PHYTOCHEMICAL INVESTIGATION OF THE TOMATILLO FRUIT (*PHYSALIS IXOCARPA* BROT., *SOLANACEAE*).

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ABSTRACT

The occurrence of alkaloids, witanolides, saponosides and flavonoids was investigated in the fruits of tomatillo (*Physalis ixocarpa* Brot.) cv. Bujna and Rendidora. The tomatillo is commonly cultivated in Mexico and has been introduced on a small scale in Poland. The chromatographic analysis of alkaloids showed only trace amounts of compounds reacting with the Dragendorff reagent. In the fraction of quaternary alkaloids soluble in water, only choline was found. Witanolides were not detected in tomatillo fruits. The saponosides were found only in trace amounts and their hemolytic indexes as well as saponification numbers were very low. Flavonoids were represented by the derivatives of quercetin differing with their sugar moieties attached to the hydroxyl group at C-3. It is concluded that the toxic compounds which would be harmful to human health were not found in tomatillo fruits. The presence of choline in them, as well as of flavonoids belonging to the vitamin P group is advantageous for health.

KEY WORDS: alkaloids, witanolides, saponosides, flavonoids, *Physalis ixocarpa* Brot., tomatillo fruit

INTRODUCTION

Tomatillo is a native plant in Mexico (Hernandez-Xolocotzi 1985) and is cultivated in this country (over 12000 ha). It is cultivated also in the USSR (Medvedev 1958) and was recently introduced to Poland (Jankiewicz 1983, Borkowski 1984). Its biology was, however, investigated only recently (Saray-Meza 1977, Cartujano-Escobar et al. 1985, 1987, Mulato-Brito et al. 1985). The selection carried out in Mexico brought the cultivar Rendidora (Saray-Meza et al. 1978). The chemical composition of *Physalis ixocarpa* fruits was investigated by Souza-Novelo (1950), Ostrzycka et al. (1988) and Mahna and Gupta (1989). They investigated commonly occurring substances: saccharides, non-volatile organic acids, crude fats, total proteins, cellulose, pectic substances, phenolic substances, mineral constituents and the following vitamins: ascorbic acid, niacin, thiamine, riboflavin and have shown that tomatillo fruit contains a relatively high percentage of dry matter (7-10%) and a medium percentage of total sugars (2.8-5.7%). Citric and malic acids prevailed among the organic acids; the content of ascorbic acid was low to medium (8-21 mg.100 g⁻¹ fresh mass).

Nevertheless it seemed necessary to get more information on the chemical organic constituents of tomatillo fruit in order to assess more precisely its nutritious value and especially to be sure that the fruit does not contain toxic substances in concentrations which may be harmful to human health.

The occurrence of compounds of alkaloidal character in different *Physalis* species was investigated by several authors.

For instance, pyrrolidine alkaloids: higrine and cuscohigrine, as well as secotropan derivatives were isolated from *Physalis ixocarpa* roots. The test analyses suggest also the presence of alkaloids in the leaves of *Ph. alkekengi* L., *Ph. longifolia* Mutt. and *Ph. peruviana* L. The reports on the occurrence of these compounds in the fruit of *Ph. alkekengi*, *Ph. peruviana* and *Ph. heterophylla* are contradictory.

Investigations on the presence of steroid alkaloids in the fruits of *Ph. peruviana* and *Ph. angulata* gave negative results (Smoleński et al. 1972, 1974, 1975, Bassey and Wolley 1973, Hegnauer 1973, Beresford and Wolley 1974, Brian et al. 1979).

Concerning witanolides, about fifty compounds were found in different species of *Physalis*. In the leaves of *Ph. ixocarpa* five witanolides were identified: physalin B, ixocarpolactonide, withaphysocarpine and isocarpolacton A and B (Subramanian and Sethi 1973, Kirson et al. 1979, Maslennikova et al. 1986). Witanolides were more abundant in other species of *Physalis*: *Ph. alkekengi* L., *Ph. minima* L., *Ph. peruviana* L., *Ph. pubescens* L., *Ph. viciosa* L. (Maslennikova et al. 1986).

