Phytochemical composition of leaves and stems of *Solanum nigrum* L. and *Solanum dulcamara* L. (Solanaceae) from Bulgaria

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Abstract. The objective of this study was the determination of certain phytochemical indices (cellulose, protein, amino acids, ash, minerals) of the leaves and stems of two *Solanum* species, *S. dulcamara* L. and *S. nigrum* L., collected from their natural habitats in Bulgaria. Both species, naturally occurring in many regions of Bulgaria, are important plants in folk medicine and fall under the regulations of the Medicinal Plant Act. The analysis of cellulose, protein and ash content in the dried leaves and stems revealed differences on a plant part basis. Stems contained higher amounts of cellulose (*S. dulcamara*, 29.4%; *S. nigrum*, 39.2%) than the leaves (14.9% in both species). The stems of *S. nigrum* contained nearly twice as much protein than the leaves (15.3% vs 8.1%); the ratio was reversed in *S. dulcamara* (12.3% vs 21.3%). The dominant macroelements in *S. dulcamara* were K (25925 and 14514 mg.kg⁻¹, respectively in the leaves and stems), Ca (3588 and 326 mg.kg⁻¹) and Mg (2561 and 308 mg.kg⁻¹). In *S. nigrum*, the same macrominerals showed relatively less variation on a plant part basis. The dominant microminerals were Fe, Zn and Mn; reasonably, higher concentrations were found in the leaves. The dominant amino acids in *S. dulcamara* leaves were phenylalanine, glutamine, asparagine, tyrosine, and serine (2.2-2.5 mg.g⁻¹); in the stems - proline (7.3 mg.g⁻¹), alanine (7.2 mg.g⁻¹), asparagine (5.0 mg.g⁻¹), and lysine (4.6 mg.g⁻¹). The dominant amino acid in *S. nigrum* leaves and stems was asparagine, 5.0 mg.g⁻¹ and 20.6 mg.g⁻¹, respectively. The results supported the assumption that the studied aerial parts contained valuable phytochemicals, which could provide grounds for their practical use in specific areas, such as human and animal nutrition, cosmetics, phytopharmacy or others.

1. Introduction
Genus *Solanum* – the genus that typifies the family Solanaceae – includes over 1500 species, thus being one of the world’s largest genera in terms of species number [1-3]. The diverse *Solanum* species represent some of the most important food crops, cultivated globally or regionally (tomato, *S. lycopersicum* L.; potato, *S. tuberosum* L.; eggplant, *S. melongena* L.; the bitter tomato, *S. aethiopicum* L.; pepino, *S. muricatum* Aiton; tamarillo, *S. betacum* Cav., and others), as well as many wild growing or cultivated ornamental plants (*S. seefordianum* L., *S. laxum* Spreng., *S. pseudocapsicum* L., and others) [2]. Most of the species have been used in folk and contemporary medicine, due to the presence of various bioactive phytochemicals, including alkaloids, glycosides, saponins, flavonoids, sterols, and
many others [3]. Two Solanum species, in particular S. nigrum L. (known as black nightshade or blackberry nightshade) and S. dulcamara L. (known as bittersweet nightshade, European nightshade, climbing nightshade, or scarlet berry) are the focus of this research. Both species, native to Europe and Asia and currently naturalized worldwide, are naturally occurring plants in many regions of Bulgaria [4]; they are recognized as important plants in Bulgarian and many other traditional medicines since ancient times. Both species are listed in the Medicinal Plants Act, regulating the sustainable use of the medicinal plant resources on the territory of Bulgaria [5].

