Research Article

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Phytochemical analysis of leaves and stems of *Physalis alkekengi* L. (Solanaceae)

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Abstract: *Physalis alkekengi* L. (Solanaceae) is encountered in different regions of Bulgaria as a wild growing or ornamental plant. The objective of this work was to characterize the phytochemical composition (macro and micro components) of the leaves and stems of two local phenotypes (PA-SB and PA-NB), with the view of revealing their use potential. The dry leaves contained (DW) protein (16.25 and 19.27%), cellulose (25.16 and 25.31%), and ash (18.28 and 16.16%) and the stems contained protein (6.83 and 7.35%), cellulose (39.34 and 38.25%), and ash (15.01 and 7.48%) for PA-SB and PA-NB, respectively. The dominant amino acids (by HPLC) in the leaves of both phenotypes were arginine (21.3–22.3 mg/g) and aspartic acid (8.8–18.4 mg/g), and those in the stems were proline and aspartic acid for PA-SB (8.8, 7.7 mg/g); isoleucine and tyrosine for PA-NB (12.8, 6.6 mg/g). Mineral elements, determined by AAS (K, Ca, Mg, Na, Cu, Fe, Zn, Mn, Pb, Cr), also varied between phenotypes and plant parts. The leaves alone were further processed by extraction with *n*-hexane, for the identification of leaf volatiles (by gas chromatography-mass spectrometry). The analysis identified 28 components (97.99%) in the leaf extract of PA-SB and 32 components (97.50%) in that of PA-NB. The volatile profile of PA-SB leaves was dominated by diterpenes (49.96%) and oxygenated sesquiterpenes (35.61%), while that of PA-NB was dominated by oxygenated aliphatics (40.01%) and diterpenes (35.19%). To the best of our knowledge, the study provides the first data about the phytochemical composition of the leaves and stems of *P. alkekengi* from Bulgaria, in a direct comparison of phenotypes from two distinct wild populations, which could be of further scientific interest.

Keywords: amino acids, Chinese lantern, GC-MS volatiles, leaf concrete, minerals

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1 Introduction

Physalis alkekengi L. (Solanaceae) is an herbaceous perennial plant, with a height of about 0.40–0.60 m. The stem is slightly branched, pubescent, often with inflated

nodes. The leaves are spirally arranged, ovate, about 6–12 cm long and 4–9 cm broad, margin entire or slightly dentate, base oblique, the pedicel 0.6–1.6 cm long. The plants produce white five-lobed corolla flowers, with a width of about 1.0–1.5 cm. The fruit is a small, shiny bright orange to red globular berry completely covered at maturity by a wide papery orange-red calyx [1–4]. The notable shape and color of *P. alkekengi* calyces gave some of the common names of the plant, such as Chinese lantern and Japanese lantern. It is the only *Physalis* species that does not originate from America but is indigenous to South Europe and South Asia [2]. Nowadays, the species is naturalized worldwide, growing in the wild or planted as an ornamental in gardens or containers [5]. The plants are equally suitable for light (sandy), medium (loamy), and heavy (clay) soils but well drained and moist; they prefer either full sun or light shade and can be found in hedgerows and damp paths, from the plains to the lower slopes of the mountains. The species is considered relatively invasive, especially when cultivated for ornamental purposes, easily spreading and colonizing flowerbeds [6].

P. alkekengi is the only *Physalis* species that is native to Bulgaria, where it is found in different regions of the country at altitudes up to 1,200 m [7]. The trivial name of the plant in Bulgarian is “mehunka”, meaning “husk.” The plant is included within the scope of the Medicinal Plants Act [8].

All aerial parts of the plant have been used in folk medicine for centuries due to the presence of various bioactive metabolites. The traditional medicinal uses of *P. alkekengi* in the treatment of various conditions were supported by the data from contemporary research on the metabolic profile of plant-derived extracts and on the mechanisms of the respective pharmacological response [2,9]. The therapeutic effects of *P. alkekengi*, as stated for other species of medicinal plants [10], were related to the chemical composition of different plant tissues; high concentrations of steroids, sterols, flavonoids, phenylpropanoids, N-containing compounds, vitamins, and others have been reported [2,11–16]. The extracts from *P. alkekengi* fruit and calyx (CAS No 90082-67-0) are referenced in the Cosmetic Ingredient Database (CosIng) of the European Commission [17], as cosmetic ingredients with skin conditioning properties. Different extracts from the fruit and calyces were reported to assist in effects important for the development of personal skin care products, such as skin protection, soothing, anti-ageing, melanogenesis inhibition, and others [12,18]. It should be outlined that most of the phytochemical studies have considered only the fruit and the calyces of *P. alkekengi*, because they are the plant organs used in the traditional Chinese and other folk medicines, while the other plant tissues (roots, stems, and leaves) have been subjected to considerably less investigation [2,9]. To the best of our knowledge, there were no previous studies regarding *P. alkekengi* leaves as a plant material for obtaining the aromatic products, as well as about the volatile composition of the extracts.

Based on these considerations, we hypothesized that the phytochemical analysis of the leaves and stems of *P. alkekengi* (vegetative waste after fruit collection), as well as the processing of the leaves into an established aromatic product (leaf concrete) and the identification of its volatile composition, could add new details to the characterization of the species. We further hypothesized that there might be some origin and phenotype-based specifics within the species. Therefore, the objective of the study was the comparison between the leaves and the stems of two phenotypes of *P. alkekengi* from Bulgaria in terms of protein, amino acid, and mineral compounds, as well as the identification of the volatile composition (by gas chromatography-mass spectrometry [GC-MS] analysis) of the leaf concretes, obtained by extraction with *n*-hexane. The outcomes from the study could provide grounds for pointing out alternatives in the overall *P. alkekengi* plants’ use.

2 Materials and methods

2.1 Plant material

According to the objectives of the study, two individual samples of the aerial parts of *P. alkekengi* plants – the leaves and the stems – were analyzed (Figure 1). To follow the phenotype-related differences in the phytochemical composition, two phenotypes of the species were compared, representing distant regions of plant occurrence in Bulgaria. The

Figure 1: Fresh leaves and stems of *P. alkekengi* (photo by authors).

leaves and stems of the first phenotype (PA-NB) were collected from a natural habitat in North-Eastern Bulgaria (Ivanski village, Shumen region), with geographical coordinates 43°07′24″N 27°04′35″E. The plants of the second phenotype (PA-SB) were collected from a population in Central Southern Bulgaria (the city of Plovdiv, Plovdiv region), 42°14′26″N 24°70′24″E. The plants were identified at the Department of Botany, Plovdiv University 'Paisii Hilendarski'. Both aerial parts were air-dried and kept in cloth bags at 5–8°C until analysis.