Saponosides frequently occur in the species of *Solanaceae*, nevertheless the genus *Physalis* was not investigated for their content.

The investigations on the occurrence of flavonoids in plants of the genus *Physalis* resulted in the isolation of luteoline 7-glycoside in the leaves of *Ph. alkekengi* L. (Jana and Raynaud 1971) and of quercetin 3,7,4'-trimethylether from the herb of *Ph. angulata* (Lopez and Schiff 1976). Rutoside was found in *Ph. peruviana* L. roots (Sahai and Neogi 1984).

Since no information was found in the literature on the presence of alkaloids, witanolides, saponosides and flavonoids in *Ph. ixocarpa* fruits, investigations were undertaken on their content in two cultivars of *Ph. ixocarpa* which are proposed to be introduced into cultivation in Poland.

MATERIAL AND PHYTOCHEMICAL METHODS

Fresh fruits deprived of their calyx envelope were investigated.

They were taken from the plants of two cultivars Bujna and Rendidora which were cultivated in the Research Institute of Vegetable Crops in Skierniewice in 1987.

Alkaloids

- tertiary and quaternary alkaloids: 1.5 kg of fresh fruits of each of the two cultivars were macerated with methanol 1:10. Thereafter the procedure of Jerzmanowska (1967) was followed: the fraction of tertiary alkaloids soluble in ethyl ether (E) and other fractions of tertiary alkaloids soluble in chloroform (C) were separated as well as the quaternary alkaloids soluble in water (W).
- steroid alkaloids (S): 0.5 kg of fruits of each of the two cultivars were cut into pieces and macerated in 2% acetic acid. Afterwards 25% ammonia was added to the mixture according to the generally accepted method (Jerzmanowska 1967).
- derivatives of sekotropin (ST) were separated according to Basey and Wolley (1973): 1 kg of cut up fruits of each of the two cultivars were mixed with 50 g of Ca(OH)₂ and left for 24 hours. Thereafter the mixture was shaken 3 times with ethyl ether. The extracts received were put together and the solvent was evaporated. The residue was shaken with ethyl ether and then extracted with hot methanol. The ether extracts were fractionated on columns packed with Kieselguhr (Merck) using phosphate buffer and afterwards ethyl ether as the developing solvent. The evaporated fractions were put into neutral aluminium oxide columns and eluted with ethyl ether.

Chromatographic analysis

All alkaloid fractions received were subjected to chromatography

- Thin-layer chromatography (TLC) on Silica Gel (Merck) G and GF₂₅₄.
Solvent systems
S-1 chloroform-methanol (3:7)
S-2 chloroform-diethylamine (19:1)
S-3 n-propanol-water-ethylacetate-methanol-chloroform (4:2:1:1:1).
- TLC - Aluminum Oxide (Merck).
Solvent systems
S-4 ethanol-ammonia 25% (9:1)
S-5 ethyl ether-ethanol (9:1)
S-6 chloroform-ethanol (19:1)
- Paper chromatography (PC, Whatman No 1)
S-7 n-butanol-glacial acetic acid (10:1) saturated with water.

The chromatograms were checked in UV₂₅₄ and UV₃₆₆, as well as, in visible light after spraying with Dragendorff reagent and iodine in chloroform (Strzelecka et al. 1987).

The following substances were applied as standards: choline, tomatine, tigloidine picrate, 3-b-tigloiloxytropine picrate, cuscohygrine.

Under the influence of Dragendorff reagent the choline revealed a violet color and the other alkaloids treated with iodine in chloroform were orange yellow. The results (R_F values) are shown in Table 1.

TABLE 1. Chromatographic analysis of alkaloid fractions from fruits of *Physalis ixocarpa* Brot., cvs Bujna, Rendidora

Fraction or standard	Solvent	R _F value
E	S-1	—
C	S-1	0.20
		0.90
W	S-4	0.47
Choline	S-4	0.47
S	S-1	—
	S-3	—
ST	S-5	—
	S-6	—

— negative result

For the fraction of steroid alkaloids (tomatine as standard) the Rosenheim reactions with trichloroacetate and the reaction of Liebermann-Bürchardt (Strzelecka et al. 1987) were applied (Jerzmanowska 1967). For these fractions no positive results were obtained.

