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EVALUATION OF PHENOLIC ACID CONTENT IN *Silphium perfoliatum* L. LEAVES, INFLORESCENCES AND RHIZOMES

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ABSTRACT

Qualitative and quantitative chromatographic analysis (HPLC) of phenolic acids fraction (free phenolic acids and released after acid and alkaline hydrolysis) extracted from leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. collected in 2000 was presented in paper.

Seven phenolic acids were identified in material from *Silphium perfoliatum*: caffeic, p-coumaric, ferulic, protocatechuic,p-hydroxybenzoic, vanilic and chlorogenic. Inflorescences were characterized with the largest amounts of phenolic acids (23.721 mg·100 g^{-1} DM in total). Leaves contained 20.882 mg of phenolic acids per 100 g of material, rhizomes – 18.986 mg. It was found that caffeic acid was dominant component of phenolic acid group isolated from material. Phenolic acids released after alkaline hydrolysis were of the highest percentage in studied phenolic acids fraction; those released after acid hydrolysis were characterized with the lowest share.

Key words: *Silphium* L., *Silphium perfoliatum* L., phenolic acids, hydroxycinnamic acids, hydroxybenzoic acids, HPLC

INTRODUCTION

Asteraceae family (*Compositae*) belongs to the largest plant's families among all angiospermous of about 25 thousand species (about 250 species in Polish flora) [22]. Common cultivation plants (oil, fodder), vegetables, medical, ornamental plants and weeds are among them. Moreover, many plants of *Asteraceae* family are poorly known, including species of *Silphium* L. genus occurring in middle and eastern part of USA and Canada, in floristic provinces of prairie and Appalachian Mountains [19]. As it follows from the latest reports, *Silphium* L. genus, particularly *Silphium perfoliatum* L. species, is current object of many scientific investigations [9, 11, 12]. Interests upon this plant are associated with its meliferous, fodder, medical and ornamental properties. Authors of present paper have carried out anatomic, morphologic, cultivation and phytochemical studies for five years. They have also undertaken the attempt to evaluate the biological activity of extracts achieved from different organs of *Silphium perfoliatum* L. [15-17, 24, 25, 27-30].

At present, new cultivation plants that could be utilized by human for various purposes as alternative plants are searched for. Possibility to use *Silphium perfoliatum* L. as a species supplying raw material for herbal industry seems to be particularly interesting. It is worth mentioning that North-American Indian tribes applied various organs of *Silphium perfoliatum* L. for medical purposes [13]. Hitherto studies performed upon biological activity of ethanol extracts from *Silphium perfoliatum* L. showed their regenerative action during post-scald wounds healing at rats [18]. Anti-cholesterol action of saponozydes isolated from *Silphium perfoliatum* L. leaves (so-called "silphiozydes") was found as well. Cholesterol concentration in rat's blood decreased by 12% and 19% depending on a dose and the time course of experiment [21]. Moreover, Davidjanc *et al.* [10] found that saponins from *Silphium perfoliatum* L. leaves inhibited the development of phytopathogenic fungi *Drechslera Graminea* (Rabh) Ito, *Rhizopus nodosus* Namysl and *Rhizopus nigricans* Ehr.

Due to the lack of wider and systematic studies upon the phytochemical composition of *Silphium perfoliatum* L., corresponding investigations were undertaken. Qualitative and quantitative HPLC analyses of phenolic acids fraction occurring in *Silphium perfoliatum* L. were presented in paper.

