Extracts from physalis leaves (Physalis peruviana L.) for prospective application in medicine and cosmetics

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Abstract

Introduction. The aim of research is to characterize the chemical composition of physalis leaves and the obtaining of extracts rich in bioactive compounds intended for medical and cosmetic applications.

Materials and methods. Extraction of dried physalis leaves was carried out under the following conditions: hydromodule – 1:10 (w/v), solvents – 95, 70, 50 and 30 vol.% ethanol, temperature – 20, 40and 60°C, and duration – 1, 3 and 5 h. The content of polyphenols, flavonoids and triterpenes in the leaves and in the obtained extracts was determined by HPLC.

Results and discussion. The analyzed physalis leaves from variety Plovdiv and from the bio-farm were with 8.32% and 8.79% moisture, respectively. The plant materials contained 9.62% and 10.58% tannins, respectively. Extract color varied by solvent concentration: yellow-orange (with 30% ethanol), yellow-brown (50% ethanol), green-brown (70% ethanol), and brown (95% ethanol). The experimental data and the derived equations showed that the two main factors – temperature and duration, had a strong influence on the content of extracted tannins. The optimal conditions of the process were: 5-hour extraction at a temperature of 60°C, with 30 and 50% ethanol for the leaves from Plovdiv genotype, and with 50 and 70% ethanol – for the bio-farm genotype. Twelve phenolic acids were identified in the leaves and extracts from Plovdiv genotype and 10 – in those from the bio-farm genotype. Rutin was the dominant flavonoid in the leaves and extracts from both genotypes. The major triterpene in the leaves and in the extracts was oleanolic acid, followed by betulin.

The extracts from physalis leaves are rich in bioactive substances (phenolic acids, flavonoids and triterpenes), and have the prospective for possible application in medicinal and cosmetic products.

Conclusions. This study provides for the first time data about the optimal conditions for the extraction of *Physalis peruviana* leaves, as well as information about the content of certain biologically active components in the leaves and in the obtained extracts. These are the first results reported about physalis genotypes grown in Bulgaria.

Introduction

The genus *Physalis* (family Solanaceae) comprises nearly 120 wild and cultivated species common to many countries around the world [1]. Among them, the most widely distributed and commercially important is *Physalis peruviana* L. [2], also known as Cape gooseberry, goldenberry, Inca berry, or simply physalis. The edible part of the plant is the fruit – ovoid in shape, cherry-sized, yellow-to-orange-fleshed and juicy. It is described as tomato-like in flavor and appearance, though the taste (sweet and sour) is much richer with a hint of tropical luxuriance [3]. The fruit is hidden in an inflated calyx or fruit basket, protecting it from insects, birds, diseases and harsh climate conditions [2, 3].

While physalis fruit, along with many other subtropical berries [4], has been long acknowledged as a source of valuable bioactive and nutritional substances, there is scarce data about the chemical composition of physalis leaves or leaf-derived bioactive products.

In a study by Ertürk et al. [5], the total phenolic and flavonoid content of an ethanol extract from physalis leaves were estimated to 1.368 mg GA/g and 0.635 mg QE/g, respectively. Wu et. al. [6] obtained ethanolic, aqueous and supercritical CO_2 extracts from physalis leaves. Total flavonoid and phenol contents were 37.39 mg/g and 18.57 mg/g, respectively, in the aqueous extract, and 94.97 mg/g and 85.81 mg/g – in the ethanolic extract. The presence of phytochemicals with different biological activities (alkaloids, saponins, tannins, steroids, terpenoids, and flavonoids) was detected in aqueous extracts from physalis leaves, although it was not quantified [7]. Cirigliano et al. [8] studied crude physalis extracts and the data indicated that the extract and its two major withanolides (withanolide E and 4-β-hydroxy withanolide E) could be used to develop baits to control the fruit fly *Ceratitis capitata*. An extract of physalis leaves showed antibiotic activity against Gram-positive bacteria from genus *Staphylococcus* [9].