S. dulcamara is a perennial climbing vine or semi-woody shrub, reaching a height of 1-2 m or more. The leaves are dark green, alternate, petiolate, arrowhead shaped, lobed at the base; different leaf forms may be found on the same plant. The flowers are in loose clusters of 10-20, on short stems, star-shaped, with five purple petals and stamens fusing in a yellow cone. The fruit is an oval or egg-shaped berry with some 30 yellow flattened seeds, about 10 mm long, ripening from green to bright red. The plant is native to Bulgaria, growing in the wild, at an altitude up to 1000 m, found in damp shady habitats, leafy shrubs, forest edges, along creeks. The flowering period is June-August [4]. The chemical composition of various plant parts, fruit, leaves, stems, and roots, has been discussed in several studies, especially in terms of alkaloid identification, bioactivity and isolation [6, 7]. All parts of the plant contain various glycoalkaloids, solanine, solasodine, solamarine, solamargine, tomatidine, and others. Pharmacological studies have revealed promising biological activities of many of these alkaloids both in vitro and in vivo; they have been used as lead molecules in novel drug synthesis [3]. The traditional medicine use of S. dulcamara is related mostly to its anti-inflammatory and diaphoretic action; therefore, it is a remedy in the treatment of colds, rheumatism, skin inflammations, dermatitis, chronic eczema, and others. The topical use of S. dulcamara leaf and stem infusions or decoctions for the treatment of skin conditions, such as eczema, acne and inflammations, is officially recognized [8]; Solanum Dulcamara Stem Extract (CAS No 84696-50-4) is a cosmetic ingredient with skin conditioning properties [9]; saponins extracted from S. dulcamara leaves are reported to reduce the skin irritation potential of bodywash gels by about 50% [10]. The consumption of fresh fruit or the internal application of S. dulcamara extracts, at higher doses, may be poisonous to people, with solanine intoxication symptoms including nausea, diarrhoea, vomiting, stomach cramps, headache, hallucinations, and others.

S. nigrum is one of the most variable species in the genus, with morphologically distinct genotypes. Typically, it is an annual (rarely biennial) herbaceous plant, 0.2 - 1.0 m high, reproducing by seeds. The leaves are alternate, petiolate, 2-8 cm long, ovate to heart-shaped, with wavy or large-toothed edges, rarely smooth-edged, dark green, smooth or finely hairy. The flowers are small, white (rarely orange-red), star-shaped, with five petals and prominent yellow anthers. The flowers are carried in loose umbels, usually 5-10 flowers. The berries are globose, 5-13 mm wide, matt black when ripe, containing numerous tiny flattened seeds. The flowering period (in Bulgaria) is June – August. The plant grows as weed in abandoned, weeded, uncultivated areas, along villages and creeks, and is found throughout the country up to 1200 m altitude [4]. Several bioactive phytochemicals, such as alkaloids (solaceine, solanidine, solasonine, solamargine, solanine, and others), phenolics, saponins, polysaccharides, glycoproteins, steroidal derivatives, and others have been identified in S. nigrum aerial parts [3, 11-13], as well as essential oil (in whole berries, seeds and leaves) and glyceride oil (seeds) [14, 15]. Although the plant is generally considered toxic to humans and animals, ripe fruit and cooked leaves of S. nigrum are used as food in some regions of the world and edible varieties of the plant are cultivated. The nutritional potential of S. nigrum fruit and leaves has been evaluated; both aerial parts have been reported to contain protein, carbohydrates, minerals, vitamins, and other phytoneutrients [16-18]. The plant is widely used in many traditional medicines, as analgesic, sedative, antispasmodic, diuretic, antiptyretic; different extracts have shown anti-inflammatory, anticancer, antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and other activities [11].

As a general observation, the studies on S. dulcamara and S. nigrum composition, focused on aspects other than their alkaloid content are limited, especially those in Bulgaria. We hypothesized that the investigation of the phytochemical composition of plants growing in Bulgaria (leaves and stems) would reveal some new aspects of species’ phytochemistry and potential of use. Therefore, the objective of
this study was the determination of certain indices of the phytochemical composition (cellulose, protein, amino acids, ash, minerals) of the leaves and stems of two Solanum species, *S. dulcamara* and *S. nigrum*, collected from their natural habitats in Bulgaria. To the best of our knowledge, there are no previous reports on this matter; therefore, the outcomes from the study might contribute to a deeper understanding of *S. dulcamara* and *S. nigrum* potential for use in phytopharmacy, cosmetics, foods, feeds, and other areas.

