CHROMATOGRAPHICAL ANALYSIS OF PHENOLIC ACIDS IN SOME SPECIES OF *POLYGONUM* L. GENUS
PART 1
QUALITATIVE ANALYSIS BY TWO-DIMENSIONAL THIN LAYER CHROMATOGRAPHY (TLC)

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ABSTRACT

The Two-Dimensional Thin Layer Chromatography method has been used for the separation and identification of phenolic acids from six taxons of *Polygonum* L. genus. The following acids were found: caffeic, p-coumaric, ferulic, p-hydroxybenzoic, m-hydroxybenzoic, vanillic, syringic, p-hydroxyphenylacetic, o-hydroxyphenylacetic, synapic, melillotic, salicylic, gentisic, elagic, gallic, chlorogenic, protocatechuic and homoprotocatechuic.

Gallic, ferulic, vanillic, p-coumaric and p-hydroxybenzoic acids were isolated from herb *Polygonum convolvulus* L. using column chromatography.

KEY WORDS: *Polygonum*, Phenolic acids, 2D-TLC.

INTRODUCTION

Polygonum L. genus is represented by about 150 species, distributed widely in the world (Graham 1958). Most of them grow in temperate climate of Europe, Asia and North America. A big variety of the species occurs in East Asia – about eighty species are listed in Japan (Kitamura and Murata 1961) – but they are also met in Africa. Sometimes, according to its geographical origin, the papers report diffrent synonymus names for the plant. The taxons whose classification is discussed are *Polygonum lapathifolium* L., *Polygonum nodsum Pers.*, and *Polygonum tomentosum Schrank*. Some autors eg. Ascherson and Grabener (1908-1913) describe *P.tomentosum* and *P.nodosum* as species, others regard them as synonyms or subspecies of *P. lapathifolium* (Szafer 1921; Timson 1963; Weeb and Chater 1964). In the second edition of "Flora polska" entitled "Flora Polski" (Jasiewicz 1992) the classification of *Polygonum convolvulus* L. was. changed. The species was classified as *Bilderdykia convolvulus* (L.) Dumort syn. *Polygonum convolvulus* L. Since in phytochemical literature the name *Polygonum convolvulus* L. is used most often,in this paper, I am going to stick to the traditional term for the species.

Three species which grow in Poland deliver pharmacopoeal drugs, they are: *Herba Polygoni avicularis*, *Herba Polygoni hydropiperis*, and *Rhizome bistorta*. The raw materials of the plants show diuretic, cholagogic, antihemorrhagic and antiseptic activity (Ożarowski 1980) Aqueous ethanol extract from bistort root showed anti-inflamatory activity (Duwiejua et al. 1994), whereas water extract from smartweed of *Polygonum hydropiper* L. showed antimutagenic effect (Sato et al. 1990). Herb of *Polygonum persicaria* L. and herb of *Polygonum bistorta* L. were used as therapeutic agent in folk medicine (Hagers Handbuch... 1997). In Chinese medicine herb *Polygonum cuspidatum* is frequently used as laxative and anticancer drug. Antimutagenic activity of this herb is due to its active component emodin (Lee and Tsais 1991). Dried roots of *Polygonum cuspidatum* have been used for the treatment of suppurative, dermatitis, gonorrhoea, favus and hiperlipemia in Chinese and Japanese traditional medicine (Jayasuriya et al. 1992). In China herb *Polygonum multiflorum* is used for constipation. Similarity to P. cuspidatum benzo [a] pyrene, 1,6 dinitropyrene and 3,9-dinitrofluoranthene isolated form *P. multiflorum* exhibit antimutagenic activity (Horikawa et al. 1994).

It very often happens that therapeutically active components of plants are not exactly known. Some of their therapeutical features may be attributed to phenolic compounds. Phenolic acids – componuds found in pharmacopeial species of *Polygonum* L. – have already been described in literature as exhibiting diverse physiological and pharmaceutical activity. Fifteen phenolic acids in *Polygonum ariculare* L, *Polygonum hydropiper* L. and *Polygonum bistorta* L. were identified by paper chromotagraphy method (Świątek and Dombrowicz 1987). In the leaves of *Polygonum amphibium* L. infected with the rust, fourteen phenolic acids were indicated (Smolarz and Waksmundzka-Hajnos 1993).

From the remaining species phenolic acids were investigated only in *Polygonum convolvulus* L. Caffeic, chlorogenic, o-dihydroxybenzoic and p-coumaric acids were showed (Constantinescu and Petrescu 1975).

TABLE 1. The occurence of phenolic acids in the herbs of the investigated plants. A-fraction of free phenolic acids; B-fraction of phenolic acids released after acid hydrolysis; C-fraction of phenolic acids released after alcaline hydrolysis.