2.2 Methods of analysis

The moisture content of the raw materials was determined by drying to constant weight, and the results from the chemical analyses were given further on a dry weight (DW) basis.

The ash content was determined according to AOAC [19], by mineralization of the samples at 550°C for 5 h.

The cellulose content was determined applying a slight modification of the method described previously [20], in which hydrolysis was completed with 16.5 mL of 80% CH_3COOH and 1.5 mL of concentrated HNO_3 at boiling for 1.5 h, and the dried solid residue (105°C for 24 h) was quantified.

The protein content was determined according to the standard method [19], on a UDK 152 system (Velp Scientifica Srl, Usmate Velate, Italy). The HPLC analysis of amino acids followed a previously described procedure [21], using an Elite LaChrome (Hitachi, Tokyo, Japan) unit (DAD; reverse phase C18 AccQ-Tag column, 3.9 mm × 150 mm).

Mineral elements were determined by atomic absorption spectrophotometry using a Perkin Elmer/HGA 500 (Norwalk, CT, USA) instrument. The identification and quantification of metal ions were performed according to the analytical procedure described in ref. [21].

The dried leaves of *P. alkekengi* were extracted with a non-polar solvent, *n*-hexane, to obtain the respective crude extracts (leaf concretes) [22]. Two-fold extraction was carried out, at 40°C temperature and hydromodule 1:30 (raw material:solvent, w/v) for 1 and 0.5 h, respectively. The solvent was completely evaporated from the combined extracts using a rotary vacuum evaporator, at temperature not exceeding 40°C.

The GC-MS analysis of the leaf concretes was carried out on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C inert XL EI/CI mass selective detector (Agilent Technologies Inc., Santa Clara, CA, USA). The column was HP-5 ms (30 m × 250 mm × 0.25 μm, i.d.); the carrier gas was helium, run at a constant rate of 1 mL/min. Oven temperature increase was at a rate of 5°C/min, from 35°C (held for 3 min) to 250°C (held for 3 min); the total run time was 49 min, and the split mode was 30:1. The MS conditions were as follows: ionization voltage 70 eV, electron impact mode, scan range 50–500 m/z, MS source temperature 230°C, injector temperature 150°C, and detector temperature 250°C. MS data were processed using the tools of 2.64 AMDIS software (NIST, Gaithersburg, MD, USA). The identification of volatiles was according to their retention times, the calculated retention (Kovat's) indices (RI), and the obtained MS fragmentation data, compared with mass spectra library data [23,24, own library]. A standard mixture of *n*-alkanes (C_8–C_{40}) in *n*-hexane was used to calculate the relative RIs and analyzed under the same conditions as described above. Volatile contents were given as percentage of the total ion current (TIC) after normalization of peak areas in the TIC chromatograms.

2.3 Statistics

All analyses were repeated three times and the data were presented as the mean values ± the standard deviation (SD).

3 Results and discussion

Both aerial parts in the study, the leaves and the stems of *P. alkekengi*, practically represent a waste plant material remaining each season after the collection of the edible fruit, and the obtained results suggested that they could be regarded as resources of functional phytochemicals.

3.1 Basic chemical indices of *P. alkekengi* leaves and stems

The basic chemical indices of the analyzed plant materials are presented in Table 1. The moisture content of the air-dried leaves and stems was relatively low (9.37–12.11%), thus facilitating the safe storage of the plant materials [25]. Data revealed that there were no significant differences between the phenotypes with regard to the analyzed macro components – protein, cellulose, and ash. The two aerial parts differed by chemical composition; the leaves reasonably were with higher moisture content

Table 1: Principle chemical indices of *P. alkekengi* leaves and stems

Index	PA-SB[1]		PA-NB	
	Stems	Leaves	Stems	Leaves
Moisture, %	10.24 ± 0.08[2]	12.11 ± 0.15	9.37 ± 0.05	11.33 ± 0.08
Protein, % (DW)[3]	6.83 ± 0.03	16.25 ± 0.11	7.35 ± 0.05	19.37 ± 0.11
Cellulose, % (DW)	39.34 ± 0.22	25.16 ± 0.12	38.25 ± 0.27	25.31 ± 0.15
Ash, % (DW)	15.01 ± 0.08	18.28 ± 0.10	7.48 ± 0.05	16.16 ± 0.11

[1]PA-SB – phenotype from Central Southern Bulgaria; PA-NB – phenotype from Northern Bulgaria. [2]Data are presented as mean value ± SD (n = 3). [3]DW – on a DW basis.

and contained more protein and ash and less cellulose than the stems of the respective phenotype.

The results from the identification of the amino acid composition of leaf and stem protein, after protein hydrolysis, are presented in Table 2. Seventeen amino acids were identified in each of the plant samples. As seen from the data, there was variation in the individual amino acid distribution, both on a phenotype and plant part basis. The most abundant amino acids in the leaves of the two phenotypes were arginine (22.3 mg/g), aspartic acid (8.8 mg/g), serine (8.5 mg/g), and alanine (8.2 mg/g) (in PA-SB) and arginine (21.3 mg/g), aspartic acid (18.4 mg/g), glutamine (11.2 mg/g), and alanine (11.0 mg/g) (in PA-NB), respectively. As seen from the table, the leaves of PA-NB phenotype contained correspondingly high levels of other essential amino acids, as well, such as lysine (10.6 mg/g), phenylalanine (10.0 mg/g), and isoleucine (7.5 mg/g). The dominant amino acids in the stems of PA-SB were proline (8.8 mg/g), aspartic acid (7.7 mg/g), and histidine (6.4 mg/g) and those in the stems of PA-NB were isoleucine (12.8 mg/g) and tyrosine (6.6 mg/g). As a general observation, the amino acid concentrations were higher in the leaves than in the stems, especially for amino acids, such as arginine, phenylalanine, lysine, alanine, valine, and others; some exceptions with higher stem contents were histidine and proline (in PA-SB) and tyrosine and isoleucine (in PA-NB). Therefore, the amino acid composition of *P. alkekengi* foliage protein was influenced by the phenotype factor, a finding that might be regarded in future use considerations. Data in Table 2 revealed substantial protein quality in *P. alkekengi* leaves and stems, due to the high proportion of essential amino acids. The ratio between essential and non-essential amino acids in the studied aerial parts of PA-SB and PA-NB phenotypes varied in the range from 0.8:1 to 1.1:1.