MATERIALS AND METHODS

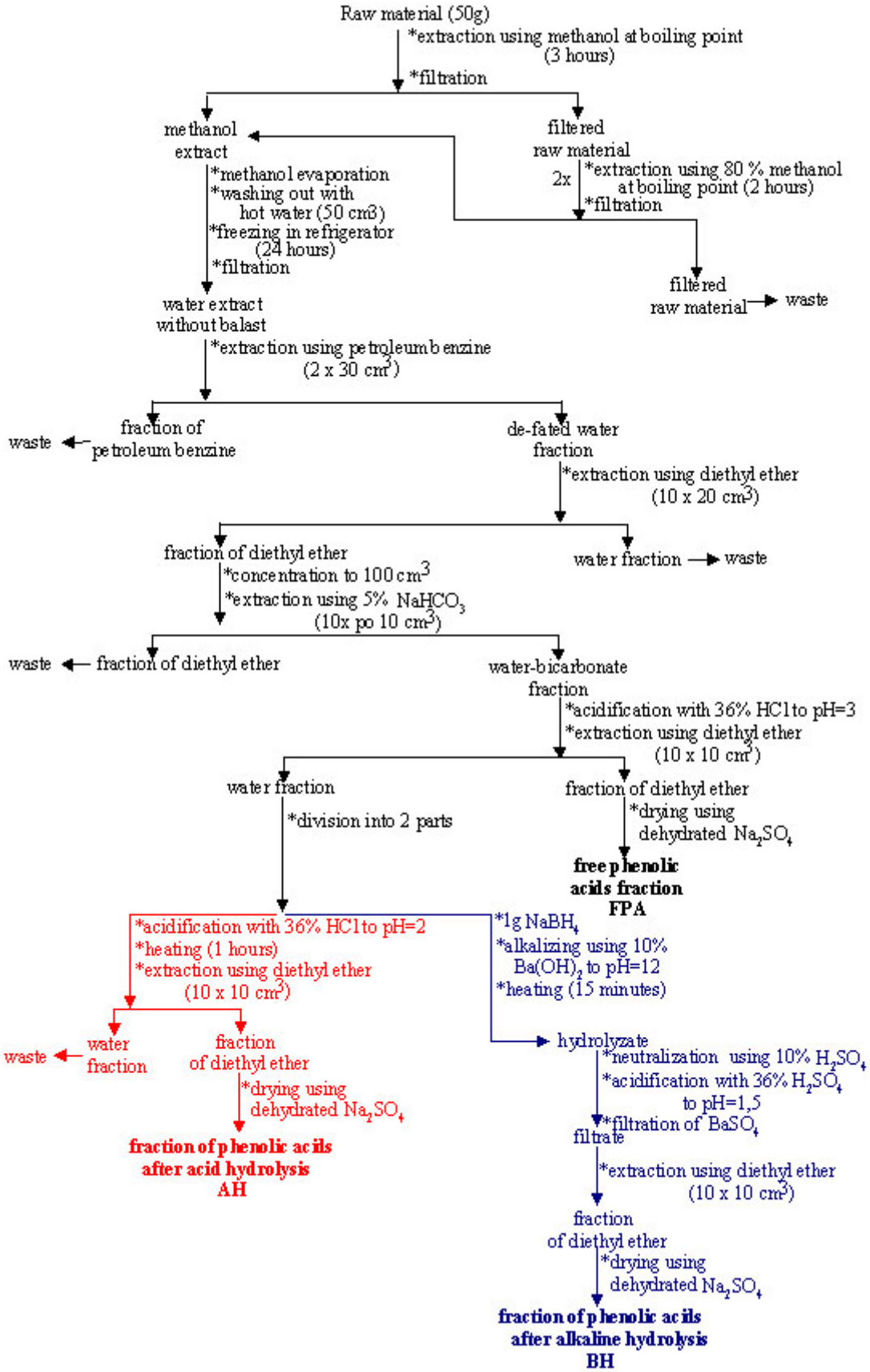
Leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. from experimental cultivation of Department of Vegetable and Medical Plants, University of Agriculture, Lublin, were material for study. Leaves and inflorescences were collected in July 2000, and rhizomes in October 2000. They were dried in shadow and air and then grinded.

Isolation and purification of phenolic acids was made according to the method worked out on a basis of literature data [14, 20, 23] and the scheme of procedures is presented on figure 1.

Particular fractions of FPA, AH and BH were dissolved in 10 cm^3 of methanol and then analyzed by means of HPLC technique.

Qualitative and quantitative HPLC analysis was performed in reversed phase system applying liquid chromatograph of LaChrom-Merck type equipped with DAD detector (L-7450), pump (l-7100), degasifier (L-7612), 20 µl dosing loop, thermostat (L-7360), Rheodyne injector, and a LiChrospher 100 RP-C_{18} column of (250×4 mm) filled with a stationary phase of 5 µm diameter. Analysis was performed at 25°C. As a mobile phase methanol + water (25:75, v/v) with 1% addition (v/v) acetic acid addition was used. Flow rate was 0.8 cm^3/min. Phenolic acid identification was performed comparing their retention times (t_r) with these for standards and spectroscopically determining their spectra in UV (220-400 nm). Contents of particular phenolic acids in raw material were calculated on a base of calibration curves plotted as dependence of area surface under peaks for standard phenolic acids (methanol solutions) on their concentration in a range: 0.5-2.5 mg/100 cm^3.

