



Polyphenols and triterpenes in leaves and extracts from three *Nicotiana* species

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ABSTRACT

Genus *Nicotiana* (Solanaceae) includes 76 species, among which only *Nicotiana tabacum* L. (common tobacco) is recognized as economically important. *Nicotiana* species synthesize various secondary metabolites with biological or ecological activities, but many of them still have not been sufficiently studied. The objective of current study was to determine the content of selected groups of metabolites (e.g., phenolic acids, flavonoids, and triterpenes) in the leaves and extracts of three *Nicotiana* species (*Nicotiana alata* Link and Otto, *Nicotiana rustica* L., and *N. tabacum* L.), grown side by side. The results from the high-performance liquid chromatography identification of polyphenols and triterpenes in leaves and extracts showed considerable variations between the species. All of them, however, were characterized as plant materials containing various specialized and bioactive metabolites, which substantiate their consideration as a valuable source for obtaining different phytoproducts. In terms of the studied groups of bioactive metabolites, the results from the study suggest that 50% and 70% ethanol are the most suitable solvent concentrations for the extraction of *N. alata*, *N. rustica*, and *N. tabacum* leaves. The high content of polyphenols and triterpenes in the extracts provides grounds for their use in various cosmetic and pharmaceutical products, which could be the subject of future research.

1. INTRODUCTION

Genus *Nicotiana* (Solanaceae) includes 76 naturally occurring species, many of which have centuries-old history of use by the indigenous people of the Americas and Australia - for medicinal, religious, ornamental, pesticide, and various other purposes [1-4]. At present, *Nicotiana* species are distributed worldwide, but the only economically important among them are *Nicotiana tabacum* L. and to a lesser degree *Nicotiana rustica* L., which are cultivated for tobacco products intended for smoking, chewing, snuffing, etc. In a recent review by Jassbi *et al.* [3], *Nicotiana* genus is evaluated as “one of the most comprehensively studied flowering plant genera, with numerous studies having produced a large body of literature on its evolution, cytology, taxonomy, and biogeography” and “perhaps the most extensively studied genus at the genetic, genomic, and phenotypic levels.” Species in the genus expose great morphological diversity, reasonably accompanied by a comprehensively documented chemical and functional diversity [2,3,5].

The sectional classification of the *Nicotiana* species regarded in the current study according to Knapp *et al.* [2] is as follows: *Nicotiana alata* Link and Otto belongs to section *Alatae* (chromosome number $n = 9$),

N. rustica L. - to section *Rusticae* ($n = 24$), and *N. tabacum* L. - to section *Nicotiana* ($n = 24$).

N. alata is a perennial herbaceous plant, reaching a height of up to 40–70 cm, which native geographical distribution is in Uruguay, Brazil, and Argentina [2]. Leaves are relatively small (typically <30 cm), spatulate, sessile, with winged petioles. One of the most distinctive features of the species (also referred to as “jasmine tobacco” or “flowering tobacco”) is its beautiful, abundant, and scented flowers, due to which *N. alata*, nowadays, is grown worldwide as an ornamental plant, found in many varieties and hybrids with varying floral characteristics [2,6]. The flowers of *N. alata* are relatively more studied than the leaves, and scientific evidence about the chemical constituents (besides nicotine) of the leaves is hardly available.

N. rustica is an annual herbaceous plant, which grows from 0.6 m to 1.5 m tall, with broad, ovate, and petiolate leaves [1,2]. The species is native to the Andean region and has been historically used as a sacred plant in religious ceremonies and as a medicine by the American Indians [4,7]. Nowadays, *N. rustica* is naturalized worldwide, being cultivated for smoking, chewing, or snuff tobacco products [4,8,9]. Unlike *N. alata*, *N. rustica* leaves carry high concentrations of nicotine and are considered useful for obtaining biopesticides [9-11].