Witanolides

The samples of 0.5 kg of fresh, dissected fruits of both cultivars were extracted 5 times with boiling methanol. Concentrated extracts were digested with hot, distilled water and the separated ballast substances were drained off. The filtrates were extracted four times with n-hexane, and thereafter three times with chloroform (Sahai 1985). Chloroform extracts were washed with water and dried with anhydrous Na₂SO₄. After evaporation they were used for qualitative reaction (according to Liebermann-Bürchardt) and for chromatographic analysis.

Two-dimensional chromatographic analysis.

Silica gel thin layer plates (DC Alufolien Kieselgel-60-Merck) were developed applying the following solvent systems:

- chloroform-methanol (9:1) – 1st direction
- benzen-ethylacetate (3:7) – 2nd direction

The chromatograms were visualised with the reagents commonly applied for witanolides (Maslennikova et al. 1977, Rozkrutova 1985):

- 25% SbCl₃ in chloroform (105°C)
- 10% H₂SO₄ (105°C).
- Dragendorff's reagents.

Saponosides

Extracts for investigation: dissected fresh fruits of cv. Bujna (1.8 kg) and cv. Rendidora (1.7 kg) were extracted 5 times over a boiling water bath, with the mixture of n-butanol-methanol (1:1) (Ellnain-Wojtaszek et al. 1976). The extracts were evaporated to a sticky syrup consistence, deballasted with methanol and filtered. Two extracts, 1 and 2, were obtained (18.8 g and 19.9 g respectively) with the dry matter content 11.6% and 16.4% respectively.

Hydrolysis: 5 g of each of two extracts were hydrolyzed with 5 ml 1M HCL over boiling water bath for 8h and afterwards shaken with ethylacetate in order to obtain aglycone fractions 1 and 2.

The qualitative reactions were done with the extracts 1 and 2 and with their aglycone fractions. The reaction of Liebermann-Bürchardt (Strzelecka et al. 1987) was positive as well for the extracts as for the aglycone fractions.

For evaluation of the saponification number (S.n.) the extracts 1 and 2 were evaporated up to dryness and were used in

quantities of 1.729 g and 2.799 g respectively, calculated in terms of dry crude material (method of evaluation according to Strzelecka et al. 1987). The S.n. for extracts 1 and 2 were 23.12 and 12.5 respectively.

Hemolytic index (H.i.). The extracts 1 and 2 dried up to constant mass were used in quantities of 3.458 g and 5.598 g respectively, calculated in terms of dry crude material. This amount was 20 times greater than that used normally for saponoside raw materials. In the case of the aglycone fraction, 10 times greater concentrations were used than for the extracts. The procedure used was according to Mazurek (see Strzelecka et al. 1987). The extracts did not hemolyze the erythrocytes. For the aglycones 1 and 2, the H.i. was 0.86 and 0.54 respectively.

Thin layer chromatography. Silica Gel (Merck) developing phase: ethylacetate-methanol-water (15:3:2). This was the most selective method among those investigated.

Reagents for qualitative tests:

- defibred bovine blood in 0.9% NaCl (2:8)
- distilled water
- methanol solution of phosphomolybdic acid (105°C)
- 10% H₂SO₄ in 50% ethanol (105°C)
- 25% SbCl₃ in chloroform (105°C)
- iodine vapor.

The results of the chromatographic analysis are shown in the Table 2.