Figure 1. Scheme of procedures at isolation and separation of phenolic acids from leaves, inflorescences and rhizomes of *Silphium perfoliatum* L.



RESULTS AND DISCUSSION

Results of HPLC analyses referring to isolated phenolic acids fraction occurring in *Silphium perfoliatum* L. rhizomes are presented on figure 2. Figure 3 presents spectra making possible to identify studied compounds occurring in leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. Numerical data referring to the amount of phenolic acids in particular fractions (FPA, AH, BH) achieved from leaves, inflorescences and rhizomes are listed in table 1.

Figure 2. HPLC chromatograms of phenolic acids fraction released from *Silphium perfoliatum* L. rhizomes: FPA – free phenolic acids, AH – phenolic acids releasing after acid hydrolysis, BH – phenolic acids releasing after alkaline hydrolysis; Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – protocatechuic, E – p-hydroxybenzoic

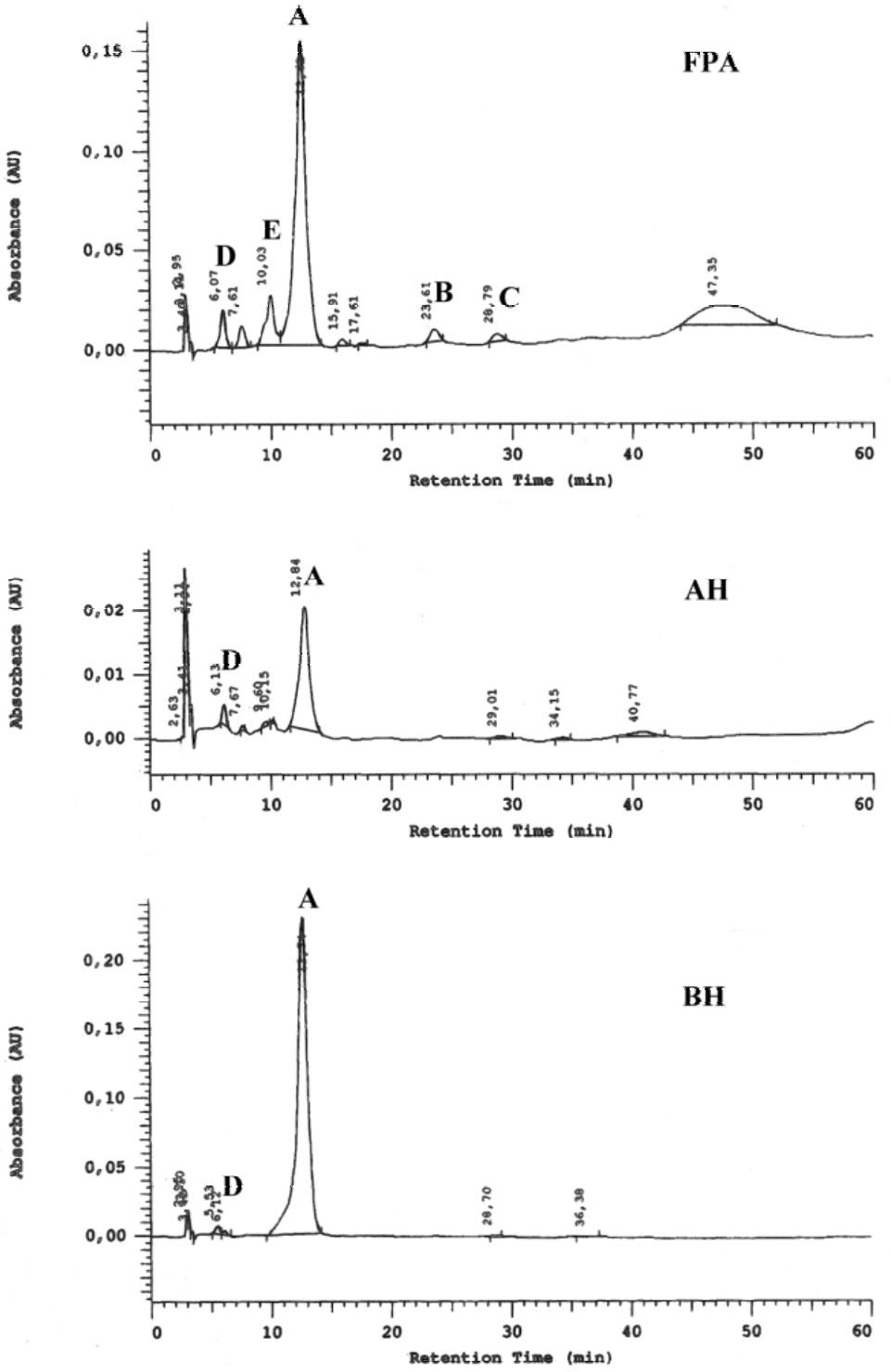


Figure 3. UV spectra (220-400 nm) of standard phenolic acids (WZ) and isolated from *Silphium perfoliatum* L. (PR): Phenolic acids: A – caffeic, B – p-coumaric, C – ferulic, D – protocatechuic, E – p-hydroxybenzoic, F – vanilic, G – chlorogenic