Table 2: Amino acid composition of *P. alkekengi* leaves and stems

Amino acid, mg/g (DW)[1]	PA-SB[2]		PA-NB	
	Stems	Leaves	Stems	Leaves
Aspartic acid	7.65 ± 0.05[3]	8.84 ± 0.06	3.97 ± 0.04	18.38 ± 0.08
Serine	4.94 ± 0.03	8.54 ± 0.06	2.17 ± 0.02	8.77 ± 0.06
Glutamine	3.51 ± 0.03	6.76 ± 0.06	4.25 ± 0.03	11.20 ± 0.08
Glycine	0.16 ± 0.00	3.89 ± 0.02	0.78 ± 0.01	2.01 ± 0.01
Histidine	6.41 ± 0.05	0.12 ± 0.00	3.91 ± 0.04	5.29 ± 0.04
Arginine	2.20 ± 0.02	22.34 ± 0.19	0.17 ± 0.00	21.27 ± 0.18
Threonine	2.29 ± 0.02	5.49 ± 0.04	1.01 ± 0.01	4.77 ± 0.04
Alanine	4.23 ± 0.03	8.22 ± 0.07	2.35 ± 0.01	10.99 ± 0.09
Proline	8.79 ± 0.07	6.06 ± 0.05	2.69 ± 0.02	10.56 ± 0.08
Cysteine	0.03 ± 0.00	0.04 ± 0.00	1.60 ± 0.01	1.33 ± 0.01
Tyrosine	1.70 ± 0.01	4.62 ± 0.03	6.56 ± 0.05	4.96 ± 0.04
Valine	2.34 ± 0.01	5.24 ± 0.04	1.39 ± 0.01	7.16 ± 0.06
Methionine	0.45 ± 0.00	0.97 ± 0.01	0.43 ± 0.00	1.08 ± 0.01
Lysine	4.16 ± 0.03	6.42 ± 0.04	2.37 ± 0.02	10.58 ± 0.10
Isoleucine	2.43 ± 0.02	5.15 ± 0.04	12.83 ± 0.11	7.54 ± 0.06
Leucine	0.45 ± 0.00	1.01 ± 0.01	0.22 ± 0.00	1.28 ± 0.01
Phenylalanine	2.65 ± 0.02	6.73 ± 0.04	1.48 ± 0.01	10.01 ± 0.09

[1]DW – on a DW basis. [2]PA-SB – phenotype from Central Southern Bulgaria; PA-NB – phenotype from Northern Bulgaria. [3]Data are presented as mean value ± SD (n = 3).

Table 3: Mineral composition of *P. alkekengi* leaves and stems

Mineral, mg/kg (DW)[1]	PA-SB[2]		PA-NB	
	Stems	Leaves	Stems	Leaves
K	34340.00 ± 221.22[3]	39768.00 ± 234.07	28271.07 ± 113.14	36495.15 ± 184.72
Ca	4410.00 ± 23.04	7897.00 ± 32.75	9099.08 ± 79.21	28184.47 ± 143.02
Mg	1520.00 ± 8.99	4505.00 ± 13.21	4624.45 ± 13.67	8791.26 ± 21.87
Na	55.80 ± 0.51	23.48 ± 0.20	164.80 ± 1.05	118.93 ± 1.11
Cu	3.66 ± 0.03	10.25 ± 0.08	17.48 ± 0.13	16.99 ± 0.09
Fe	7.68 ± 0.06	143.22 ± 1.08	177.79 ± 1.01	344.66 ± 3.02
Zn	72.32 ± 0.42	18.32 ± 0.11	142.83 ± 1.22	32.52 ± 0.18
Mn	7.38 ± 0.04	16.14 ± 0.08	35.46 ± 0.19	67.96 ± 0.29
Pb	<0.1[4]	<0.1	2.00 ± 0.01	1.41 ± 0.01
Cd	<0.01[5]	<0.01	<0.01	<0.01
Cr	14.24 ± 0.09	12.42 ± 0.09	0.40 ± 0.01	0.29 ± 0.01

[1]DW – on a DW basis.
[2]PA-SB – phenotype from Central Southern Bulgaria; PA-NB – Phenotype from Northern Bulgaria.
[3]Data are presented as mean value ± SD (n = 3).
[4]Not quantified.
[5]Not detected.

The results about the individual mineral composition of the leaves and the stems of *P. alkekengi* are presented in Table 3. Data revealed that both aerial parts contained considerable amounts of micro and micro minerals, as well as some differentiation between the two phenotypes in the study. The dominant macro mineral in all samples was K; the leaves of PA-NB contained comparable amounts of Ca, as well. The distribution of K and Mg was predominantly in the leaves, and that of Na was predominantly in the stems. In general, PA-NB phenotype contained higher levels of Ca, Mg, and Na, compared to PA-SB phenotype (both leaves and stems). The same trend was observed for the accumulation of micro minerals, as well, and the leaves and stems of PA-NB were richer in Cu, Fe, and Zn than the respective plant parts of PA-SB. The heavy metals had different presence in the two phenotypes, too, obviously reflecting the influence of soil and environmental conditions of the respective plant habitats [26]. Pb was identified only in the aerial parts of PA-NB, while Cr was practically found only in PA-SB leaves and stems. Cd was not detected in either of the plant samples. It is hard to parallel those results to previous data; as stated earlier, the phytochemical research on the species has regarded almost exclusively fruit and calyx constituents [2].

3.2 Volatiles (by GC-MS) in *P. alkekengi* leaf concretes

The diversification of plant species available for obtaining natural bioactive extracts, as a better alternative of synthesized components, has become essentially important for the fragrance industry and phytopharmacy [27], and rigorous research has been conducted in the last decades [28]. Therefore, the leaves of *P. alkekengi* alone were further processed by extraction with *n*-hexane to obtain the respective leaf concretes, and the general descriptions of the obtained aromatic products are presented in Table 4.

The results from the identification of GC-MS volatiles in the two concretes from *P. alkekengi* leaves revealed significant variations between the phenotypes, both in the presence of individual components (Figure 2 and Table 5) and in their distribution by chemical classes (Figure 3).

In the concrete from PA-SB, leaves were identified 28 components (97.99% of the total composition); 12 of them were with over 1% content. The major components (over 3% content) were seven, as follows: phytol acetate (48.60%), (2*E*,6*E*)-methyl farnesoate (8.38%), (2*E*,6*E*)-farnesoic acid (5.88%), (2*E*,6*E*)-farnesyl acetate (5.79%), (5*E*,9*E*)-farnesyl

Table 4: Principle indices of the obtained concretes from *P. alkekengi* leaves

Index	PA-SB[1]	PA-NB
Yield of concrete, % (DW, w/w)	1.02 ± 0.01[2]	4.81 ± 0.03
Appearance of concretes	Waxy, thick masses	
Color of concretes	Dark yellow-orange	

[1]PA-SB – Phenotype from Central Southern Bulgaria; PA-NB – phenotype from Northern Bulgaria.
[2]Data are presented as mean value ± SD (n = 3).