2. Materials and methods

2.1. Plant material

The plants of *S. dulcamara* were collected in the region of Plovdiv, Central South Bulgaria (N 42°08’39.5” E 24°42’23.0”) and those of *S. nigrum* - near Kapitan Dimitrievo village, Peshtera municipality (N 42°07’26” E 24°18’52”), both in July 2020 (Figure 1 and Figure 2). The identification of the plants were confirmed at the Botany Department of Plovdiv University “Paisii Hilendarski”. Leaves were carefully detached from the stems and both plant parts were air-dried in the shade.

![Figure 1. Leaves and upper stems of *Solanum dulcamara* L.](image1)

![Figure 2. Leaves and upper stems of *Solanum nigrum* L.](image2)

2.2. Chemical analyses

The moisture content of the samples was determined by drying to constant weight at 105°C; all results were given on a dry weight (DW) basis.

The ash content was determined by mineralization of the samples, performed for 5 hours at 550°C in a muffle furnace [19].

Cellulose content was determined by the method described in [20], slightly modified. In brief, cellulose and hemicellulose were hydrolyzed with 16.5 mL 80% CH₃COOH and 1.5 mL concentrated HNO₃ for 1.5 h, the solid residue filtrated from the suspension was dried at 105°C for 24 h and weighed.

The determination of the total protein content was according to the AOAC Method 976.06 [21], using an UDK 152 System (Velp Scientifica, Italy). The derivatization of the free amino acids from protein hydrolysis was carried out with the AccQ-Fluor kit (WATO52880, Waters Corporation, USA). The
separation of AccQ-Fluor amino acid derivatives was performed on an ELITE LaChrome HPLC chromatograph (Hitachi) using a diode array detector (DAD) and a reverse phase C18 AccQ-Tag column (3.9 mm × 150 mm), operated at a temperature of 37°C; the mobile phases were WATO52890 buffer (Waters Corporation, USA) and 60% acetonitrile; the detection wavelength was 254 nm.

For the determination of mineral elements the air-dried samples of S. dulcamara and S. nigrum leaves and stems were mineralized at 450°C; the residue was dissolved in concentrated HCl, evaporated to dryness, and the remainder was subsequently dissolved in 0.1 mol.L⁻¹ HNO₃ solution. Mineral contents were determined on a Perkin Elmer/HGA 500 (Norwalk, USA) atomic absorption spectrophotometer (AAS); the instrumental parameters for the flame AAS were: Na, 589.6 nm; K, 766.5 nm; Mg, 285.2 nm; Ca, 317.0 nm; Zn, 213.9 nm; Cu, 324.7 nm; Fe, 238.3 nm; and Mn, 257.6 nm. Identification of metals was carried out by comparison to a standard solution of metal salts, and metal concentrations were calculated from a calibration curve, built by using a standard 1 μg.mL⁻¹ solution.

All analyses were performed in a threefold repetition and data were presented as mean value ± standard deviation.

3. Results and discussion
The results from the analysis of cellulose, protein and ash content in the leaves and stems of S. dulcamara and S. nigrum, as well as the initial moisture content are presented in Table 1.

| Index (%) | S. dulcamara | S. nigrum |
|-----------|--------------|-----------|
| Moisture  | 6.28±0.05⁴  | 7.30±0.06  |
| Ash       | 12.58±0.11   | 7.50±0.06  |
| Protein   | 21.32±0.18   | 12.36±0.11 |
| Cellulose | 14.93±0.13   | 29.42±0.21 |

⁴ All data are presented as mean value ± standard deviation (n=3).

As seen from Table 1, the results revealed differences in the chemical indices of both species on a plant part basis. As anticipated, stems contained significantly higher amounts (2-3 times) of cellulose than the leaves, while there was no uniform distribution of protein and ash levels between the two plant parts in the species. Interestingly, the stems of S. nigrum contained about twice as much protein than the leaves (15.3% vs 8.1%), while the ratio was reversed in S. dulcamara (12.3% vs 21.3%). In general, our results were in compliance with previous findings for S. nigrum leaf proximates; for example, 10.2 - 15.3% ash; 23.8 - 24.9% crude protein, and 6.8 - 9.2% crude fiber [16, 18, 22].