In folk medicine, the juice of physalis leaves has been used in the treatment of worm and bowel complaints, while heated leaves are applied as a poultice [1, 10, 20].

Although physalis is not a popular crop in Bulgaria, an original local variety of *P. peruviana* named Plovdiv has been selected and officially recognized by the national authorities in 2006 [11].

The above-presented brief review on available data clearly identifies the lack of sufficient scientific evidence about the chemical composition of physalis leaves and about the obtaining of extracts rich in bioactive compounds intended for medical and cosmetic applications, which is set as the objective of current study.

Materials and methods

Plant Material

Leaves of two genotypes of cultivated phyisalis (*Physalis peruviana* L.) were investigated. The first genotype was the only local Bulgarian variety of physalis named Plovdiv and was grown in the region of Plovdiv city, located in Central South Bulgaria [11]. The second genotype was grown and provided by a certified bio-farm (Versol Ltd.), located in Lik village, North-West Bulgaria. Fresh leaves were picked in the period September-October, and then were air-dried in the shade. Dried leaves were isolated in plastic bags and stored at a temperature of 5-8°C until processing.

Chemicals

HPLC grade methanol and acetonitrile, as well as phenolic acid and flavonoid standards were purchased by Sigma (Sigma-Aldrich Chemie GmbH, Germany).

Chemical analyses

The moisture content of the leaves was determined by drying (at 105°C) to constant weight [12], and all data in the study were calculated on a DW basis. The content of tannins was determined by titration of hot water extract with potassium permanganate solution using indigo carmine as indicator [12]. The HPLC analysis of polyphenols, flavonoids and triterpenes in the plant material and in the extracts was according to Marchev et al. [13, 14].

Obtaining of extracts

Extraction was carried out in laboratory conditions, in a batch static mode, at a ratio of raw material to solvent = 1:10 (w/v). Four solvents were used for the extraction, representing different ethanol concentrations: 95, 70, 50 and 30 vol.%. The solvent, its concentrations and the hydromodule were chosen on the basis of authors' own published data. The influence of the technological factors – temperature and duration of extraction, was examined by mathematical modeling of the experiment (Table 1).

Table 1

Mathematical modeling of the experiment

Variant	Duration, (x_1), h	Temperature, (x_2), °C
1	20	1
2	20	3
3	20	5
4	20	7
5	40	1
6	40	3
7	40	5
8	40	7
9	60	1
10	60	3
11	60	5
12	60	7

Process effectiveness was evaluated in terms of the quantity of extracted tannins [15].

Statistical analysis

Based on experimental data, the equations of tannin extraction were derived, and their coefficients were verified for significance by Student's test and for adequacy – by Fisher's test. All experiments were for performed at least three times. Statistical significance was assessed by Student's-test or ANOVA. Differences between means were considered statistically significant if $p > 0.05$. The figures were created with MicroCal™ Origin 9.1 software.