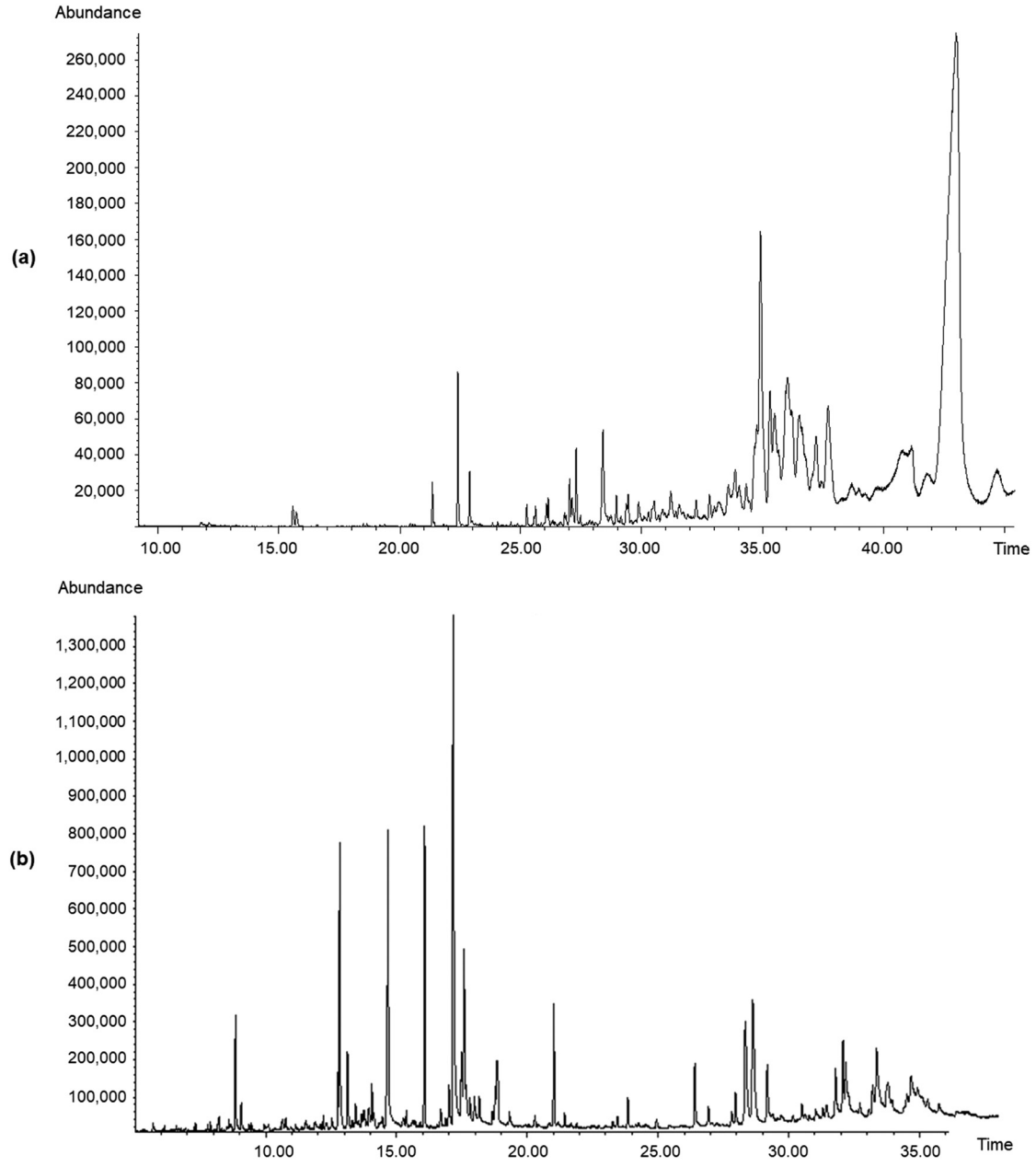

Figure 2: TIC chromatograms (GC-MS) of concretes from *P. alkekengi* leaves: (a) phenotype from Central Southern Bulgaria, PA-SB, and (b) phenotype from North-Eastern Bulgaria, PA-NB.

acetone (4.89%), 1-octadecene (4.67%), and myristic acid (3.48%). The volatile profile of PA-SB leaves was dominated by diterpenes (49.96% of the identified composition) and oxygenated sesquiterpenes (35.61%).

In the concrete from the leaves of the second phenotype, PA-NB, 32 components (97.50% of the total composition) were identified; 19 were over 1%. The major compounds (with over 3% content) were as follows: phytol (17.90%), *n*-hexadecanol (10.72%), methyl hexadecanoate (9.25%), (2*E*,6*E*)-farnesoic acid (8.14%), 3α-acetoxy-manool (7.35%), 3α-14,15-dihydro-manool oxide (5.56%), oleic acid (4.69%), methyl octadecanoate (3.41%), 3α-hydroxy-manool (3.23%), and 4,8,12,16-tetramethylheptadecan-4-olide (3.16%). The dominant groups of volatiles in the PA-NB leaf concrete were oxygenated aliphatics (40.01%) and diterpenes (35.19%). As seen from Table 5, only four compounds were identified in both concretes, (2*E*,6*E*)-farnesoic acid, (2*Z*,6*E*)-farnesyl acetate, phytol, and phytol acetate, but at greatly different concentrations. The rest of the major and minor components in the two concretes were phenotype specific. Correspondingly, there were significant differences in the distribution of most of the chemical classes

Table 5: Volatile composition (by GC-MS) of the obtained *P. alkekengi* leaf concretes

