PHENOLIC ACIDS IN LEAVES OF *SECAMONE AFZELII* (RHOEM.) SCHULT. (ASCLEPIADACEAE)

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ABSTRACT

The analysis of the sets of free and liberated by hydrolysis phenolic acids in the leaves of, *Secamone afzelii* (Rhoem.) Schult. was conducted by 2D-TLC and RP-HPLC methods. Sixteen phenolic acids were identified: chlorogenic, gallic, protocatechuic, homoprotacatechuic, caffeic, gentysic, α-resorcylic, p-hydroxyphenylacetic, p-hydroxybenzoic, p-coumaric, o-hydroxyphenylacetic, syryngic, vanillic, synapic, ferulic and salicylic. By means of the RP-HPLC the contents of major phenolic acids was described. It is fairly diversified in cases of different compounds ranging from 0.05 up to 16.89 mg%.

KEY WORDS: *Secamone afzelii* (Rhoem.) Schult., Asclepiadaceae, phenolic acids, 2D-TLC and RP-HPLC analysis.

INTRODUCTION

The natural environment of *Secamone afzelii* (Rhoem.) Schult. (F. Asclepiadaceae) is south-western Africa.

This interesting plant is used by native people as an antiinflammatory, antibacterial and tonic drug. Latex of *S. afzelii* is traditionally used in various skin diseases (boils, abscesses and eruptions). Bitter sap of stems and leaves of *S. afzelii* is used as a stomachic and purgative and diuretic drug. Crushed *S. afzelii* is used for cooking food for gonarrhoea patients (Abbiw 1990).

The species has so far not been phytochemically investigated. Only the occurence of friedelin was reported in the root of this plant (El-Said et al. 1971).

MATERIAL AND METHODS

Plant material

Leaves of *Secamone afzelii* were collected in September 1995 in Mampong Akwapim in Ghana (West Africa) and identified in the Centre for Scientific Research into Plant Medicine by Ministry of Health in Ghana. A voucher specimen has been deposited at the herbarium of the Department of Pharmaceutical Botany in Lublin, Poland.

Extraction and isolation

The air-dried leaves (70 g) were extracted with hot MeOH and the dry extract dissolved in H_2O and successively extracted with Et_2O. Consequently, in the typical way for this group of compounds, free phenolic acids fraction (A), phenolic acids released after acidic hydrolysis fraction (B) and the ones released after basic hydrolysis (C) were isolated.

Fraction of free phenolic acids (A) was isolated according to Ibrahim and Tower's method (1960). The process of hydrolysis of bound phenolic acids were performed according to Schmidtlein, Herrman (1975) and Świątek et al. (1984).

- acidic hydrolysis: 2M HCl, 100°C, 1h;
- alkaline hydrolysis: 1% $Ba(OH)_2$, $NaBH_4$, pH12-13, 100°C, 15 min.

Fractions B and C were isolated in an analogous way as fraction A-out of the acidic and alkaline hydrolysate.

The obtained fractions A, B, C were dissolved in 2 cm^3 of ethanol. The 2D-TLC and RP-HPLC methods, described in detail by Nowak and Zgórka (1997), were used for analysis of these fractions.

In the TLC-analysis the following phases were used:

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 cellulose plates (Merck, Armstad, FRG) were conditioned for about 5 min. with vapors above benzene-methanol-acetic acid (90:5:5) and then developed in the first direction (Smolarz, Waksmundzka-Hajnos 1993). Derivatisation was then performed by spraying with typical reagents (Randerath 1962, Krzaczek et al. 1979).

The obtained results of TLC analysis are presented in Table 1 and Fig. 1.