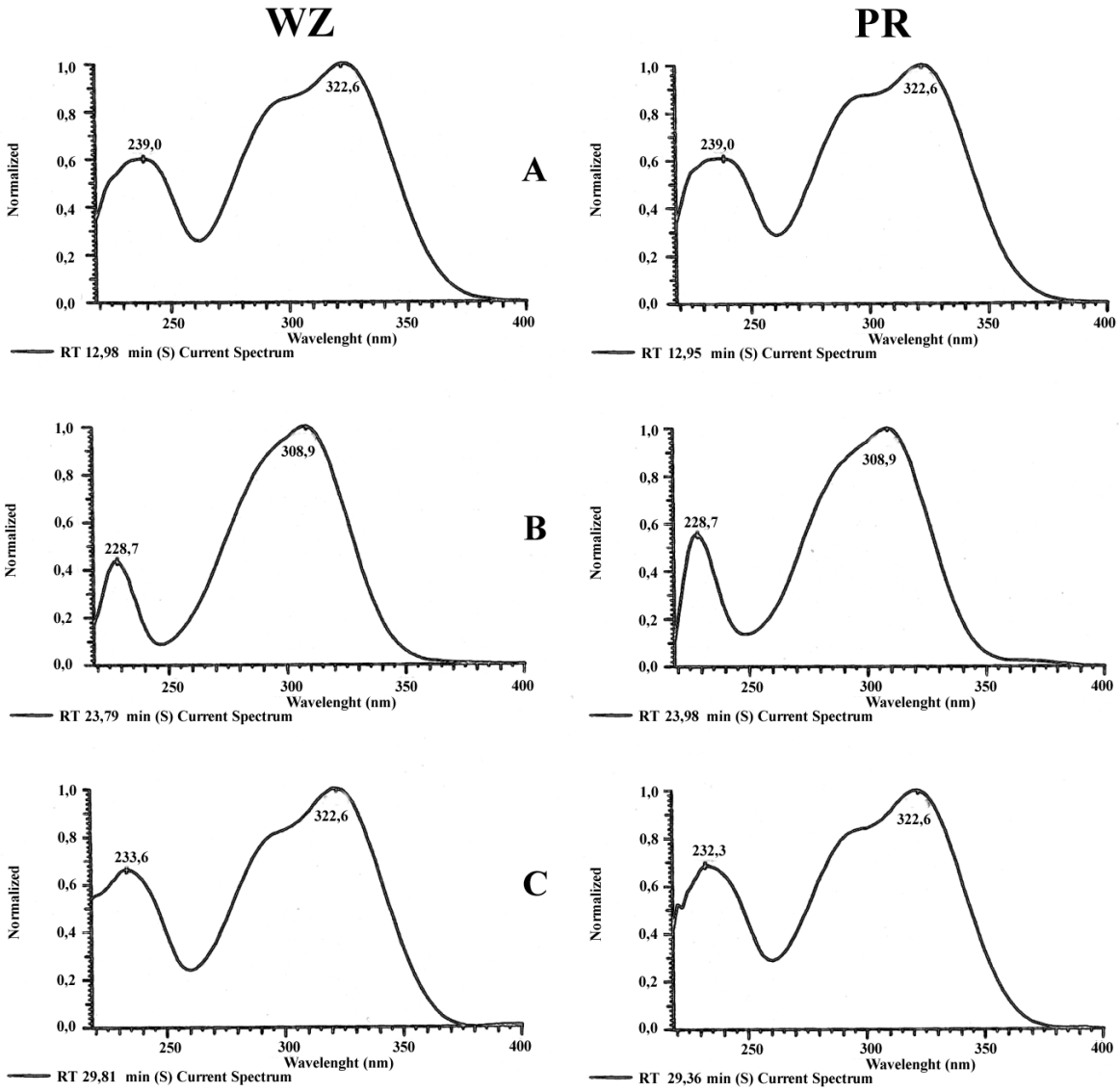


Fig. 3. cont.