The results from the identification of mineral elements in the leaves and stems of S. dulcamara and S. nigrum are presented in Table 2.
Table 2. Mineral elements in *S. dulcamara* and *S. nigrum* leaves and stems.

| Mineral (mg kg\(^{-1}\)) | *S. dulcamara* | *S. nigrum* |
|---------------------------|----------------|-------------|
|                           | Leaves         | Stems       | Leaves         | Stems       |
| K                         | 25925±138.23\(^a\) | 14514±89.62 | 25252±114.25   | 17176±98.36 |
| Ca                        | 3588±21.55     | 326±1.78    | 2446±13.21     | 2739±14.23  |
| Mg                        | 2561±13.12     | 308±1.23    | 4980±21.18     | 1662±9.47   |
| Na                        | 21.15±0.11     | 29.07±0.13  | 170.25±0.98    | 362.18±1.68 |
| Cu                        | 9.98±0.06      | 5.66±0.03   | 8.80±0.04      | 2.97±0.01   |
| Fe                        | 55.01±0.03     | 23.80±0.11  | 301.71±1.74    | 163.94±0.78 |
| Zn                        | 20.86±0.12     | 6.32±0.01   | 51.09±0.39     | 21.76±0.12  |
| Mn                        | 19.91±0.08     | 10.47±0.04  | 98.00±0.64     | 19.68±0.11  |
| Pb                        | < 0.1\(^b\)    | < 0.1       | 2.21±0.01      | 2.23±0.01   |
| Cd                        | < 0.01\(^c\)   | < 0.01      | 2.35±0.01      | 0.79±0.00   |
| Cr                        | < 0.1          | < 0.1       | 3.63±0.01      | 3.68±0.01   |

\(^a\) All data are presented as mean value ± standard deviation (n=3).

\(^b\) Not quantified.

\(^c\) Not detected.

The data in Table 2 suggested that *S. dulcamara* and *S. nigrum* leaves and stems contained various macro- and microminerals, with some quantitative differences between the two plant parts studied.

The dominant macrominerals in the leaves and stems of *S. dulcamara* were K (25925 and 14514 mg kg\(^{-1}\), respectively), Ca (3588 and 326 mg kg\(^{-1}\)) and Mg (2561 and 308 mg kg\(^{-1}\)); they were found in significantly higher concentrations in the leaves opposed to the stems, especially in the case of Ca and Mg. In *S. nigrum*, the dominant macrominerals, K, Ca and Mg, showed relatively less variation on a plant part basis; moreover, Ca content was practically identical in the leaves and stems (2446 and 2739 mg kg\(^{-1}\), respectively). The opposite distribution, however, was found for Na, with higher stem concentrations in both species, and especially in *S. nigrum*. The profile of the dietary macroelements in *S. nigrum* leaves agreed well with previous data, although numerical differences were observed, explicable by leaf origin [16-18, 22].

Similarly, some variations in the group of microelements between the two plant parts in each of the species were observed. The dominant microminerals in all samples were Fe, Zn and Mn; reasonably, higher levels were found in the leaves. An interesting observation was the high Fe content in *S. nigrum* leaves and stems (301.7 and 163.9 mg kg\(^{-1}\), respectively), which supported previous findings about the dominant share of Fe in the trace elements of *S. nigrum* leaves, reporting values of 852.67 - 2251.33 mg kg\(^{-1}\) (variation on a seasonal basis) [23] and 130.1 mg kg\(^{-1}\) [17, 22]. The presence of heavy metals, Pb, Cd and Cr, identified in *S. dulcamara* leaves and stems (varying in the range from 0.8 to 3.7 mg kg\(^{-1}\)) was probably related to the environmental factors of plant habitat, via the soil-to-plant uptake route, as already reported [18, 23]. The ratios Na/K and Zn/Cu, important in the prevention of high blood pressure and cardiac abnormalities, were also favorable and substantially lower than the recommended threshold values, Na/K<0.6 and Zn/Cu<16 [17, 18]. The obtained data supported the assumption that the leaves and stems of *S. dulcamara* and *S. nigrum* were rich sources of dietary macro and microminerals.