TABLE 1. The occurence of phenolic acids in leaves of *Secamone afzelii*

	Phenolic acids		A	B	C
1	chlorogenic		+	+	–
2	gallic		+	+	–
3	caffeic	trans	+	+	+
		cis	–	–	+
4	protocatechuic		+	+	+
5	homoprotocatechuic		+	–	–
6	α-resorcylic		+	+	+
7	gentysic		+	+	+
8	p-hydroxybenzoic		+	+	+
9	p-hydroxyphenylacetic		+	+	–
10	p-coumaric	trans	+	+	+
		cis	+	+	+
11	o-hydroxyphenyloacetic		+	+	–
12	syringic		+	+	+
13	vanillic		+	+	+
14	synapic	trans	–	+	–
		cis	–	+	–
15	ferulic	trans	+	+	+
		cis	+	+	+
16	salicylic		+	+	+

Explanations:
A – free phenolic acids fraction
B – phenolic acid fraction after acid hydrolysis
C – phenolic acid fraction after alkaline hydrolysis
+ present, – absent

The HPLC analysis was performed in HP-1050 chromatograph (Hewlett-Packard, Palo-Alto, CA, USA) equipped with a 20 µl sample injector (Rheodyna) and a variable wavelength UV detector (HP-1050) operated at 254 nm, steel column (200 x 4,6 mm I.D.) packed with Hypersil ODS (Shandon, Cheshire, UK), dp = 5 µm.

The mobile phase consisted of methanol:water (25:75) with 1% v/v acetic acid.

The results of HPLC-quantitative analysis are given in Table 2.

The example of a chromatogram of HPLC of phenolic acids from fraction A of *Secamone afzelii* is presented in Fig. 2.

RESULTS AND DISCUSSION

This paper is the first attempt at revealing the qualitative and quantitative composition of phenolic acids in leaves of *Secamone afzelii*.

Very rich qualitative composition of examined compounds in this plant material was observed. Sixteen phenolic acids were identified (TLC, HPLC): chlorogenic, gallic, protocatechuic, homoprotocatechuic, caffeic, gentysic, α-resorcylic, p-

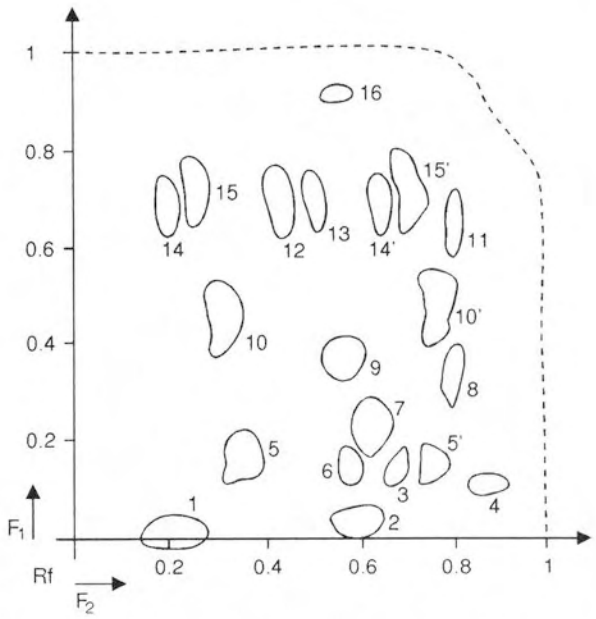

Fig. 1. The 2D-TLC chromatogram of phenolic acids of *Secamone afzelii*. Numbers of spots are given in Table 1. The cis isomers are marked with an apostroph.

TABLE 2. The contents of determined phenolic acids in leaves of *S. afzelii* [mg/100 g dry material]

	Phenolic acids	A	B	C
1	Protocatechuic	0.57	16.86	1.64
2	p-hydroxybenzoic	0.83	3.72	1.75
3	Vanillic	0.16	1.51	2.31
4	Caffeic	0.05	0.76	tr
5	Syringic	0.07	2.68	5.48
6	p-Coumaric (trans)	0.74	0.52	7.32
7	p-Coumaric (cis)	0.33	2.03	5.58
8	Ferulic (trans)	0.31	1.18	3.09
9	Ferulic (cis)	0.13	3.19	9.58