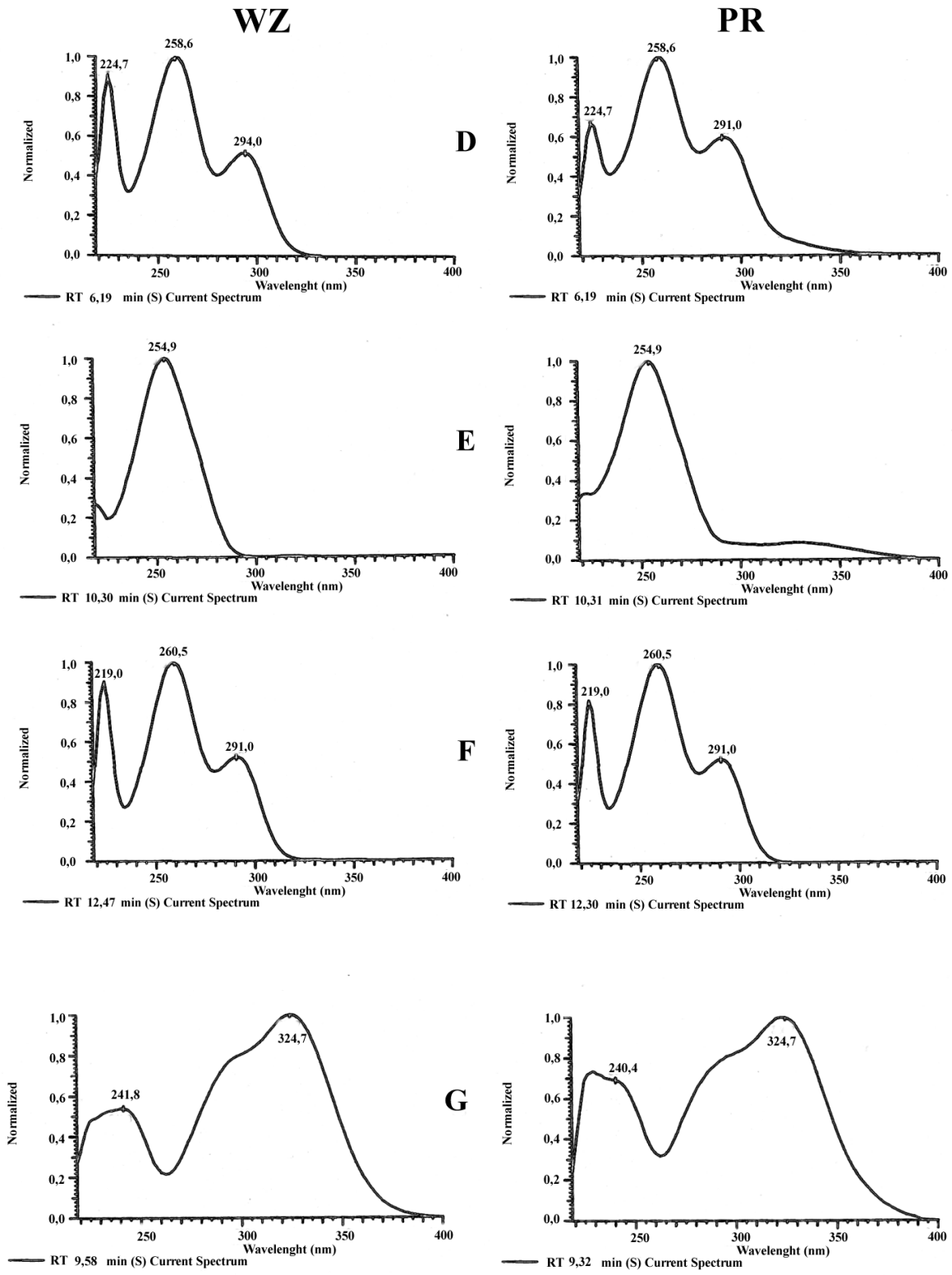

WZ
PR
D
E
F
G
Normalized
Wavelenght (nm)
224,7
258,6
294,0
291,0
254,9
219,0
260,5
241,8
324,7
240,4
RT 6,19 min (S) Current Spectrum
RT 10,30 min (S) Current Spectrum
RT 10,31 min (S) Current Spectrum
RT 12,47 min (S) Current Spectrum
RT 12,30 min (S) Current Spectrum
RT 9,58 min (S) Current Spectrum
RT 9,32 min (S) Current Spectrum

Table 1. Contents of phenolic acids contained in leaves, inflorescences and rhizomes from *Silphium perfoliatum* L. determined using HPLC technique

Phenolic acids	Amounts of phenolic acids (mg· 100 g^{-1} DM)											
	LEAVES				INFLORESCENCES				RHIZOMES			
	FPA	AH	BH	Σ	FPA	AH	BH	Σ	FPA	AH	BH	Σ
X* HYDROXYCYNNAMIC ACIDS												
A. Caffeic	2.196	0.176	12.868	15.240	2.566	0.304	13.220	16.090	4.206	1.057	12.666	17.929
B. p-Coumaric	0.351	-	1.010	1.361	0.087	-	0.174	0.261	0.476	-	-	0.476
C. Ferulic	-	-	-	-	0.326	-	0.130	0.456	0.190	-	-	0.190
Σ X*	2.547	0.176	13.878	16.601	2.979	0.304	13.524	16.807	4.872	1.057	12.666	18.595
Y*HYDROXYBENZOIC ACIDS												
D. Protocatechuic	1.537	0.439	0.527	2.503	2.131	0.826	0.913	3.870	0.147	0.052	0.052	0.251
E. p-Hydroxybenzoic	0.834	-	-	0.834	1.348	0.174	0.174	1.696	0.140	-	-	0.140