No	Compound	RI[1]	Content (% of TIC)[2]	
			PA-SB[3]	PA-NB
1	Methyl octanoate	1,122	0.25 ± 0.00[4]	nd[5]
2	Octyl formate	1,129	0.20 ± 0.00	nd
3	Geranyl formate	1,298	nd	0.23 ± 0.00
4	Geranyl acetone	1,423	nd	0.27 ± 0.00
5	*n*-Tridecane	1,300	0.28 ± 0.00	nd
6	Methyl decanoate	1,330	1.16 ± 0.01	nd
7	Neryl acetate	1,330	0.38 ± 0.00	nd
8	Neryl acetone	1,360	0.18 ± 0.00	nd
9	α-Zingiberene	1,494	0.14 ± 0.00	nd
10	β-Curcumene	1,515	0.33 ± 0.00	nd
11	6-Methyl-α-Ionone	1,520	0.27 ± 0.00	nd
12	Methyl dodecanoate	1,524	nd	0.21 ± 0.00
13	Dihydroactinidiolide	1,531	nd	2.97 ± 0.02
14	α-Cadinene	1,537	0.24 ± 0.00	nd
15	α-Calacorene	1,546	0.42 ± 0.00	nd
16	(*Z*)-Cadinene ether	1,552	0.37 ± 0.00	nd
17	(*E*)-Nerolidol	1,560	0.71 ± 0.01	nd
18	Butyl decanoate	1,572	nd	1.30 ± 0.01
19	Viridiflorol	1,591	1.76 ± 0.01	nd
20	Ethyl dodecanoate	1,594	nd	0.28 ± 0.00
21	tau-Cadinol	1,640	0.54 ± 0.00	nd
22	α-Bisabolol	1,685	0.45 ± 0.00	nd
23	(2*Z*,6*E*)-Farnesol	1,719	0.67 ± 0.00	nd
24	Butyl laurate	1,772	nd	0.98 ± 0.01
25	Myristic acid	1,779	3.48 ± 0.03	nd
26	(2*E*,6*E*)-Methyl farnesoate	1,785	8.38 ± 0.06	nd
27	1-Octadecene	1,790	4.67 ± 0.04	nd
28	(2*E*,6*E*)-Farnesoic acid	1,816	5.88 ± 0.05	8.14 ± 0.07
29	Isobutyl phthalate	1,819	nd	2.32 ± 0.02
30	(2*Z*,6*E*)-Farnesyl acetate	1,841	2.61 ± 0.02	1.17 ± 0.01
31	(2*E*,6*E*)-Farnesyl acetate	1,840	5.79 ± 0.04	nd
32	*n*-Hexadecanol	1,874	nd	10.72 ± 0.11
33	(5*E*,9*Z*)-Farnesyl acetone	1,881	2.86 ± 0.01	nd
34	(5*E*,9*E*)-Farnesyl acetone	1,912	4.89 ± 0.04	nd
35	Methyl hexadecanoate	1,921	nd	9.25 ± 0.08
36	*n*-Hexadecanoic acid	1,958	nd	0.74 ± 0.01
37	Phytol	2,104	0.37 ± 0.00	17.90 ± 0.14
38	Methyl linoleate	2,095	nd	0.89 ± 0.01
39	*cis*-Vaccenic acid	2,115	nd	0.80 ± 0.01
40	Methyl octadecanoate	2,120	nd	3.41 ± 0.03
41	4,8,12,16-Tetramethylheptadecan-4-olide	2,124	nd	3.16 ± 0.03
42	Linoleic acid	2,133	nd	0.48 ± 0.00
43	Oleic acid	2,140	nd	4.69 ± 0.04
44	Monoethylhexyl phthalate	2,163	nd	0.61 ± 0.00
45	Ethyl octadecanoate	2,196	nd	0.25 ± 0.00
46	Phytol acetate	2,220	48.60 ± 0.38	0.28 ± 0.00
47	3α-Hydroxy-Manool	2,297	nd	3.23 ± 0.03
48	*n*-Tricosane	2,300	2.14 ± 0.02	nd
49	3α-14,15-Dihydro-Manool oxide	2,338	nd	5.56 ± 0.05
50	3α-Acetoxy-Manool	2,362	nd	7.35 ± 0.06
51	*n*-Tetracosanol	2,422	nd	1.85 ± 0.01
52	*n*-Pentacosane	2,500	nd	2.28 ± 0.02
53	*n*-Hexacosane	2,600	nd	2.81 ± 0.02
54	*n*-Heptacosane	2,700	nd	1.17 ± 0.01

(*Continued*)

Table 5: *Continued*

No	Compound	RI[1]	Content (% of TIC)[2]	
			PA-SB[3]	PA-NB
55	*n*-Octacosane	2,800	nd	0.82 ± 0.01
56	Squalene	2,877	nd	1.39 ± 0.01
Total identified, %			97.99	97.50

[1]RI – retention (Kovat's) index.
[2]TIC – total ion current.
[3]PA-SB – phenotype from Central Southern Bulgaria; PA-NB – Phenotype from Northern Bulgaria.
[4] Data are presented as mean value ± SD (n = 3).
[5]nd – below 0.05% of TIC or not detected.

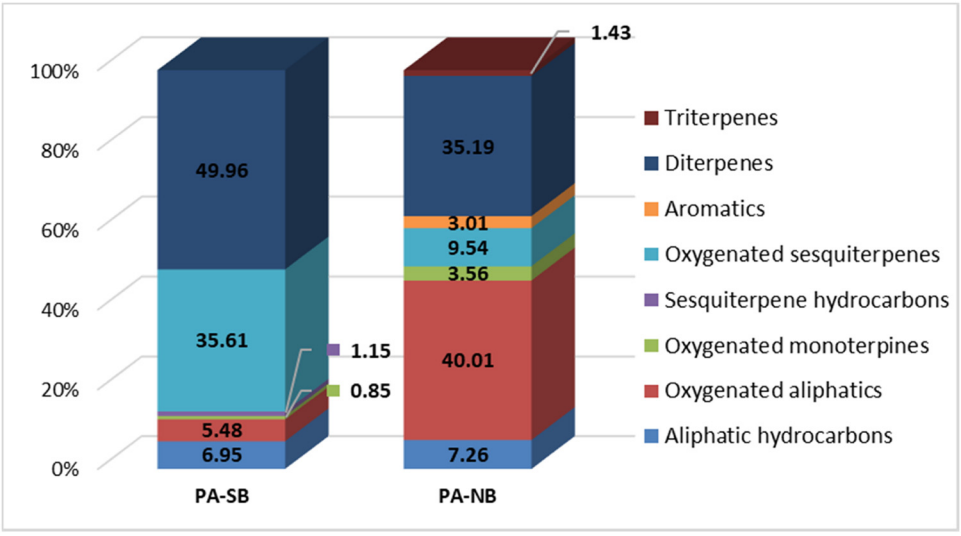

Figure 3: Distribution of compounds (GC-MS) in concretes from *P. alkekengi* leaves: PA-SB – phenotype from Central Southern Bulgaria; PA-NB – phenotype from North-Eastern Bulgaria.

on a phenotype basis, as well. As seen from Figure 3, the major differences were with regard to the presence of oxygenated aliphatics (5.48% in PA-SB vs 40.01% in PA-NB) and oxygenated sesquiterpenes (35.61% in PA-SB vs 9.54% in PA-NB); significant variation was found in the share of oxygenated monoterpenes and aromatic compounds, as well. Therefore, the results suggested that the phenotype factor (environment, harvest period, probably genetics, and other details) should be considered important in the assessment of *P. alkekengi* leaves as a resource for obtaining aromatic products for cosmetic use, as it strongly affects the volatile composition of the products. Of course, additional research devoted to that subject is needed, to make conclusions that are more definite [10,11].