N. tabacum L. (common tobacco, cultivated tobacco, or simply: Tobacco) is an herbaceous annual or perennial plant, which is one of the most commercially important technical crops around the world [3]. The species has been developed into numerous cultivars, varieties, and commercial types, representing an enormous diversity in plant

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habitus, leaf shape, size, yield, chemical, or smoking properties, etc. *N. tabacum* is one of the most thoroughly studied plant species, with >2500 metabolites identified in the leaves [3,5,12].

Although many *Nicotiana* species - inclusive of *N. alata*, *N. rustica*, and *N. tabacum* are considered as traditionally intended for smoking [4], they all are capable of intensive biosynthesis of secondary (or specialized) metabolites with various biological or ecological activities. The metabolites (including alkaloids, terpenoids, phenolics, flavonoids, isoprenoids, and many other classes of chemicals) isolated from leaves, flowers or other plant organs inspire innovative, much broader application by people of the species of *Nicotiana* genus [3,5,12].

Therefore, the objective of current study was to characterize selected groups of metabolites (phenolic acids [PAs], flavonoids, and triterpenes) in the leaves and ethanol extracts of three *Nicotiana* species (*N. alata*, *N. rustica*, and *N. tabacum*) grown side by side.

2. MATERIALS AND METHODS

2.1. Plant Material

The study was carried out with cured leaves of three *Nicotiana* species - *N. alata*, *N. rustica*, and *N. tabacum*. The latter of the species was represented by a local variety named "Plovdiv 7" belonging to the Basma variety group of Oriental (also known as aromatic or Turkish) tobacco. The species were grown side by side, in the region of Plovdiv, South Bulgaria, crop year 2017. Leaves were picked at maturity, sun-dried following the established technology, and stored until processing at a temperature of 5–8°C to avoid undesirable changes.

The moisture content of the plant materials was determined by drying to constant weight at a temperature of 105°C [13]. Before phytochemical analyses, the samples were dried at 40°C for 6 h, ground in a laboratory mill, and then finely powdered by a laboratory homogenizer.

2.2. Determination of Polyphenols

Triple extraction of 0.5 g finely powdered tobacco with 70% methanol (Sigma-Aldrich, St. Louis, MO, USA) was carried out, each at 70°C for 3 h in an ultrasonic bath. The combined extract was evaporated at 60°C on a rotary evaporator, and the residue was dissolved in methanol (Sigma-Aldrich, St. Louis, MO, USA). The obtained methanol solution was adjusted to 5 mL in a volumetric flask (IsoLab, Germany), and filtered before injection through a 0.45 µm syringe filter (Chromafix Xtra PTFE-45/25, Macherey-Nagel, Germany) [14].

2.2.1. High-performance liquid chromatography (HPLC) analysis of PAs

Analysis of PAs was carried out on a Waters 1525 Binary Pump HPLC system (Waters, Milford, MA, USA), equipped with Waters 2487 Dual λ Absorbance detector. The separation of PAs was on a SUPELCO Discovery HS C18 column (5 µm, 250 mm×4.6 mm), operated at a temperature of 26°C, at detection wavelengths of 280 nm and 320 nm, and with mobile phases 2% acetic acid, v/v (Solvent A) and 0.5% acetic acid: acetonitrile (1:1, v/v) (Solvent B). The elution gradient was according to Marchev *et al.* [15]: 0–30 min - Solvent B increased from 5% to 35% at a flow rate of 0.8 mL/min; 30–45 min - from 35% to 70% (0.4 mL/min); 45–50 min - from 70% to 80% (1.2 mL/min); 50–60 min - from 80% to 100% (1.2 mL/min); and 60–65 min - dropped down from 100% to 5% (at 0.8 mL/min), and then maintained to 70 min for column equilibration. The determination of PAs was done by building calibration curves, with rosmarinic, gallic, protocatechuic, salicylic, chlorogenic, vanillic, caffeic, syringic,

ferulic, sinapic, *p*-coumaric, and cinnamic acids used as standards (all purchased from Sigma-Aldrich, St. Louis, MO, USA).