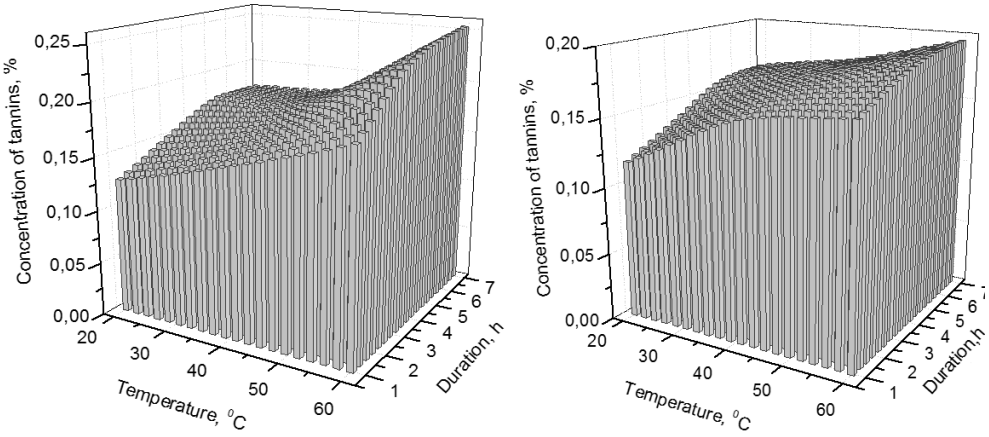
Results and discussion

Obtaining and characteristics of ethanol extracts from physalis leaves

The analyzed physalis leaves from variety Plovdiv and from the bio-farm were with 8.32% and 8.79% moisture level, respectively. The plant materials contained 9.62% and 10.58% tannins, respectively for the two genotypes.

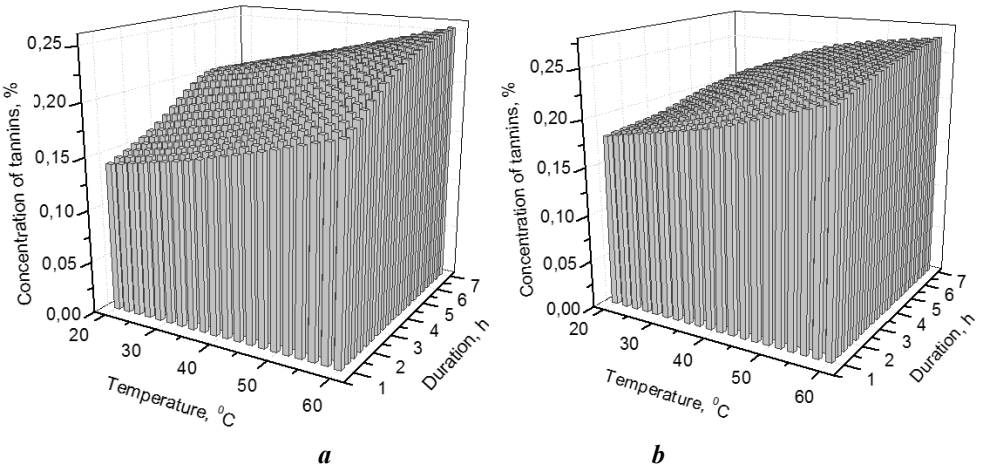
The obtained ethanol extracts from physalis leaves were liquids, and their color varied by solvent concentration: yellow-orange (with 30% ethanol), yellow-brown (50% ethanol), green-brown (70% ethanol), and brown (95% ethanol).

Figures 1– 4 present the results about the tannin content in the extracts from the two physalis genotypes, according to the scheme of the experiments that have been carried out. The results show that the elevation of temperature from 20 to 60 ºC, and the extension of process duration from 1 to 5 h, both increase the content of extracted tannins, independent of ethanol concentration or the origin of the leaves. Extraction for 7 h resulted in an insignificant increase in the amount of extracted tannins.