To the best of our knowledge, there were no previous data about the identification of *P. alkekengi* leaf volatiles, as well as previous attempts to obtain certain aromatic products for cosmetic use (leaf concretes), moreover, in a comparative mode regarding different phenotypes. As already stated, the identification of biologically active constituents in *P. alkekengi* tissues has been focused on fruit and calyces, as they are used in folk medicine, while phytochemical investigations of other plant parts (leaves, stems, roots) are rather scarce [29]. For those reasons, it is hard to make direct comparison between our results and published data, although some studies have identified similar chemical classes and individual volatile components in different *P. alkekengi* tissues, such as terpenoids, phenylpropanoids, aliphatic derivatives, organic acids, sucrose esters, and others [2,13,14,30–32].

The results characterized the leaves of *P. alkekengi* as good sources for obtaining aromatic products for cosmetic purposes, despite the significant difference in the concrete-yielding potential of the studied phenotypes. The

registered concrete yields were sufficiently high (1.02%, PA-SB and 4.81%, PA-NB), approximating those from other Solanaceae species currently being used as plant materials for obtaining similar concentrated aromatic products [22,33]. The different concrete yields could be reasonably related to the impact of environmental factors, as the soil and climatic conditions of the regions of plant populations are considerably different [34]; some genetic differences might also be involved, although genotype characterization was not regarded in this study. Both leaf concretes were undistinguishable in terms of visual and olfactory assessment: semi-solid waxy masses with yellow-orange color and specific odor. The color and the appearance of the concretes correlated well with the chemical composition of the extracts (as presented below); the color was due to the extraction of natural pigments, and the semi-solid waxy texture was due to the presence of aliphatic hydrocarbons, with equal share in the two extracts.

The presence of various aroma active volatile classes identified in the study spoke in favor of the potential of *P. alkekengi* leaf extracts for cosmetic and perfumery purposes [28,33]. As seen from the data (Table 5 and Figure 3), the aroma-active terpenoid derivatives (in particular, diterpenes and oxygenated sesquiterpenes) and oxygenated aliphatic derivatives (alcohols, acids, and esters) were the major chemical classes in both concretes. Several individual constituents with established aroma-contributing properties were found in significant concentration in those classes; for example, farnesyl acetate (with floral type odor), farnesyl acetone (with fruity, winey, creamy odor), phytol acetate (with waxy, fruity, balsamic odor), methyl farnesoate, 3α-hydroxy-manool, 3α-14,15-dihydromanool oxide, 3α-acetoxy-manool, farnesoic acid, myristic acid, hexadecanol, methyl hexadecanoate, methyl octadecanoate, and others; a number of minor components with known aroma-active potential were also present [35]. In addition, the leaf concretes did not contain fragrance substances referenced as human allergens [28,36], with the single exception of farnesol identified as a minor component (0.67%) in PA-SB concrete. Considering the safe future cosmetic use of *P. alkekengi* leaf concretes, attention should be paid to the presence of phthalates in the extract from PA-NB leaves (isobutyl phthalate, 2.32% and monoethylhexyl phthalate, 0.61%), as they are specified in the list of banned ingredients of cosmetic products [36]. The identified phthalate esters in the concretes might be contaminants but also natural plant constituents, as previously reported [37]. Phthalates have been found as naturally occurring constituents in different edible and essential oils, and a number of edible plants; phthalate contamination in plants has been reported to occur through different transfer mechanisms, most probably through migration and accumulation from the surrounding environment into the roots and the aerial parts [38].

Finally, it should be outlined that the ecological impact of medicinal plants collection and processing is an important aspect in the general discussion about the prospective use of medicinal plants as sources of bioactive compounds. World Health Organization reports that various plant fractions and their dynamic constituents are utilized as traditional medicines by 80% of the world population. Medicinal plants are used for the treatment of different infections and received worldwide attention. As a medicinal plant within the scope of the national legislation [8,39–42], *P. alkekengi* is not under a special regime of protection and use, and there are reasonable grounds to suggest that the possible utilization of its aerial parts (e.g. for pharmaceutical and perfumery products) would not be problematic in terms of disturbances of the populations and species conservation [8,43,44]. Moreover, the plant is vigorous, highly adaptive, rapidly multiplying, invasive in some environments, disease resistant, and with a good cultivation potential [2,5,6,31,45]. Thus, in a broader sense, the natural occurrence and the targeted cultivation of *P. alkekengi* could provide sufficient availability of plant biomass; moreover, such considerations are in line with the contemporary requirement for the sustainable utilization of the natural Bulgarian medicinal flora [44].

Therefore, the results from the non-targeted GC-MS analysis, as well as those from the rest of the phytochemical assays in this study, could be considered confirmative for the potential of *P. alkekengi* leaves and stems for obtaining functional derivatives [22,27,46,47]. Similar practices have already been established in the utilization of various plant organs and wastes, including a number of medicinal and cultivated plants from the Solanaceae family, such as *Solanum nigrum* L., *Solanum dulcamara* L., *Datura stramonium* L., and *Nicotiana tabacum* L. [15,48–51].

4 Conclusions

The study revealed the distribution of selected phytochemical indices (e.g. protein and amino acids, cellulose, minerals) in two separate parts of *P. alkekengi* L. plants, the leaves and stems, which represent a waste material after fruit collection. The results from the phytochemical analysis supported the assumption that the regarded aerial parts had potential for utilization in different areas.

The leaves, in particular, were suitable for processing into aromatic products, e.g. leaf concretes, due to the high yield (1.0–4.8%) and the specific profile of GC-MS volatiles, including a number of aroma-active compounds (such as terpenoids, oxygenated aliphatics, and others). The direct comparison between two different phenotypes in the study also revealed certain phenotype-related differences in the chemical indicators of *P. alkekengi* leaves and stems, thus suggesting that plant origin might be a significant factor in the complex phytochemical evaluation of the species, although further research is definitely needed. The data aquired by the study also supplement the knowledge about the medicinal plants in Bulgaria as resources of functional constituents with biological activity, and they might be useful in expanding the scope of available natural products for phytopharmacy and cosmetics.

