THE PHENOLIC ACIDS OF SOME SPECIES OF THE *OENOTHERA* L. GENUSTADEUSZ KRZACZEK¹, ANNA BOGUCA-KOCKA¹, RENATA ŚNIEŻKO²¹Department of Pharmaceutical Botany, Medical Academy,
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ABSTRACT

The occurrence and approximative quantitative proportions of the phenolic acids in four species of the *Oenothera* L. genus was determined by the method of TLC and HPLC. In all species of *Oenothera* L. genus the permanent occurrence of acids: 2-hydroxy-4-methoxybenzoic, salicylic, ferulic, syringic, vanillic, p-coumaric, p-hydroxybenzoic, p-hydroxyphenylacetic, γ -resorcil, gentysic, protocatechuic, caffeic and gallic has been confirmed. Whereas the other phenolic acids: o-coumaric, o-hydroxyphenylacetic and pirocatechuic were found in some species of the *Oenothera* L. genus only.

KEY WORDS: *Oenothera* species, phenolic acids.

INTRODUCTION

The present interest in the *Oenothera* L. is the effect of the mixture of fatty acids, contained in the oil obtained from seeds. *Oenothera* is known in therapeutics for a long time but so far its use has been connected with non-oil substances contained in its roots or herbs. The dietetic values of this plant were also important. The roots of *Oenothera* were often used, because their taste resembled that of ham (Madaus 1938). In Ukraine, Belorussia and France the fresh raw roots are recommended as a tasty, valuable vegetable (Franke 1981). The decoction of dry roots is mainly adhibited in the case of problems respiratory tracts, and the decoction of dry herbs in cases of diarrhoea and nephritis (Lamer-Zarawska and Hojden 1989). In other areas of the world the herb of *Oenothera* is used as an anti-diarrhoeic remedy (Hoppe 1975) and infusion of herbs is recommended in the case of problems with chronic inflammation of urinary passages and alimentary canal, in asthma and pertussis (Milispangh 1974).

The repeated tests and new interest in this plant by Heiduszek and Lüft in 1919 (Lamer-Zarawska and Hojden 1989) showed, that the seeds of *Oenothera* contain γ -linoleic acid. The average oil content in seeds of this plant is 24% (Hudson 1984), but some sources say, that there are varieties, which contain 30% of oil (Wolf 1983). This oil contains triglycerides (97-98%), unsaponifiable substances (1,5-2,0%) and polar lipides (0,5-1,0%) with γ -linoleic acid (7-14%) and linolenic acid (65-85%) (Hudson 1984). The seeds of the *Oenothera* contain also proteins (15,2%), starch, dextrin and sugars (5,6%), cellulose (27%), lignin (16%) and water (8,3%) (Hudson 1984).

Chromatographic methods showed, that leaves of *Oenothera biennis* contain phenolic acids, flavonoids (Szepczyńska and Wolbiś 1992) and caoutchouc (Hegnauer

1969), whereas herbs contain organic acids and tannins (Hegnauer 1969). Some pharmacological properties of seeds of this plant may depend on the presence of phenolic acids (Lamer-Zarawska and Hojden 1989) – this is why our studies on the presence of phenolic acids in seeds and in herbs of four *Oenothera* species has been undertaken.

MATERIALS AND METHODS

Herbs and seeds of four species of *Oenothera* L. – *Oenothera biennis* L., *Oenothera lamarckiana* L., *Oenothera suaveolens* Desf. ex Pers. and *Oenothera hookeri* Torr. – were used in our study. The pure genetic plants grew in the experimental garden of the Institute of Developmental Biology in Kolonia and later they were cultivated owing to self-fertilization in the experimental garden of the Institute of Biology Maria Curie-Skłodowska University in Lublin.

The plant materials were dried in natural conditions and later were pulverized in an electrical mill.

Phenolic acids were isolated according to the method of Ibrahim and Towers (1960) as used by Krzaczek (1984). At the beginning 60 g of seeds and 36 g of herbs from each species were extracted twice with sevenfold vol/vol 80% methanol at boiling point of the dissolvent for 1 hr. Later the extracts were mixed and methanol was distilled. The remains were diluted with boiling water and they stayed in room temperature for 24 hrs.