No	Compound	Commonly used names	*P. persicaria* L.			*P. mite* Schrank			*P. convolvulus* L.			*P. lapathifolium ssp.tomentosum* (Schrank) Dans			P. lapathifolium ssp. nodosum (Pers) Dans			*P. amhibium* L.		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	galloilogallic acid	ellagic	+	+			+			+		+	+		+	+		+	+	
2	3,4,5 trihydroxybenzoic acid	gallic		+	+				+	+	+	+	+	+		+	+	+	+	+
3	caffeoylquinic acid	chlorogenic	+			+	+					+			+			+		
4	3,4 dihydroxybenzoic acid	protocatechuic	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+
5	3,4 dihydroxyphenylacetic acid	homopotocatechuic		+						+			+							
6	3,4 dihydroxycinnamic acid	caffeic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	2,5 dihydroxybenzoic acid	gentysic	+	+		+			+	+		+		+	+	+		+	+	
8	4 hydroxyphenylacetic acid	p-hydroxyphenylacetic		+		+	+	+	+	+	+	+	+	+	+	+	+		+	
9	4 hydroxybenzoic acid	p-hydroxybenozic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	3 hydroxybenzoic acid	m-hydroxybenzoic					+			+			+		+	+				
11	4 hydroxycinnamic acid	p-coumaric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	2 hydroxyphenylacetic acid	o-hydroxyphenylacetic							+	+	+									
13	2 hydroxy-dihydrocinnamic acid	melillotic	+			+			+										+	
14	3,4,5 trimethoxybenzoic acid	syringic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	3 methoxy 4 hydroxybenzoic acid	vanillic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	3,5 dimethoxy 4 hydroxycinnamic acid	synapic	+		+		+	+	+	+	+			+			+	+		+
17	3 methoxy 4 hydroxycinnamic acid	ferulic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18	2 hydroxybenzoic acid	salicylic							+											

MATERIAL AND METHODS

Material

Herbs from six taxons of *Polygonum* L. genus: – *P. persicaria* L., *P. mite* Schrank, *P. amphibium* L., *P. convolvulus* L., (*Bilderdykia convolvulus* (L.) Dumort), *P. lapathifolium* L. ssp. *tomentosum* (Schrank), Dans and *P. lapathifolium* L. ssp. *nodosum* (Pers.) Dans were used in my study. The plant material was selected from different localities in Samokręski near Lublin.

Samples

The dried herbs (100g) were extracted twice with boiling 80% methanol. The obtained extract was purified and free phenolic acids fractions were isolated in classical way (Ibrahim and Towers 1960).

The processes of acid hydrolysis of phenolic acids bounded with sugar were performed according to Schmidtlein and Hermann (1975) and Świątek and Dombrowicz (1987). The alcaline hydrolysis were preformed with $Ba(OH)_2$ in reductive medium $NaBH_4$ (Schmidtlein and Herrmann 1975). The samples of free phenolic acids (A), phenolic acids liberated by acid hydrolysis (B) and phenolic acids liberated by alcaline hydrolysis (C) were dissolved in methanol and investigated by 2D-TLC method.

Two-dimensional thin layer chromatography

Chromatography was performed on 100 × 100 × 0,1 mm cellulose plates (E. Merck, Darmstadt, FRG)

After spotting of the phenolic acids standard solution or plant extract, the plates were developed; the development was performed "face down" in DS horizontal chamber (Dzido 1993) in the following phases (Smolarz and Waksmundzka-Hajnos 1993):

I direction:

benzene – methanol – acetic acid – acetonitryle (80+10+5+5 v/v),

II direction:

sodium formate – formic acid – water (10+1+200 w/v/v).

The plates were conditioned for about 5 min. with vapours above benzene – methanol – acetic acid (94:1:5 v/v) and developed in the first direction.

Chromatographic derivatization

After drying, the chromatograms were observed in UV light (λ=366 nm) before and after treatment with ammonia vapour. Derivatisation was then preformed by spraying with one of the reagents:

1. diasotized sulphanilic acid in 20% sodium carbonate solution;
2. diasotized p-nitroaniline in 10% sodium carbonate solution;
3. 2% aqueous solution of ferric chloride.

After derivatisation chromatograms were observed in daylight.

Column chromatography

The fraction of free phenolic acids (A) from herb *Polygonum convolvulus* L. was separated by preparative column chromotography on silica gel (Merck, FRG) by means of the following eluents: hexane, hexane and chlorofrom (3:1; 1:1; 1:3) chloroform, and chlorofrom with ethyl acetate. (1:1)

The isolated phenolic acids were identified by cochromatography with authentic samples and UV spectra. The spectral analyses of identified compounds were recorded in methanol and with the addition of 0.1 M NaOH and 1% $AlCl_3$ (Specord UV/VIS). The results of the spectral analysis are given in Table 2.