2.2.2. HPLC analyses of flavonoids

Column and HPLC system were the same as in the PA analysis. The mobile phases in the gradient elution were 2% acetic acid, v/v (Solvent A) and methanol (Solvent B), and gradient setup was as follows: 0–10 min - Solvent B increased from 30% to 50% at a flow rate of 1.0 mL/min; 10–15 min - maintained; 15–16 min - increased to 52% (at 0.8 mL/min); 16–30 min - from 52% to 80% (0.8 mL/min); and 30–35 min - reduced to 30% (1.0 mL/min), and then maintained to 40 min to equilibrate the column [15]. The detection of flavonoids was carried out at 308 and 380 nm, and the standards used to build the calibration curves were myricetin, kaempferol, quercetin, hesperidin, and apigenin (Sigma-Aldrich, St. Louis, MO, USA).

2.2.3. HPLC analyses of flavonoid glycosides

The quercetin glycosides rutin and hyperosid were analyzed on the same HPLC system and column, with mobile phases 2% acetic acid, v/v (Solvent A) and acetonitrile (Solvent B), and detection wavelength 370 nm, and the following elution gradient: 0–15 min - 20% Solvent B; 15–17 min - 50% Solvent B; and 17–20 min - 20% Solvent B [16]. Calibration curves were built using rutin and hyperosid standards (Sigma-Aldrich, St. Louis, MO, USA).

2.3. HPLC Analysis of Triterpenes

About 1.0 g of homogenized tobacco were subjected to triple ultrasound-assisted extraction with acetone, each for 30 min at a temperature of 45°C and a hydro module of 1:20 (w/v). The combined extract was evaporated at 60°C on a rotary vacuum evaporator, and then the residue was transferred to 1 mL of methanol [14].

The same HPLC unit as that described in section 2.2.1 was used for the analysis of triterpenes, with the mobile phase being a methanol:0.01M potassium dihydrogen phosphate (pH 2.8) = 88:12 solution (v/v), and the flow rate gradient set up as follows: 0–18 min - at 0.8 mL/min; 18–19 min - at 0.6 mL/min; 19–30 min - at 0.6 mL/min; 30–31 min - at 0.8 mL/min, and 31–40 min - at 0.8 mL/min. Detection was carried out at 210 nm, and quantification - against a calibration curve, with betulin, betulinic, carnosic, ursolic, and oleanolic acids (97%) (Extrasynthese, France) used as standards [15].

2.4. Obtaining of *Nicotiana* Leaf Extracts

Before processing dried leaves were cut and fractionated to obtain uniform samples with a particle size of about 10 × 15 mm. The extraction was carried out under the following technological parameters: Raw material: solvent - 1:10 (w/v), solvent - ethanol in four concentrations (30, 50, 70, and 95%, v/v), duration - 5 h, and temperature - 60°C. Extraction conditions were selected according to the previous data from process modeling [17], which defined the applied combination of temperature and duration as the optimal in terms of tannin yield in the extracts from the studied *Nicotiana* species. Ethanol in different concentrations is recognized as the most popular polar solvent for obtaining biologically active extracts from medicinal and aromatic plants intended for food, cosmetic, and other purposes [13,18,19], as well as for processing tobacco leaves [17,19,20].

3. RESULTS AND DISCUSSION

3.1. Results

The moisture content of the analyzed plant materials was as follows: *N. alata* - 10.17%, *N. rustica* - 9.17%, and *N. tabacum* - 7.84%.

Data about the content of polyphenols and triterpenes in the leaves of the three studied *Nicotiana* species are presented in Table 1, and data from the analysis of the respective ethanol extracts are presented in Table 2.

The obtained results undoubtedly revealed that all *Nicotiana* species in the study contained significant amounts of the selected groups of metabolites (PAs, flavonoids, flavonoid glycosides, and triterpenes), which were, in turn, transferred into the obtained extracts. At the same time, there were observed considerable numerical and compositional variations in polyphenol and triterpene distribution between the species, concerning both the leaves and the ethanol extracts.