Then the sediment was filtered through filter paper. The filtrate was purified by repeated washing with petroleum ether. The purified aqueous solution was extracted with diethyl ether exhaustively. The ether layer was washed out with 5% aqueous solution of NaHCO₃. The carbonate layer was acidified with 10% HCl to pH 3 and free acids were extracted by diethyl ether. The extract was dried with anhydrous Na₂SO₄

TABLE 1. The occurrence of phenolic acids in fractions of species *Oenothera* L. genus.

Explanation: + small amount, ++ average amount, +++ large amount.

A – free phenolic acids fractions, B – acid hydrolysis after phenolic acids fractions, C – alkaline hydrolysis after phenolic acids fractions s – fraction from seeds, h – fraction from herb

PHENOLIC ACIDS	<i>Oenothera biennis</i>						<i>Oenothera lamarckiana</i>						<i>Oenothera suaveolens</i>						<i>Oenothera hookeri</i>					
	As	Ah	Bs	Bh	Cs	Ch	As	Ah	Bs	Bh	Cs	Ch	As	Ah	Bs	Bh	Cs	Ch	As	Ah	Bs	Bh	Cs	Ch
1. gallic	+++	+	++	++	++	+++	+++		++	++	+++	+++	+++	+	+	++	++	+++	+++	+	+	++	++	+
2. caffeic	++	+	++	++	+	+++	+++	+	+	++	++	+++	+++	+	++	+++	+++	+++	++	+++	++	+++	++	+++
3. proto-catechuic	+	++	+	+	+	+	+		+	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+
4. gentysic				++		+				++		+		+		+++		+		+		+++		+++
5. γ -rezorcil				+						+						+						+		
6. p-hydroxy-phenylacetic			+	++					+	+					+	+			+		+	++		
7. p-hydroxy-benzoic	+++	+	++	+	+	++	++	++	+	++	+	++	+++	+	++	++	++	+	++	++	++	+	+	++
8. p-coumaric	+	+	+	+	+	++	++		+	+	++	++	++	++	+	+	++	+++	+++	+	++	+	+++	++
9. pirocatechuic	+	+								+			+	+		+			+					
10. o-coumaric	+						+						+				+							
11. o-hydro-xyphenylacetic										+						+						+		
12. vanillic	+++	+	++	++	++	++	++	+	++	++	++	+	+++	+	++	++	++	+	+++	+	++	++	++	+
13. syringic		+	+	++	+	+			+	+	++	++	++		+	+	+	++	++	+	+	+	++	++
14. ferulic	++	++	++	++	+++	+++	++	+++	++	+++	+++	+++	+++	++	++	+++	+++	+++	+++	++	++	+++	+++	+++
15. salicylic	++	++	+		+		+		+				+	++		++		+	++		+		+	
16. 2-hydroxy-4-metoxy-benzoic	+		+		+		+		+	+	+	+	+		+		+		+	+		+		

and the ether was evaporated. The remnants were dissolved in 3 ml of ethanol and were indicated: A_s fraction (from seeds) and A_h fraction (from herbs) and were used for chromatography. The residual aqueous eluates after A_s and A_h fraction extraction were divided into two parts, they were separated in alkaline and acid hydrolysis (Schmidtlein and Herrman 1985).

One half of the aqueous solution was subjected to acid hydrolysis with 98% H₂SO₄ (6 ml/100 ml of extracts) in boiling water both for 2 hr. Next the eluent was extracted by diethyl ether exhaustively and were indicated: B_s fraction (from seeds) and B_h fraction (from herbs). The remaining half of the aqueous solution was subjected to alkaline hydrolysis with sodium borohydride (0,8 g/100 ml of extracts) and alkalinized with a saturated solution of barium hydroxide to pH 12-13. The obtained solution was warmed in boiling water both for 15 min. and next was extracted by diethyl ether exhaustively. The fractions of phenolic acids liberated the alkaline hydrolysis after were indicated: C_s fraction (from seeds) and C_h (from herbs) – analogous as above.

The A_s, A_h, B_s, B_h, C_s and C_h fractions were investigated with two methods: two-dimensional thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC).

1. Two-dimensional TLC (Smolarz and Waksmundzka-Hajnos 1993) on plates with 0.1 mm layer of cellulose pow-

der coated. The cellulose-coated plates were run in DS-type chambers in the following phases:

I direction: benzene - methanol - acetic acid - acetone nitril
80 : 10 : 5 : 5 and
II direction: sodium formate - formic acid - water
10 : 1 : 200.