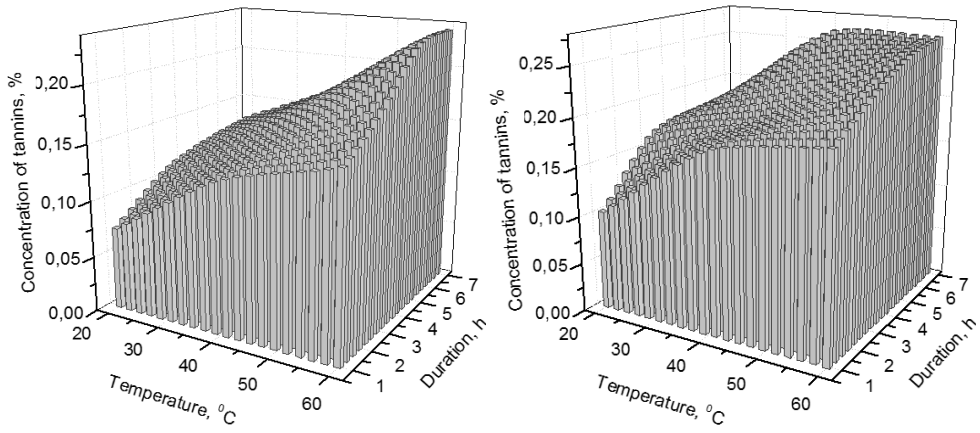
a *b*

Figure 1. Content of tannins (%) in extracts from physalis leaves with 30% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



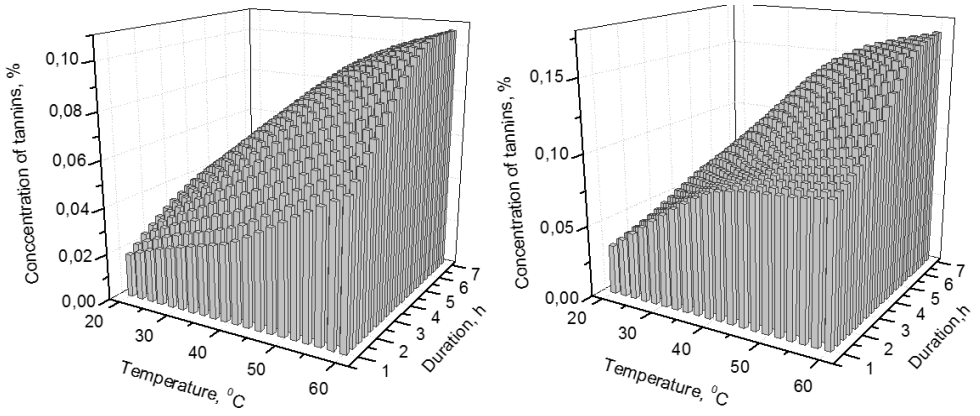
a *b*

Figure 2. Content of tannins (%) in extracts from physalis leaves with 50% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



a *b*

Figure 3. Content of tannins (%) in extracts from physalis leaves with 70% ethanol: a – Plovdiv genotype; b – bio-farm genotype.



a *b*

Figure 4. Content of tannins (%) in extracts from physalis leaves with 95% ethanol: a – Plovdiv genotype; b – bio-farm genotype.

Reasonably, the two main factors of the extraction process – duration (x_1) and temperature (x_2), had a strong influence on the content of extracted tannins. The relation between tannin content (y,%.) and process parameters (x_1, h and x_2, °C) is confirmed by the obtained equations, which were verified as adequate and with significant coefficients:

Solvent	Equation
	Leaves from Plovdiv genotype
30% ethanol	$y=0.177 + 0.036x_1 + 0.023x_2 + 0.002x_1x_2 + 0.015x_1^2 - 0.006x_2^2$
50% ethanol	$y = 0.190 + 0.027x_1 + 0.028x_2 - 0.003x_1x_2 + 0.005x_1^2 + 0.002x_2^2$
70% ethanol	$y = 0.151 + 0.047x_1 + 0.026x_2 + 0.006x_1x_2 - 0.003x_1^2 - 0.001x_2^2$
95% ethanol	$y = 0.063 + 0.026x_1 + 0.019x_2 + 0.006x_1x_2 - 0.001x_1^2 - 0.005x_2^2$
	Leaves from the bio-farm genotype
30% ethanol	$y = 0.167 + 0.022x_1 + 0.014x_2 - 0.004x_1x_2 - 0.006x_1^2 - 0.002x_2^2$
50% ethanol	$y = 0.225 + 0.032x_1 + 0.013x_2 + 0.001x_1x_2 - 0.003x_1^2 - 0.006x_2^2$
70% ethanol	$y = 0.204 + 0.042x_1 + 0.029x_2 - 0.005x_1x_2 - 0.012x_1^2 - 0.006x_2^2$
95% ethanol	$y = 0.088 + 0.038x_1 + 0.025x_2 + 0.011x_1x_2 - 0.008x_1^2 + 0.007x_2^2$