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References

[1] TeBeest M, Van Den Berg R, Brandenburg W. A taxonomic analysis of the species of *Physalis* L. (Solanaceae) based on morphological characters. In: Sivadasan M, Mathew P, editors. Biodiversity, taxonomy and conservation of flowering plants. Calicut, Kerala, India: Mentor Books; 1999. p. 85–97.

[2] Li A, Chen B, Li G, Zhou M, Li Y, Ren D, et al. *Physalis alkekengi* L. var. *Franchetii* (Mast.) Makino: An ethnomedical, phytochemical and pharmacological review. J Ethnopharmacol. 2018;210:260–74. doi: 10.1016/j.jep.2017.08.022.

[3] Shah P, Bora K. Phytochemical and therapeutic potential of *Physalis* species: A review. IOSR J Pharm Biol Sci. 2019;14(4):34–51. doi: 10.9790/3008-1404033451.

[4] Mazova N, Popova V, Stoyanova A. Phytochemical composition and biological activity of *Physalis* spp.: A mini-review. Food Sci Appl Biotechnol. 2020;3(1):56–70. doi: 10.30721/fsab2020.v3.i1.80.

[5] Popa-Mitroi D, Popa-Mitroi G, Nicu C, Manda M. Study on behavior of *Physalis alkekengi* L. species in spontaneous flora and culture in order to evaluate its decorative quality. South West J Hortic Biol Env. 2012;3(2):185–202.

[6] Vogl-Lukasser B, Vogl C, Gütler M, Heckler S. Plant species with spontaneous reproduction in homegardens in Eastern Tyrol (Austria): Perception and management by women farmers. Ethnobot Res Appl. 2010;8:11–5.

[7] Delipavlov D, Cheshmedzhiev I. Key to the plants in Bulgaria. Plovdiv, Bulgaria: Academic Publishing House of the Agricultural University; 2003 (in Bulgarian).

[8] Parliament of the Republic of Bulgaria. Medicinal plants Act of the 38th Parliament of the Republic of Bulgaria of 23 March 2000. State Gaz. 2000:29;9–30 (in Bulgarian).

[9] Shokooh A, Badi H, Abdossi V, Mehrafarin A. Overview on the agronomic, phytochemical and therapeutic traits of bladder cherry (*Physalis alkekengi* L.). J Med Plants. 2020;18(72):1–13. doi: 10.29252/jmp.4.72.S12.1.

[10] Ali M, Ullah H, Bari WU, Ul Islam N, Zahoor M, Ullah R, et al. Phytochemical isolation and biological screening of Cotoneaster microphyllus. Int J Food Prop. 2021;24(1):1318–34.

[11] Zaman S, Zahoor M, Shah SW, Ullah Z, Ullah R, Alotaibi A. Pharmacognostic evaluation of *Artemisia maritima* L. a highly medicinal specie of genus *Artemisia*. Saudi J Biol Sci. 2022;29(10):103419.

[12] Liu XG, Jiang FY, Gao PY, Jin M, Yang D, Nian ZF, et al. Optimization of extraction conditions for flavonoids of *Physalis alkekengi* var. *franchetii* stems by response surface methodology and inhibition of acetylcholinesterase activity. J Mex Chem Soc. 2015;59(1):59–66. doi: 10.29356/jmcs.v59i1.16.

[13] Zhang W, Tong W. Chemical constituents and biological activities of plants from the genus *Physalis*. Chem Biodivers. 2016;13(1):48–65. doi: 10.1002/cbdv.201400435.

[14] Ben Ayed R, Moreau F, Ben Hlima H, Rebai A, Ercisli S, Kadoo N, et al. SNP discovery and structural insights into OeFAD2 unravelling high oleic/linoleic ratio in olive oil. Comput Struct Biotechnol J. 2022;20:1229–43. doi: 10.1016/J.CSBJ.2022.02.028.

[15] Assouguem A, Kara M, Ramzi A, Annemer S, Kowalczyk A, Ali EA, et al. Evaluation of the effect of four bioactive compounds in combination with chemical product against two spider mites tetranychus urticae and eutetranychus orientalis (Acari: Tetranychidae). Evidence-Based Complement. Altern. Med; 2022.

[16] Zhang Q, Hu XF, Xin MM, Liu HB, Sun LJ, Morris-Natschke S, et al. Antidiabetic potential of the ethyl acetate extract of