TABLE 2. Chromatographic analysis of saponoside fractions of *Physalis ixocarpa* Brot. fruits, cvs Bujna and Rendidora

	No of the spot	Rf	Reactions with developing reagents*					
			a	b	c	d	e	f
Extract 1	1	0.15	+-	w	b	y	ggn	cy
	2	0.23	+-	w	by	yg	gv	y
	3	0.32	+-	w	b	y	gv	cy
	4	0.37	+-	w	b	y	gv	cy
	5	0.86	+	w	b	ygn	ggn	cy
	6	0.93	+	w	bgn	gv	gv	y
	7	0.98	+	w	nb	gv	gv	y
Aglycon fraction of 1	5	0.83	+	w	bgn	ggn	ggn	cy
	6	0.91	+	w	nb	gv	gv	y
	7	0.96	+	w	nb	gv	gv	y
Extract 2	1	0.15	+-	w	b	y	ggn	cy
	2	0.22	+-	w	b	yg	ggn	y
	3	0.30	+-	w	b	yg	gv	cy
	4	0.34	+-	w	b	yg	gv	cy
	5	0.86	+	w	nb	g	ggn	cy
	6	0.91	+	w	nb	gv	gv	y
	7	0.96	+	w	nb	gv	gv	y
Aglycon fraction of 2	5	0.83	+	w	bgn	ggn	ggn	cy
	6	0.91	+	w	nb	gv	gv	y
	7	0.96	+	w	nb	gv	gv	y

w – white not wetting with water, b – blue, v – violet
y – yellow, g – grey, gn – green, nb – navy blue, c – clear
+ hemolysis
+- weak hemolysis

* For composition of reagents see text

Flavonoids

Fresh fruits of cv. Bujna (2.3 kg) and Rendidora (1.5 kg) were dissected and extracted 4 times with boiling methanol. The procedure of Mabry et al. (1970) was followed.

Chromatographic analysis

Chromatography was performed on paper (Whatman No 1 and 3) or on column (Cellulose CF 11) in the following solvent systems

S-8 glacial acetic acid – water (15:85)

S-9 isopropanol – formic acid – water (2:5:5)

S-10 ethyl acetate – methanol – water (100:5:5)

S-11 ethyl acetate – pyridine – water (8:2:1).

The chromatograms were observed in the UV₃₆₆ before and after spraying with AlCl₃ and KOH. The saccharides were detected in day light after spraying with aniline hydrogen phthalate and heating at 105°C.

The flavonoid fraction was subjected to column packed with cellulose powder and eluted with S-10. The collected fractions were analyzed applying paper chromatography in S-8 and afterwards the respective fractions were joined and evaporated. The fractions containing flavonoids were further separated with preparative chromatography (Whatman No 3 paper) in S-8. The spots of separated compounds were localized in UV₃₆₆ light and then were cut off and eluted with hot methanol. Chromatographically homogenous fractions of flavonoid compounds 1-6 (Table 3) were received and after dissolving them in 5 ml methanol were used for identification.

Identification of flavonoid compounds:

Acid hydrolysis: 1 ml 1% HCl was added to 1 ml of the compounds 2-6 and the mixture was heated at 100°C for 1 h. The hydrolysate was then shaken with ethyl ether and ethyl acetate. Joined ether and ethyl acetate extracts were used after their concentration for the analysis of aglycons (PC, Whatman No 1, S-9). The residue was analysed for sugar moieties (PC, Whatman No 1, S-11, flow procedure). The Rf values of separated flavonoid compounds, their fluorescence in UV₃₆₆, as well as, the products of hydrolysis are presented in the Table 3.

TABLE 3. Characteristics of tomatillo flavonoids (*Physalis ixocarpa* Brot.)

Compound	Rf values in S-8	Occurrence:		Fluorescence in UV ₃₆₆	Products of hydrolysis	
		cv. Bujna	cv. Rendidora		aglycone	sugars
1	0.02	+	-	yellow	quercetin	-
2	0.09	+	+	brown	-	-
3	0.43	+	+	brown	quercetin	Glc
4	0.58	+	+	brown	quercetin	Glc, Gal
5	0.63	+	+	brown	quercetin	Glc, Arab
6	0.74	+	-	brown	-	-