The highest concentration of tannins in the extracts was obtained under the following conditions: 5-hour extraction at a temperature of 60°C, with 30 and 50% ethanol for the leaves from variety Plovdiv, and with 50 and 70% ethanol – for the leaves from the bio-farm. The lowest concentration of tannins was obtained with 95% ethanol, regardless of leaves' origin. The differences in tannin concentration in the extracts obtained with the four ethanol concentrations reflect the significant influence of the solvent, and can be explained with the different selectivity of water-ethanol mixtures used in the extraction of bioactive molecules. The same pattern of the influence of process temperature, duration and ethanol concentration on the content of tannins in liquid extracts has been reported in other studies on essential oil-bearing and medicinal plants, such as tobacco [15], mint [16], thyme [17], and rosemary [18].

Polyphenols in physalis leaves and extracts

The results about the content of phenolic acids and flavonoids in the leaves and extracts of *P. peruviana* are shown in Tables 2 and 3.

Data show that in the leaves and extracts from Plovdiv genotype were identified 12 phenolic acids, and the dominant was rosmarinic acid, followed by salicylic, sinapic and ferulic acids. In the leaves and extracts from the bio-farm genotype were identified 10 phenolic acids, among which dominated protocatechuic acid, followed by chlorogenic, sinapic and vanillic acids. In the leaves and extracts from both genotypes, rutin was the dominant flavonoid, which corresponded to the results obtained by Ertürk et. al. [5].

On a genotype basis, data about flavonoids showed higher levels of the flavon glycoside hesperetin in the leaves of variety Plovdiv, but lower – in the extracts, compared, respectively, to the leaves and extracts from the bio-farm genotype. The concentration of the flavon glycoside myricetin in the leaves and extracts from the bio-farm genotype were characteristically higher.

The content of salicylic, caffeic, p-coumaric, sinapic, ferulic and rosmarinic acids in the samples from Plovdiv genotype was significantly higher than the respective content in the leaves and extracts from the second genotype. Gallic acid, rosmarinic acid, luteolin, kaempherol, and apigenin were not identified in the leaves and extracts from the bio-farm genotype. Despite that, the contents of chlorogenic acid and myricetin were higher than those in the leaves and extracts from variety Plovdiv.

Table 2

Content of polyphenols in leaves and extracts from Plovdiv genotype (*P. peruviana*)

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Gallic acid	-[a)]	53.91±0.51	16.75±0.15	-	-
Protocatechuic acid	142.77±1.39	-	39.39±0.38	7.33±0.07	2.34±0.02
Salicylic acid	1161.62±10.54	26.21±0.25	254.77±2.50	194.10±1.90	1.20±0.01
Chlorogenic acid	193.67±1.89	17.40±0.16	19.98±0.18	31.32±0.30	1.61±0.01
Vanillic acid	236.47±2.30	19.92±0.18	62.85±0.60	51.27±0.50	17.08±0.16
Caffeic acid	170.61±1.69	34.49±0.33	45.48±0.44	33.30±0.30	16.82±0.15
Syringic acid	63.05±0.62	16.36±0.15	18.21±0.17	9.74±0.09	1.57±0.01
p-Coumaric acid	665.00±6.62	119.56±1.10	136.63±1.34	125.92±1.21	58.40±0.51
Sinapic acid	776.05±7.74	183.59±1.78	203.10±2.00	159.07±1.54	65.01±0.61
Ferulic acid	722.23±7.20	113.62±1.10	120.33±1.19	97.83±0.93	54.59±0.50
Cinnamic acid	24.63±0.20	18.79±0.17	6.79±0.06	1.36±0.01	0.52±0.00
Rosmarinic acid	2316.41±22.11	322.03±3.18	508.56±5.00	529.27±5.20	238.49±2.30
Myricetin	17.94±0.17	5.12±0.04	5.70±0.05	4.99±0.04	-
Hesperetin	40.85±0.39	8.77±0.08	10.01±0.09	12.77±0.11	3.22±0.03
Quercetin	6.87±0.06	2.61±0.02	2.54±0.02	2.44±0.02	2.43±0.02
Luteolin	1.44±0.01	1.04±0.01	0.62±0.00	0.38±0.00	0.37±0.00
Kaempferol	3.62±0.03	1.98±0.18	1.48±0.01	1.57±1.50	1.40±0.01
Apigenin	31.09±0.30	tr[b)]	tr	tr	tr
Rutin	4996.37±48.50	738.54±7.25	999.09±9.91	1040.12±10.00	395.68±3.80
Hyperoside	226.96±2.20	-	34.41±0.31	36.11±0.32	15.02±0.14