3.2. DISCUSSION

3.2.1. *N. alata*

Analytical data revealed that the group of PAs in the leaves of *N. alata* was dominated by caffeic (1202.60 µg/g) and chlorogenic (974.12 µg/g) acids, that of flavonoids - by myricetin (48.73 µg/g), the flavonoid glycosides - by rutin (441.37 µg/g), and the triterpenes - by

betulin (216.63 µg/g). Ursolic acid was not detected in *N. alata* leaves, while carnosic and oleanolic acids were found only in trace amounts. There were numerical differences from data reported in previous studies on *N. alata* leaf composition [6], which could be explained by the influence of crop year, environmental, and other factors. The concentrations of the individual groups of polyphenol compounds varied considerably in the four ethanol extracts, as well, which are explicable by the different selectivity of the solvent. In terms of PAs, the highest concentration in the extract obtained with 30% ethanol was found for sinapic and chlorogenic acids; in that with 50% ethanol - for chlorogenic, syringic and sinapic acids; in the extract with 70 % ethanol - for chlorogenic, caffeic and vanillic acids, and in the extract with 95% ethanol - for syringic acid. The group of flavonoids in the extract obtained with 30% ethanol from *N. alata* leaves was dominated by apigenin and luteolin, with 50% ethanol - by hesperetin, with 70% ethanol - by myricetin, and with 95% ethanol - by apigenin and myricetin. Among the flavonoid glycosides, hyperosid was not identified in the extracts, and rutin concentration was highest in the extract obtained with 50% ethanol. The extraction of triterpenes from *N. alata* leaves was also strongly influenced by the solvent, with the highest concentration of triterpenes being registered in the extract obtained with 70% ethanol - betulin (121.22 µg/mL), oleanolic acid (107.18 µg/mL), and betulinic acid (82.82 µg/mL), respectively. Ursolic acid was not identified in either of the extracts, while in the extract with 30% ethanol no triterpenes was detected at all.

Table 1: Polyphenols and triterpenes (µg/g DW) in the leaves of three *Nicotiana* species

Compound	NA ¹	NR ²	NT ³
Phenolic acids			
Gallic acid	53.52±0.48 ⁴	42.12±0.22	90.75±0.67
Protocatechuic acid	41.81±0.41	795.26±5.52	1334.94±8.47
Salicylic acid	299.94±1.28	239.85±1.36	403.09±1.62
Chlorogenic acid	974.12±7.46	1100.71±7.88	2377.07±7.88
Vanillic acid	664.25±5.47	163.90±1.02	225.70±1.44
Caffeic acid	1202.60±8.63	306.61±2.07	396.50±1.53
Syringic acid	275.93±2.14	300.30±1.65	263.93±1.43
p-Coumaric acid	110.85±1.03	1362.75±8.41	173.25±1.11
Sinapic acid	303.35±1.28	1162.60±7.99	3666.37±9.54
Ferulic acid	622.02±5.48	536.24±4.25	288.07±1.04
Cinnamic acid	30.90±0.27	16.21±0.21	48.24±0.51
Rosmarinic acid	443.12±2.98	2404.19±9.33	2103.41±8.57
Flavonoids			
Myricetin	48.73±0.33	80.08±0.42	107.76±1.08
Hesperetin	nd ⁵	nd	nd
Quercetin	10.85±0.09	24.57±0.11	20.13±0.13
Luteolin	9.07±0.08	70.70±0.32	96.00±0.41
Kaempferol	10.70±0.09	22.86±0.18	19.99±0.11
Apigenin	5.37±0.02	48.95±0.31	319.96±2.08
Flavonoid glycosides			
Rutin	441.37±3.02	4324.88±9.31	8030.36±9.77
Hyperoside	nd	210.07±2.06	40.44±0.28
Triterpenes			
Carnosic acid	tr ⁶	87.62±0.75	tr
Betulin	216.63±2.01	22.27±0.14	51.41±0.24
Betulinic acid	84.66±0.76	61.06±0.37	tr
Oleanolic acid	tr	78.31±0.34	5.54±0.01
Ursolic acid	nd	67.34±0.23	16.71±0.18

