of 2 mg PEE and ChE I and was equal to 20, 29, 32 and 51% after 30, 60, 120 and 180 min. When 5 mg of WE or EAE were added the inhibition was similar to the effect observed with 5 mg of PEE and ChE I. ChE II, EEE and WR had the lowest effect on oxidation of OP oil.

Tannin, catechins, gallic and elagic acids, sugars (glucose, galactose, fructose) amino acids (glycine, alanine, proline, histidine, tryptophan, tyrosine, glutamic acid), vitamins K and C were identified in *Erodium cicutarium* extracts. Standard samples of all these substances were tested for their antioxidative activity. Only polyphenolic compounds (tannin, gallic acid, (+)-catechin and vitamin C exhibited strong antioxidative properties (Table III). In the presence of 1 mg of tannin, inhibition of oxidation was 87, 83, 92 and 100% after 30, 60, 120 and 180 min of incubation. When 1 mg of gallic acid was added the inhibition was 88, 87, 85 and 87% in the same time. (+)-Catechin inhibited 50, 46, 51 and 58% of the reaction and ascorbic acid 41, 21, 31 and 27%.

At the present stage of investigation it is difficult to demonstrate which chemical substances present in EC extracts are active agents responsible for their antioxidative activity.

After GC-MS analysis it was observed that fractions PEE, BE, ChE I are rich in saturated and unsaturated fatty acids and carbohydrates of vari-

Table II. The influence of various extracts from *Erodium cicutarium* on the oxidation of OP oil. Inhibition of the reaction was calculated according to the equation showed in section Materials and Methods.

Type of extract		Time of reaction [min] 30	60	120	180
		% of inhibition			
Petroleum ether extract (PEE)	1 mg	76	50	51	35
	2 mg	62	70	58	58
	5 mg	81	95	88	79
Benzene extract (BE)	1 mg	47	37	29	37
	2 mg	68	48	36	44
	5 mg	58	52	48	59
Chloroform extract I (ChE I)	1 mg	100	63	58	61
	2 mg	100	63	60	65
	5 mg	100	93	90	82
Water extract (WE)	2 mg	34	34	46	49
	5 mg	88	100	95	92
Chloroform extract II (ChE II)	1 mg	9	22	13	15
	2 mg	17	24	17	26
	5 mg	21	34	43	52
Ethyl acetate extract (EAE)	1 mg	20	18	25	33
	2 mg	20	29	32	51
	5 mg	84	93	88	100
Ethyl ether extract (EEE)	1 mg	0	3	5	0
	2 mg	0	3	8	11
Water residue (WR)	1 mg	0	13	0	5
	2 mg	13	5	21	14
	5 mg	19	13	3	0

ChE I, chloroform extract obtained from raw material (see preparation of extracts – section Materials and Methods). ChE II, chloroform extract obtained from hot water fraction (see preparation of extracts – section Materials and Methods).

Table III. Antioxidant activity vitamin C and some polyphenols identified in OP extracts. The rate of the reaction was expressed as μg of malonaldehyde formed in the sample after 30, 60, 120, and 180 min incubation.

Type of compound		Time of reaction [min] 30	60	120	180
		Malonaldehyde formed in the reaction mixture [μg]			
Tannin	control	1.41	1.63	2.25	2.93
	1 mg	0.17	0.28	0.17	0.00
	2 mg	0.00	0.00	0.00	0.00
	5 mg	0.00	0.00	0.00	0.00
Gallic acid	control	1.46	1.80	2.59	3.77
	1 mg	0.17	0.23	0.39	0.48
	2 mg	0.00	0.00	0.00	0.00
	5 mg	0.00	0.00	0.00	0.00
(+)-Catechin	control	1.12	1.46	1.85	2.52
	1 mg	0.56	0.78	0.90	1.06
	2 mg	0.00	0.00	0.06	0.11
	5 mg	0.00	0.11	0.00	0.22
Ascorbic acid	control	0.95	1.35	1.63	1.85
	1 mg	0.56	1.06	1.12	1.35
	2 mg	0.00	0.00	0.00	0.00

ous length. The substances with potential strong antioxidative activity have not been detected yet.

Hydrophylic fractions WE and EAE contain polyphenolic compounds and vitamin C. One can speculate that these substances are responsible for antioxidative properties of WE and EAE extracts but explanation of this problem needs more detailed investigation.

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