[a)] not identified; [b)] trace amount

These results reveal the presence of various classes of bioactive polyphenols in the leaves and in the leaf extracts obtained from the two genotypes of physalis grown in Bulgaria. The observed differences in the profile and content distribution of polyphenols are attributed to the impact of plant genotype. The results agreed well with previous findings about polyphenol content in *Physalis* leaves, for example: total phenolic concentration of 30.9-129.2 mg/g [19] and 129 mg/g [26]; total flavonoid concentration of 37.39-226.19 mg/g [6] and 23.036 mg/g [26]. Studies on the determination of flavonoid and phenolic acid content in different *Physalis* species has been focused exclusively on the fruits, but current results reveal that leaves' potential of accumulating these secondary metabolites is even higher than that of the fruits [26]. Expectedly, the numerical results for the polyphenol content varied from data found in the scientific literature for other plant materials [22, 23, 24, 25]. The established differences in terms of leaf chemical chemical composition between the present investigation and the reported data may be due to the environmental conditions under which the plants were grown, as well as to the impact of species, origin and extraction technique.

Table 3

Content of polyphenols in leaves and extracts from bio-farm genotype (*P. peruviana*)

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Gallic acid	-[a)]	-	-	-	-
Protocatechuic acid	933.22±9.28	-	-	-	-
Salicylic acid	-	47.94±0.45	49.45±0.45	67.64±0.65	52.26±0.51
Chlorogenic acid	324.33±3.20	28.65±0.26	21.29±0.20	25.83±0.24	17.64±0.16
Vanillic acid	248.56±2.41	3.68±0.03	68.62±0.65	67.55±0.36	40.74±0.38
Caffeic acid	44.90±0.40	13.01±0.11	31.60±0.30	37.75±0.36	25.16±0.22
Syringic acid	39.25±0.38	1.13±0.01	8.47±0.08	6.01±0.05	2.60±0.02
p-Coumaric acid	79.65±0.75	15.13±0.104	13.16±0.11	11.01±0.10	6.14±.05
Sinapic acid	282.59±2.79	39.06±0.37	181.69±1.78	13.18±0.11	5.55±0.04
Ferulic acid	108.89±1.00	21.71±0.20	27.22±0.25	45.32±0.43	15.26±0.14
Cinnamic acid	8.45±0.08	0.84±0.00	1.77±0.01	3.29±0.03	1.94±0.01
Rosmarinic acid	-	-	-	-	-
Myricetin	142.09±1.40	33.67±0.31	32.27±0.30	36.50±0.33	33.31±0.30
Hesperetin	32.58±0.30	49.42±0.47	24.52±0.21	14.31±0.12	15.65±0.14
Quercetin	27.17±0.25	16.32±0.15	-	-	-
Luteolin	-	-	-	-	-
Kaempferol	-	-	-	-	-
Apigenin	-	-	-	-	-
Rutin	2953.44±2.90	272.04±2.70	431.82±4.30	537.00±5.30	333.79±3.27
Hyperoside	209.11±0.19	37.28±0.18	28.77±0.09	50.53±0.33	28.48±0.09

[a)] not identified

Triterpenes in physalis leaves and extracts

The results about the content of triterpenes in the leaves and extracts of *P. peruviana* are shown in Tables 4 and 5.