3.2.2. *N. rustica*

With regard to *N. rustica* leaves, the most abundant free PAs were rosmarinic, p-coumaric, sinapic, and chlorogenic acids (the respective levels being within the range from 2404.19 to 1100.71 µg/g). The group of flavonoids was dominated by myricetin and luteolin (80.08 and 70.70 µg/g, respectively) and that of flavonoid glycosides - by rutin (4324.88 µg/g). Five triterpenes were identified in the leaves, and the highest amounts were those of carnosic (87.62 µg/g) and oleanolic (78.31 µg/g) acids. In the ethanol extracts, the levels of the identified compounds varied substantially by solvent concentration, due to the impact of solvent selectivity on the transfer of the soluble polyphenols and triterpenes. In terms of PAs, the extract obtained with 30% ethanol had the highest concentration of salicylic, ferulic, and rosmarinic acids; with 50% ethanol - of rosmarinic, sinapic and protocatechuic acids; with 70% ethanol - of rosmarinic and chlorogenic acids, and the extract with 95% ethanol - of rosmarinic and sinapic acids. Within the flavonoid group, the extract with 30% ethanol had the highest concentration of luteolin and myricetin, those with 50% and 70% ethanol - of apigenin and luteolin, and that with 95% ethanol - of myricetin and luteolin. All extracts contained high levels of the flavonoid glycoside rutin (varying from 232.92 to 395.19 µg/mL), while hyperoside was identified only in the extracts obtained with 50% and 70% ethanol. The highest concentration of triterpenes was found in the extract obtained with 50% ethanol - betulinic acid (59.49 µg/mL) and betulin (25.18 µg/mL), while no triterpenes were detected in the extract with 30% ethanol.

3.2.3. *N. tabacum*

Data in Table 1 about the leaves of common tobacco (*N. tabacum*) revealed that in the group of PAs the highest were the amounts of sinapic, chlorogenic, and rosmarinic acids (3666.37, 2377.07, and 2103.41 µg/g, respectively); in the group of flavonoids - of apigenin (319.96 µg/g) and myricetin (107.76 µg/g), and in the group of flavonoid glycosides - of rutin (being reasonably the highest among the studied species - 8030.36 µg/g). Again, as it was the case with *N. alata* leaf composition, there were numerical differences on polyphenol and

¹NA: *N. alata* Link&Otto, ²NR: *N. rustica* L., ³NT: *N. tabacum* L. (Oriental type, variety "Plodiv 7"), ⁴Mean±SD (n=3), ⁵tr: Trace amount, ⁶nd: Not detected

Table 2: Polyphenols and triterpenes ($\mu\text{g/mL}$) in the extracts obtained from the leaves of three Nicotiana species