F. Vanilic	0.768	-	-	0.768	1.218	0.130	-	1.348	-	-	-	-
Σ Y*	3.139	0.439	0.527	4.105	4.697	1.130	1.087	6.914	0.287	0.052	0.052	0.391
Z* DEPSIDES												
G. Chlorogenic	-	0.176	-	0.176	-	-	-	-	-	-	-	-
Σ Z*	-	0.176	-	0.176	-	-	-	-	-	-	-	-
Σ (X* + Y* + Z*)	5.686	0.791	14.405	20.882	7.676	1.434	14.611	23.721	5.159	1.109	12.718	18.986

FPA – free phenolic acids,
AH – phenolic acids releasing after acid hydrolysis,
BH – phenolic acids releasing after alkaline hydrolysis,
"-" – absent.

As it follows from table 1, fraction of FPA from leaves contained 5 phenolic acids; presence of 3 phenolic acids was found in AH and BH fractions. All leaf fractions contained 20.882 mg of phenolic acids in 100 g of dry matter, on average. Caffeic acid was dominant in studied material (15.240 mg·100 g^{-1} DM).

Fraction of FPA isolated from inflorescences of *Silphium perfoliatum* L. contained 6 phenolic acids. After acid hydrolysis (AH), presence of 4 phenolic acids and after alkaline hydrolysis (BH) – 5 phenolic acids was observed. Phenolic acids in inflorescences were 23.721 mg·100 g^{-1} DM, on average. Similarly as in the case of leaves, caffeic acid was also dominant one in total sum of compounds isolated from inflorescences.