The position of the phenolic acid spots on chromatograms was analysed using UV-light (about 366 nm) before and after exposure to ammonium vapors and daylight after spraying with the following developers:

– diazotized sulphanilic acid in 20% Na₂CO₃ (Bray 1950, Linskens 1959),
– diazotized p-nitroaniline (Randterath 1962) and
– 2% aqueous FeCl₃ solution (Jerzmanowska 1961).

The quantitative proportion of the phenolic acid found by us (Tab. 1) was stated on the ground of size of the spots and the intensity of the colour (Krzaczek and Krzaczek 1979, Świątek 1970), (Fig. 1).

2. The HPLC was performed in 1050 Hewlett Packard chromatograph with UV-Vis crystal set and Rheodyne valve, with the Hypersil ODS 200 mm length and 4,6 mm diameter column, 5 μ m thick of granules and 3396 Integrator HP at the rate of 0,5 /min tape transfer (Fig. 2). The mobile phases was methanol – water (25 : 75) with 5 % vol/vol acetic acid (Główniak and Zgórk 1993). The detection of phenolic acid

Fig. 1. Two-dimensional thin layer chromatogram of phenolic acids of species *Oenothera* L. genus. Number and staining of spots are given in Table 1. The cisomers are marked with apostroph.

Fig. 2. High performance liquid chromatography of phenolic acids from A_s fraction from seeds of *Oenothera biennis* L. The number of peaks are given in Table 1.

was registered using $\gamma=254$ nm and was identified on the ground of the corresponding standards.

RESULTS AND DISCUSSION

The TLC and HPLC chromatographic methods of tests showed the proportion of phenolic acids in four species of the *Oenothera* L.: *Oenothera biennis* L., *Oenothera lamarckiana* L., *Oenothera suaveolens* Desf. ex Pers. and *Oenothera hookeri* Torn. In general 16 phenolic acids were identified. The occurrence of acids: salicylic, p-hydroxybenzoic, protocatechuic, vanillic, gentisic, p-coumaric, caffeic, ferulic and p-hydroxyphenylacetic was found earlier in leaves of *Oenothera biennis* L. by Szepczyńska and Wolbiś (1992).

In our investigations the gallic, caffeic, protocatechuic, p-hydroxyphenylacetic, p-hydroxybenzoic, p-coumaric, vanillic, syringic and ferulic acids occurred regularly in herbs and seeds of the species being examined.

The o-coumaric acid occurred only in seeds. We have not observed the gentisic, γ -rezorcylic and o-hydroxyphenylacetic acids in seed fractions of all *Oenothera* species. The o-hydroxyphenylacetic acid was not found in *Oenothera biennis* L. and o-coumaric acid in *Oenothera hookeri* L. in no fractions from seeds and herbs.

In seeds the gallic and vanillic acids, and in herbs the caffeic and ferulic acids predominated.

Most of the phenolic acids show pharmacological activity. Considering the therapeutical effect of plant material the following acids are conspicuous:

- p-hydroxybenzoic, vanillic, p-coumaric and caffeic acids – antibacterial activity (Masquelier and Delaunay 1965)
- ferulic acid – cholagogue activity (Kohlmünzer 1985) and
- salicylic acid – antibacterial, antiphlogistic and antipyretic activities (Negver 1978).

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KWASY FENOLOWE W NIEKTÓRYCH GATUNKACH RODZAJU *OENOTHERA* L.

STRESZCZENIE

Określono występowanie i proporcje ilościowe kwasów fenolowych w czterech gatunkach rodzaju *Oenothera* L. metodami chromatografii cienkowarstwowej (TLC) i wysokosprawnej chromatografii cieczowej (HPLC). We wszystkich badanych gatunkach stwierdzono stałe występowanie następujących kwasów fenolowych: 2-hydroksy-4-metoksybenzoesowego, salicylowego, ferulowego, syringowego, wanilinowego, p-kumarowego, p-hydroksybenzoesowego, p-hydroksyfenylooctowego, γ -rezorcyłowego, gentyzowego, protokatechowego, kawowego i galusowego. Natomiast kwasy: pirokatechowy, o-kumarowy i o-hydroksyfenylooctowy występowały tylko w niektórych gatunkach rodzaju *Oenothera* L.

SŁOWA KLUCZOWE: *Oenothera*, kwasy fenolowe.