Table 4

Content of triterpenes in leaves and extracts from Plovdiv genotype *(P. peruviana)*

Compounds	Leaves, µg/g DW	Ethanol extracts, µg/mL			
		30%.	50%.	70%.	95%.
Betulin	105.83±1.00	-[a)]	-	87.24±0.86	73.98±0.72
Betulinic acid	42.36±0.40	-	-	67.41±0.66	35.04±0.34
Oleanolic acid	264.90±2.60	46.98±0.45	70.01±0.69	86.97±0.85	31.10±0.30
Ursolic acid	-	30.77±0.29	60.73±0.60	58.69±0.57	8.18±0.07

[a)] not identified

Table 5

Content of triterpenes in leaves and extracts from bio-farm genotype *(P. peruviana)*

Compounds	Leaves, μg/g DW	Ethanol extracts, μg/mL			
		30%.	50%.	70%.	95%.
Betulin	- [a)]	122.21±1.20	124.36±1.21	201.91±19.97	394.44±38.00
Betulinic acid	-	-	-	-	-
Oleanolic acid	889.83±8.71	8.15±0.08	208.93±19.89	200.87±19.54	176.52±1.68
Ursolic acid	-	-	-	-	-

[a)] not identified

Three triterpenes were identified in the leaves of Plovdiv genotype, and the dominant were oleanolic acid (264.9 μg/mg) and betulin (105.83 μg/mg). In the second genotype, only oleanolic acid was identified, but in a significantly (nearly four-time) higher concentration (889.83 μg/mg). In total, four triterpenes were identified in the extracts obtained from the leaves of Plovdiv genotype, and there was a clear differentiation between the extracts (30 and 50% ethanol vs. 70 and 95% ethanol). In the extracts obtained with the lower solvent concentrations only oleanolic and ursolic acids were identified (total content 77.75 and 130.74 μg/mL, respectively). The extracts of the second sub-group were considerably richer in triterpenoids (with a total of 300.31 and 148.30 μg/mL, respectively), and the dominant were betulin, betulinic acid and oleanolic acid. In the extracts obtained from the leaves of the bio-farm genotype only two triterpenes were identified – oleanolic acid and betulin, but in higher concentrations (three-to-four times higher). Data from the study suggest that, in terms of triterpenoid content, the more suitable solvents would be 70 and 95% ethanol.

Our results clearly differentiate between the extracts on a triterpene basis, and the differences reflect the impact of plant genotype and solvent selectivity. With regard to data from previous research on triterpenoid concentration in physalis leaves and leaf extracts, it is even harder to make comparisons, than in the case of polyphenols. To the best of our knowledge the triterpenoid content of physalis leaves, and especially – of genotypes grown in Bulgaria, has not been determined elsewhere. Similar to the observations above, current results about the triterpene content in physalis leaves and ethanol leaf extracts characterize the species as distinctive form other plants, regarding literature data [24, 25]. The differences in the triterpene composition between the present investigation and the reported data may be attributed to environmental conditions, plant material origin and other influencing factors.

Conclusion

To the best of our knowledge, data achieved by this study provide for the first time the optimal conditions for the extraction procedure of *Physalis peruviana* L. leaves, as well as information about the content of certain biologically active components in the leaves and in the obtained extracts. These are the first results reported about physalis genotypes grown in

Bulgaria. The extracts contained phenolic acids, flavonoids and triterpenes, and have the prospective for possible application in different medicinal and cosmetic products, but additional investigations are undoubtedly required.

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