Regarding the relatively high protein content cited above and their use prospective, the two plant parts of *S. dulcamara* and *S. nigrum* were also analyzed in terms of their amino acid composition and the results are given in Table 3.
Table 3. Amino acids in *S. dulcamara* and *S. nigrum* leaves and stems.

| Amino acid     | *S. dulcamara* | *S. nigrum* |
|----------------|----------------|-------------|
|                | Leaves        | Stems       | Leaves    | Stems |
| Asparagine     | 2.41±0.02a    | 4.99±0.03   | 5.03±0.04 | 20.58±0.14 |
| Serine         | 2.20±0.02     | 1.71±0.01   | 0.07±0.00 | 1.70±0.01  |
| Glutamine      | 2.46±0.02     | 2.36±0.02   | 5.75±0.04 | 1.96±0.01  |
| Glycine        | 0.02±0.00     | 0.01±0.00   | nd        | 0.39±0.00  |
| Histidine      | 0.08±0.00     | 0.05±0.00   | 0.02±0.00 | 1.99±0.02  |
| Arginine       | 0.84±0.01     | 1.51±0.01   | 2.55±0.02 | 1.53±0.01  |
| Threonine      | 1.08±0.01     | 3.97±0.02   | 2.27±0.02 | 1.72±0.01  |
| Alanine        | 0.67±0.00     | 7.23±0.05   | 0.15±0.01 | 0.27±0.00  |
| Proline        | 0.90±0.01     | 7.35±0.05   | 2.51±0.02 | 3.72±0.02  |
| Cysteine       | ndb           | nd          | nd        | nd        |
| Tyrosine       | 2.32±0.02     | 2.52±0.02   | 2.48±0.02 | 0.46±0.00  |
| Valine         | 0.60±0.00     | 2.63±0.02   | 1.72±0.01 | 1.48±0.01  |
| Methionine     | 0.33±0.00     | 0.29±0.02   | 0.28±0.00 | 0.07±0.00  |
| Lysine         | 0.42±0.00     | 4.57±0.03   | 2.37±0.01 | 1.99±0.01  |
| Isoleucine     | 0.61±0.00     | 2.58±0.02   | 2.50±0.02 | 1.93±0.01  |
| Leucine        | 0.11±0.00     | 0.50±0.00   | 0.38±0.00 | 0.27±0.00  |
| Phenylalanine  | 2.55±0.02     | 3.35±0.03   | 3.22±0.02 | 1.67±0.01  |

a All data are presented as mean value ± standard deviation (n=3).
b Not detected.

As seen from Table 3, the dominant amino acids in *S. dulcamara* leaves were phenylalanine, glutamine, asparagine, tyrosine, and serine, in identical concentrations (2.2 - 2.5 mg.g⁻¹). In turn, the stems contained higher amounts of proline (7.3 mg.g⁻¹), alanine (7.2 mg.g⁻¹), asparagine (5.0 mg.g⁻¹), and lysine (4.6 mg.g⁻¹), thus suggesting different nutritional value and use potential of the two plant parts.

The dominant amino acid in *S. nigrum* was asparagine, 5.0 mg.g⁻¹ and 20.6 mg.g⁻¹, respectively in leaves and stems; glutamine was the other major amino acid in the leaves, 5.8 mg.g⁻¹. Cysteine was not identified in either of the species. Our results differed numerically from the data about some of the essential and functional amino acids in *S. nigrum* leaves from Nigeria, explicable by different plant origin [18].

4. Conclusions

The results from the analysis of the leaves and stems of two *Solanum* species naturally occurring in Bulgaria, *S. dulcamara* and *S. nigrum*, revealed some new aspects in their phytochemical composition. For the first time data about the protein, cellulose, mineral, and amino acid composition of the respective plants in Bulgaria were provided, differentiated on a plant part basis (leaves and stems). Those first results supported the assumption that the studied aerial parts of the two species contained valuable phytochemicals, which could provide grounds for their practical use in specific areas, such as human and animal nutrition, cosmetics, phytopharmacy or others. Of course, further research on *S. dulcamara* and *S. nigrum* chemical composition and biological activities is a must, in order to reveal deeper their use potential, which is set as the objective of our future work.
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