RESULTS AND DISCUSSION

From the investigations carried out by two-dimensional thin layer chromatography method it follows that herbs of the investigated raw material contain derivatives: benzoic, cinnamic and phenylacetic acids which are presented in the Table 1. They are the following acids: ellagic, chlorogenic, protocatechuic, homoprotocatechuic, caffeic, gentisic, p-hydroxypheny-

TABLE 2. UV spectral analysis of phenolic acids isolated from herb of *Polygonum convolvulus* L.

Compound	Values λ max (nm)		
	MeOH	MeOH+NaOH	MeOH+$AlCl_3$
p-hydroxybenzoic acid	256	257	261
ferulic acid	286, 318	302, 347	334
vanillic acid	258, 282	256, 284	–
p-coumaric acid	300	333	320
gallic acid	272	286	318
unidentified	262	312	284

lacetic, o-hydroxyphenylacetic, p-hydroxybenzoic, m-hydroxybenzoic, p-coumanic, melillotic, syringic, vanillic, synapic, ferulic, and salicylic.

The caffeic, p-hydroxybenzoic, p-coumaric, syringic, vanillic, ferulic and protocatechuic acids are commonly present both in elementary and combined state. Frequently in free phenolic acid fractions and after acid hydrolysis gentisic, ellagic, gallic acids were present; only in *Polygonum mite* Schrank gallic acid was absent. Only in *Polygonum convolvulus* L. the free chlorogenic acid was not found. Melillotic acid – an uncommon compound in plant – was detected in *Polygonum persicaria*. L., *P. convolvulus* L., *P. amphibium* L. and *P. mite* Schrank., and other in farmacopoeial species too (Świątek and Dombrowicz 1987). The rarely occuring, in raw material, m-hydroxybenzoic acid was found after acid hydrolysis fraction from *P. mite* Schrank, *P. convolvulus* L., *P. lapathifolium ssp. tomentosum* (Schrank) Dans, and *P. lapathifolium ssp. nodosum* (Pers) Dans. Synapic acid occured in different state in all investigated herbs.

Free salicylic acid was detected in *P. convolvulus* L. From phenylacetic acid derivatives, p-hydroxyphenylacetic acid was present in all analysed herbs, whereas o-hydroxypenylacetic acid was found only in *P. convolvulus* L.

The investigations show, that in genus *Polygonum* L. 18 phenolic acids are discovered. Most of the identified phenolic acids are present in both as bounded and liberated forms.

Phenolic acids from the free phenolic acid fraction (A) of *Polygonum convolvulus* L., were separated by means of preparative column chromatography on silica gel G. In the result six compounds were isolated. The investigation of the obtained compounds was based on cochromatography with standard samples by 2D-TLC method on celullose. Next UV spectra in methanol and after addition of 0.1 H NaOH and 1% $AlCl_3$ for isolated compounds and standard samples were made.

The UV spectra were recorded at 220-350 nm. The results are presented in Table 2.

On the basis of the comparison of UV spectra from isolated phenolic acids with UV spectra standard samples p-hydroxybenzoic acid, p-coumaric acid, vanillic acid, ferulic acid and gallic acid were identified.

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CHROMATOGRAFICZNA ANALIZA KWASÓW FENOLOWYCH W KILKU GATUNKACH Z RODZAJU *POLYGONUM* L. CZ. 1 JAKOŚCIOWA ANALIZA METODĄ DWUKIERUNKOWEJ CHROMATOGRAFII CIENKOWARSTWOWEJ

STRESZCZENIE

W sześciu taxonach z rodzaju *Polygonum* L. analizowano skład kwasów fenolowych przy użyciu dwukierunkowej chromatografii cienkowarstwowej. Wykazano obecność następujących kwasów: kawowego, p-kumarowego, ferulowego, p-hydroksybenzoesowego, m-hydroksybenzoesowego, wanilinowego, syryngowego, p-hydroksyfenylooctowego, o-hydroksyfenylooctowego, synapowego, melilotowego, salicylowego, gentyzowego, elagowego, galusowego, chlorogenowego, protokatechowego, homoprotokatechowego. Z ziela *P. convolvulus* L. wyizolowano przy użyciu chromatografii kolumnowej oraz zidentyfikowano kwas galusowy, ferulowy, wanilinowy, p-kumarowy i p-hydroksybenzoesowy.

SŁOWA KLUCZOWE: *Polygonum*, kwasy fenolowe, 2D-TLC.