Five phenolic acids were identified in FPA fraction of *Silphium perfoliatum* L. rhizomes. After acid (AH) and alkaline hydrolysis (BH), presence of 2 phenolic acids was found. Rhizomes contained 18.986 mg of phenolic acids in 100 g od dry matter, on average. Caffeic acid was dominant among all compounds isolated from rhizomes.

Phenolic acids in leaves, inflorescences and rhizomes released after alkaline hydrolysis had the highest share in sum of studied compounds, and those released after acid hydrolysis had the lowest percentage.

It could be stated that *Silphium perfoliatum* L. inflorescences contained the most phenolic acids, and rhizomes were characterized with the lowest content of compounds studied. Moreover, it was proved that caffeic acid dominated among all phenolic acids. It occurred mainly in bonded form and was released due to acid hydrolysis. The presence of significant amounts of caffeic acid can determine biological activity of extracts achieved from *Silphium perfoliatum* L. organs.

Wojcińska and Drost-Karbowska [26] in studies upon phenolic acids in disk and ray flowers of *Silphium perfoliatum* L. found the presence of following acids: caffeic, p-coumaric, ferulic, protocatechuic, p-hydroxybenzoic, vanillic and syringic. Authors stated that total content of all phenolic acids fractions (determined using HPLC technique) amounted to 20.202 mg·100 g^{-1} DM, including 16.217 mg·100 g^{-1} DM of free phenolic acids and 1.306 mg·100 g^{-1} DM of phenolic acids released after acid and 2.679 mg·100 g^{-1} DM after alkaline hydrolysis. They also proved that protocatechuic (7.170 mg·100 g^{-1} DM), caffeic (4.379 mg·100 g^{-1} DM) and p-coumaric acids (4.392 mg·100 g^{-1} DM) were dominant in group of phenolic acids. It is worth mentioning that caffeic acid occurred mainly in bonded form and it was released mostly after alkaline hydrolysis, which is consistent with present results.

Recently in phytochemical studies, much attention has been paid to phenolic acids as substances having immunostimulative effects [1, 2]. Esther and glicoside connections of caffeic acid are of particular importance. In angiogenesis test (LIA), among others, caffeic acid at dose of 0.02 mg/kg/day/mouse showed the influence on angiogenic activity of mononucleous blood leukocytes at healthy humans [2]. At the same time, in SRBC test it was found that caffeic acid affected the production of IgG class antibodies at dose of 0.02 $mg \cdot kg^{-1}$. *In vitro* studies of mouse's tymocytes resistance to steroids revealed strong tymomimetic action of caffeic acid in the range of 0.1-100 $\mu g \cdot ml^{-1}$ of culture. Investigations prove that caffeic acid also shows antiseptic, antiviral, choleretic and cholekinetic properties [2, 3].

Caffeic acid and its derivatives in ester and heterozyde form (so-called "caffeoylics") are of a special meaning in *Asteraceae* family [4, 5]. Among others, Echinacea genus is characterized with the occurrence of caffeic acid esters with sugars glicoside-like bonded with 3,4-dihydroxyphenylethylalcohol, i.e. so-called "phenylpropanoids", whose representative is echinacoside [6]. Products of caffeic acid condensation of tannin character are also present in *Lamiaceae* family (sage, bahu and peppermint) [8]. The notion "caffeotannins" is more often used in literature. It more strictly characterizes the polyphenolic compounds occurring in *Lamiaceae* and *Asteraceae* families and showing properties of tannins. Therefore, Borkowski and Miłkowska [5] proposed to create the group of compounds derivatives of tannins – caffeotanoids including derivatives of caffeic and other phenolic acids descending from cinnamic acid and taking into account mainly their antiviral and immunostimulative actions [5, 7].

CONCLUSIONS

HPLC analysis revealed that leaves, inflorescences and rhizomes of *Silphium perfoliatum* L. contain following phenolic acids: caffeic, p-coumaric, ferulic, protocatechuic, p-hydroxybenzoic, vanillic and chlorogenic both in free and bonded form.
It was found that caffeic acid was dominant component of phenolic acid group isolated from material, particularly after alkaline hydrolysis, which can prove of the fact that it occurs in caffeoylic fraction.
Phenolic acids released after alkaline hydrolysis were of the highest percentage in studied phenolic acids fraction; those released after acid hydrolysis were characterized with the lowest share.

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