Glc – glucose, Gal – galactose, Arab – arabinose

RESULTS AND CONCLUSION

Fresh fruits of *Physalis ixocarpa* Brot. cvs. Bujna and Rendidora deprived of their husks were analysed for the presence of alkaloids, witanolides, saponosides and flavonoids. For the qualitative analysis of alkaloids a separate fraction was prepared which could contain steroid alkaloids-derivatives of secotropan and a fraction of the tertiary and quaternary alkaloids. In this last fraction the different solubility of alkaloids was also taken into consideration: the fraction of tertiary alkaloids soluble in ethyl ether, the fraction of tertiary alkaloids soluble in chloroform, and the fraction of quaternary alkaloids

soluble in water were collected. According to its color reactions and the behaviour in chromatography, the choline was found to occur in the fraction of quaternary alkaloids and trace amounts of tertiary alkaloids soluble in chloroform were found. Tertiary alkaloids soluble in ethyl ether, steroid alkaloids and alkaloid derivatives of secotropan were not found.

Negative results of the reaction of Liebermann-Bürchardt, as well as the negative chromatographic results with the reactions specific for witanolides indicated their absence in the analyzed fractions. Due to the small amount of plant material used for the isolation of witanolides the results obtained may be considered only as introductory ones.

A positive Liebermann-Bürchardt reaction for the respective extracts and for aglycone fractions (after hydrolysis of these extracts) indicates the presence of saponosides with specific steroid structures. The results of the chromatographic analysis support this suggestion. However, a negative hemolytic index for the extracts and a very low hemolytic index for the aglycones, in spite of a much greater amount of plant material than normally used for the saponoside raw material, indicates a very low content of hemolysing saponosides in tomatillo fruits. The very low saponification numbers indicate also, that saponosides occur in tomatillo fruits only in trace amounts.

The flavonoids were separated from tomatillo fruits as chromatographically homogeneous fractions. The identification of flavonoids comprised mainly the analysis of products of qualitative hydrolysis. The derivatives of quercetin substituted with different sugar moieties at C-3 were prevailing.

The detailed work on the identification of these compounds will be presented in a separate publication (Matławska et al. in press).

It can therefore be concluded that tomatillo fruits of both the investigated cultivars do not contain compounds harmful to human health. On the other hand, choline and flavonoid compounds belonging to the vitamin P group constitute advantageous ingredients in the fruits.

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BADANIA FITOCHEMICZNE OWOCÓW MIECHUNKI POMIDOROWEJ
(*Physalis ixocarpa* Brot., *Solanaceae*)

STRESZCZENIE

Owoce dwóch krajowych odmian Miechunki pomidorowej badano na obecność alkaloidów, witanolidów, saponozydów i flawonoidów. O ile flawonoidy należące do grupy witaminy P mają wszechstronny i korzystny wpływ na organizm człowieka to znaczna zawartość alkaloidów, witanolidów i saponozydów dyskwalifikowałaby owoce jako produkt spożywczy. W badanych owocach nie wykazano obecności witanolidów. Saponozydy występowały natomiast w ilościach śladowych, a uzyskane wskaźniki liczby pienienia i indeksu hemolitycznego były bardzo niskie. Wyznaczenie ich możliwe było po zastosowaniu odpowiednio 20- i 10-krotnie większej ilości surowca niż jest to wymagane przy surowcach saponinowych. We frakcjach otrzymanych tokiem przyjętym dla rozfrakcjonowania zespołu alkaloidów nie znaleziono alkaloidów sterydowych i pochodnych sekotropanu, a także alkaloidów trzeciorzędowych rozpuszczalnych w eterze etylowym. Stwierdzono jedynie obecność trzeciorzędowych alkaloidów rozpuszczalnych w chloroformie, a we frakcji zasad czwartorzędowych rozpuszczalnych w wodzie wyłącznie cholinę, którą zidentyfikowano chromatograficznie. Związki flawonoidowe w owocach obu odmian Miechunki pomidorowej to kwercetyna i jej trzy pochodne glikozydowe. Szczegółowe badania flawonoidów będą tematem oddzielnej publikacji.

SŁOWA KLUCZOWE: alkaloidy, witanolidy, saponozydy, flawonoidy, *Physalis ixocarpa* Brot., Miechunka pomidorowa