Physalis alkekengi and chemical constituents identified by HPLC-ESI-QTOF-MS. J Ethnopharmacol. 2018;225:202–10. doi: 10.1016/j.jep.2018.07.007.
[17] Cosmetic Ingredient Database (CosIng) of the European Commission; 2022. [cited 2022 March 18]. https://ec.europa.eu/growth/sectors/cosmetics_en.
[18] Namjoyan F, Jahangiri A, Azemi M, Arkian E, Mousavi H. Inhibitory effects of *Physalis alkekengi* L., *Alcea rosea* L., *Bunium persicum* B. Fedtsch. and *Marrubium vulgare* L. on mushroom tyrosinase. Jundishapur J Nat Pharm Prod. 2015;10(1):e23356. doi: 10.17795/jjnpp-23356.
[19] Association of Official Analytical Chemists (AOAC). Official methods of analysis. 20th edn. Geithersburg, MD, USA: AOAC International; 2016.
[20] Brendel O, Iannetta P, Stewart D. A rapid and simple method to isolate pure alpha-cellulose. Phytochem Anal. 2000;11(1):7–10.
[21] Popova V, Petkova Z, Ivanova T, Stoyanova M, Panayotov N, Stoyanova A. Determination of the chemical composition of seeds, peels, and seedcakes from two genotypes of Cape gooseberry (*Physalis peruviana* L.). Turk J Agric For. 2020;44(6):642–50. doi: 10.3906/tar-2003-66.
[22] Başer K, Buchbauer G. Handbook of essential oils: Science, technology, and applications. 1st edn. Boca Raton, FL, USA: CRC Press; 2010.
[23] Adams R. Identification of essential oil components by gas chromatography/mass spectrometry. 4th edn. Carol Stream, IL, USA: Allured Publishing; 2007.
[24] Shen V, Siderius D, Krekelberg W, Hatch H, editors. NIST standard reference simulation website, NIST standard reference database 173. Gaithersburg, MD, USA: National Institute of Standards and Technology. doi: 10.18434/T4M88Q.
[25] Binici H, Sat I, Aoudeh A. The effect of different drying methods on nutritional composition and antioxidant activity of purslane (*Portulaca oleracea*). Turk J Agric For. 2021;45(5):680–89. doi: 10.3906/tar-2012-60.
[26] Zia-Ul-Haq M, Ahmad S, Qayum M, Ercisli S. Compositional studies and antioxidant potential of *Albizia lebbeck* (L.) Benth. Pods and seeds. Turk J Biol. 2013;37(1):25–32. doi: 10.3906/biy-1204-38.
[27] Daley D. Plant crude drugs. In: Badal S, Delgoda R, editors. Pharmacognosy: Fundamentals, applications and strategies. London, UK: Academic Press; 2017. p. 81–9.
[28] Sarkic A, Stappen I. Essential oils and their single compounds in cosmetics – a critical review. Cosmetics. 2018;5:1–21. doi: 10.3390/cosmetics5010011.
[29] Helvacı S, Kökdil G, Kawai M, Duran N, Duran G, Güvenç A. Antimicrobial activity of the extracts and physalin D from *Physalis alkekengi* and evaluation of antioxidant potential of physalin D. Pharm Biol. 2010;48(2):142–50. doi: 10.3109/13880200903062606.
[30] Chen CY, Peng WH, Tsai KD, Hsu SL. Luteolin suppresses inflammation-associated gene expression by blocking NF-κB and AP-1 activation pathway in mouse alveolar macrophages. Life Sci. 2007;81(23–24):1602–14. doi: 10.1016/j.lfs.2007.09.028.
[31] Chen LX, Xia GY, Liu QY, Xie YY, Qiu F. Chemical constituents from the calyces of *Physalis alkekengi* var. *franchetii*. Biochem Syst Ecol. 2014;54:31–5. doi: 10.1016/j.bse.2013.12.030.
[32] Sharma N, Bano A, Dhaliwal H, Sharma V. Perspectives and possibilities of Indian species of genus *Physalis* (L.) – a comprehensive review. Eur J Pharm Med Res. 2015;2(2):326–53.
[33] Bauer K, Garbe D, Surburg H. Common fragrance and flavor materials. preparation, properties and uses. 4th edn. Weinheim, NY, USA: Wiley-VCH; 2001.
[34] Popova Z, Ivanova M, Pereira L, Alexandrov V, Kercheva M, Doneva K, et al. Droughts and climate change in Bulgaria: assessing maize crop risk and irrigation requirements in relation to soil and climate region. Bulg J Agric Sci. 2015;21(1):35–53.
[35] The Good Scents Company (TGSC); 2022. [cited 2022 May 10]. http://www.thegoodscentscompany.com/categories.html.
[36] European Commission. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. Off J Eur Union. 2009:342;59.
[37] Getahun T, Sharma V, Gupta N. Chemical composition and biological activity of essential oils from *Aloe debrana* roots. J Essent Oil Bear Plants. 2020;23(3):493–502. doi: 10.1080/0972060X.2020.1788996.
[38] Giuliani A, Zuccarini M, Cichelli A, Khan H, Reale M. Critical review on the presence of phthalates in food and evidence of their biological impact. Int J Env Res Public Health. 2020;17:5655. doi: 10.3390/ijerph17165655.
[39] Alqahtani AS, Ullah R, Shahat AA. Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia. Evidence-Based Complementary and Alternative Medicine; 2022. p. 2022.
[40] Shahat AA, Ullah R, Alqahtani AS, Alsaid MS, Husseiny HA, Al Meanazel OT. Hepatoprotective effect of *Eriobotrya japonica* leaf extract and its various fractions against carbon tetra chloride induced hepatotoxicity in rats. Evidence-Based Complemen Altern Med. 2018;2018:Article ID 3782768, 8 pages.
[41] Ullah R, Alqahtani AS. GC-MS analysis, heavy metals, biological, and toxicological evaluation of reseda muricata and marrubium vulgare methanol extracts. Evidence-Based Complemen Altern Med. 2022;2022.
[42] Ullah R, Alqahtani AS, Noman OM, Alqahtani AM, Ibenmoussa S, Bourhia M. A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. Saudi J Biol Sci. 2020;27(10):2706–18.
[43] Evstatieva L, Hardalov R, Stoyanova K. Medicinal plants in Bulgaria: diversity, legislation, conservation and trade. Phytol Balc. 2007;3(3):415–27.
[44] Tashev A, Dimitrova V. Medicinal plants of Bulgaria. Curr Perspect Med Aroma Plants. 2019;2(1):29–39.
[45] Wen X, Erşan S, Li M, Wang K, Steingass C, Schweiggert R, et al. Physicochemical characteristics and phytochemical profiles of yellow and red *Physalis* (*Physalis alkekengi* L. and *P. pubescens* L.) fruits cultivated in China. Int Food Res J. 2019;120:389–98. doi: 10.1016/j.foodres.2019.03.002.
[46] Sengul M, Ercisli S, Yildiz H, Gungor N, Kavaz A, Cetin B. Antioxidant, antimicrobial activity and total phenolic content within the aerial parts of *Artemisia absinthum*, *Artemisia santonicum* and *Saponaria officinalis*. Iran J Pharm Res. 2011;10(1):49–55.
[47] Ergun Z. Seed oil content and fatty acid profiles of endemic *Phoenix theophrasti* Greuter, *Phoenix roebelenii* O'Brien, *Phoenix caneriensis* Hort. Ex Chabaud, and *Phoenix dactylifera* L. grown in the same locality in Turkey. Turk J Agric For. 2021;45(5):557–64. doi: 10.3906/tar-2105-34.

[48] Jassbi A, Zare S, Asadollahi M, Schuman M. Ecological roles and biological activities of specialized metabolites from the genus *Nicotiana*. Chem Rev. 2017;117:12227–80. doi: 10.1021/acs.chemrev.7b00001.
[49] Campisi A, Acquaviva R, Raciti G, Duro A, Rizzo M, Santagati N. Antioxidant activities of *Solanum nigrum* L. leaf extracts determined in *in vitro* cellular models. Foods. 2019;8(2):63. doi: 10.3390/foods8020063.
[50] Assouguem A, Kara M, Mechchate H, Al-Mekhlafi FA, Nasr F, et al. Evaluation of the impact of different management methods on *Tetranychus urticae* (Acari: Tetranychidae) and their predators in citrus orchards. Plants. 2022;11:623. doi: 10.3390/PLANTS11050623.
[51] Banožić M, Babić J, Jokić S. Recent advances in extraction of bioactive compounds from tobacco industrial waste – A review. Ind Crop Prod. 2020;144:112009. doi: 10.1016/j.indcrop.2019.112009.