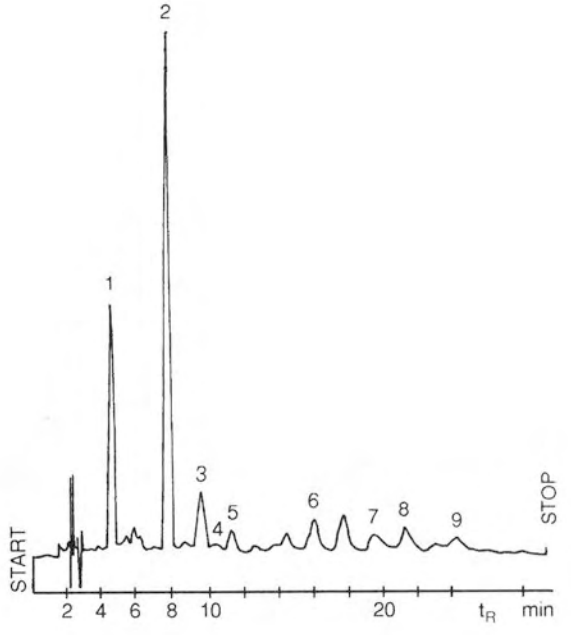

Fig. 2. The HPLC chromatogram of phenolic acids from A fraction from leaves of *S. afzelii*. The numbers of peaks are given in Table 2.

hydroxyphenylacetic, p-hydroxybenzoic, p-coumaric, o-hydroxyphenylacetic, syryngic, vanillic, synapic, ferulic and salicylic.

Most of the phenolic acids occured both in the elemental and combined state. The homoprotocatechuic acid appeared in the free state only. The synapic acid was in fraction B, in glycoside bound form only. Chlorogenic, gallic, p- and o-hydroxyphenylacetic acids occured as free and glycosides form.

By means of HPLC method the contents of major phenolic acids in the fractions studied from the leaves of *Secamone afzelii* were described. It is fairly diversified and ranging from 0.05 up to 16.86 mg/100 g of dry material. The large growth of the contents of phenolic acids in the material studied after alkaline and acidic hydrolysis was observed. Acids combined with sugars (fraction B) and appearing in esters (fraction C) were present in the plant in a much larger quantity.

For example the amount of protocatechuic acid after acidic hydrolysis increased up from 0.57 mg/100 g to 16.89 mg/100 g of dry leaves.

Most of the identified phenolic acids show a certain pharmacological activity. P-hydroxybenzoic, p-coumaric, caffeic and vanillic acids occuring in larger quantities in the studied plant have an antibacterial activity (Borkowski 1993a, b, Masqualier 1965). Ferulic and caffeic acids have both choleretic and cholekinetic activities. Protocatechuic acid shows biligenic and fungicidal activity (Negver 1978).

It seems that, phenolic acids may act synergetictly and affect the diverse activities of *Secamone afzelii*.

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FENOLOKWASY W LIŚCIACH *SECAMONE AFZELII* (RHOEM.) SCHULT. (F. ASCLEPIADACEAE)

STRESZCZENIE

Metodami 2D-TLC i RP-HPLC analizowano skład fenolokwasów wolnych i uwalnianych w wyniku hydrolizy kwasowej i zasadowej w liściach *Secamone afzelii (Rhoem.) Schult.* Zidentyfikowano 16 fenolokwasów: chlorogenowy, galusowy, protokatechowy, homoprotokatechowy, kawowy, gentyzowy, a-rezorcylowy, p-hydroksyfenylooctowy, p-hydroksybenzoesowy, p-kumarowy, o-hydroksyfenylooctowy, syryngowy, wanilinowy, synapowy, ferulowy i salicylowy. Metodą RP-HPLC określono zawartość głównych fenolokwasów, która była zróżnicowana i w przypadku poszczególnych związków wynosiła od 0,05 do 16,89 mg%.

SŁOWA KLUCZOWE: *Secamone afzelii (Rhoem.) Schult.*, *Asclepiadaceae*, fenolokwasy, analiza 2D-TLC i RP-HPLC.