Compound	30% ethanol			50% ethanol			70% ethanol			95% ethanol		
	NA ¹	NR ²	NT ³	NA	NR	NT	NA	NR	NT	NA	NR	NT
Phenolic acids												
Gallic acid	6.43±0.02 ⁴	2.73±0.00	5.06±0.02	2.77±0.00	nd ⁵	2.47±0.00	6.28±0.02	7.23±0.03	4.33±0.01	nd	3.48±0.00	nd
Protocatechuic acid	23.40±0.12	63.86	104.44±0.90	21.06±0.12	73.65±0.33	109.18±0.82	nd	73.82±0.32	103.75±0.52	18.90	48.45±0.22	70.56±0.30
Salicylic acid	14.90±0.09	269.33±1.00	32.36±0.14	21.70±0.12	28.98±0.13	46.04±0.14	19.77	20.65±0.12	38.47±0.14	4.06±0.01	nd	nd
Chlorogenic acid	64.31±0.30	50.55±0.30	208.17±0.98	98.43±0.52	54.68±0.22	213.17±0.90	94.77±0.50	90.71±0.42	194.89±0.80	40.55±0.22	74.34±0.32	100.75±0.47
Vanillic acid	54.84±0.27	61.67±0.32	34.43±0.12	nd	49.92	17.58±0.10	61.18±0.41	49.115±0.22	14.88±0.008	44.87±0.23	40.55±0.22	42.01±0.22
Caffeic acid	14.73±0.09	18.03±0.10	29.08±0.12	45.30±0.22	15.09±0.11	8.06±0.05	80.28±0.39	33.03±0.10	6.48±0.03	30.39±0.14	nd	11.78±0.09
Syringic acid	13.61±0.08	7.02±0.04	18.50±0.08	61.51±0.30	13.23±0.09	22.69±0.12	18.90±0.11	26.70±0.12	20.16±0.11	86.46±0.42	22.60±0.12	10.58±0.09
p-Coumaric acid	5.78±0.02	nd	14.70±0.07	7.01±0.03	nd	17.73±0.10	11.89±0.08	18.49±0.10	15.38±0.10	5.55±0.02	5.15±0.02	9.39±0.07
Sinapic acid	163.10±0.80	60.34±0.32	188.63	63.07±0.30	142.12±0.69	229.85±1.00	49.05±0.22	63.28±0.31	260.08±1.00	25.23±0.12	88.51±0.42	192.48±0.61
Ferulic acid	31.19±0.18	178.14±0.92	26.85	33.86±0.12	52.22±0.25	24.67±0.12	22.40±0.12	33.02±0.12	24.45±0.12	18.84±0.10	22.77±0.12	15.93±0.08
Cinnamic acid	3.37±0.00	4.96±0.01	5.54±0.01	4.19±0.01	5.22±0.02	6.58±0.02	4.43±0.01	5.37±0.02	6.47±0.02	3.47±0.00	1.4±0.00	5.73±0.02
Rosmarinic acid	nd	172.63±0.92	260.53±1.00	10.7±0.08	193.74±0.72	297.46±1.00	nd	189.31±0.82	293.24±1.00	nd	112.38±0.40	170.54±0.82
Flavonoids												
Myricetin	8.78±0.04	11.59±0.06	nd	6.46±0.02	9.91±0.04	12.10±0.06	7.86±0.03	8.49±0.04	13.18	5.91±0.02	7.54±0.03	10.21±0.04
Hesperetin	nd	nd	nd	12.38	6.51±0.03	nd						
Quercetin	3.23±0.00	3.53±0.00	3.09±0.00	3.43±0.00	4.04±0.01	nd	2.97±0.00	3.57±0.00	3.59±0.00	2.78±0.00	3.26±0.00	3.18±0.00
Luteolin	9.65±0.08	12.82±0.10	10.17±0.08	8.79±0.07	13.55±0.06	14.91±0.07	1.74±0.00	10.43±0.07	22.27±0.12	2.51±0.00	7.20±0.12	12.25±0.06
Kaempferol	2.60±0.00	3.77±0.00	3.63±0.00	2.07±0.00	5.61±0.02	4.32±0.01	2.42±0.00	3.78±0.00	3.19±0.00	2.00±0.00	1.96±0.00	1.66±0.00
Apigenin	12.65±0.06	5.59±0.02	4.82±0.01	6.08±0.02	21.02±0.12	5.92±0.02	5.18±0.02	19.39±0.10	18.11±0.10	6.21±0.02	5.37±0.02	7.86±0.02
Flavonoid glycosides												
Rutin	23.67±0.12	320.61±1.51	550.21±2.22	46.69±0.24	395.19±0.145	598.49±2.50	35.68±0.16	361.09±1.51	700.3±3.61	20.57±0.12	232.92±0.99	488.93±2.23
Hyperoside	nd	nd	13.44±0.07	nd	2.33±0.00	32.14±0.13	nd	27.83±0.12	15.73±0.08	nd	nd	tr ⁶
Triterpenes												
Camosic acid	nd	nd	13.68	6.28±0.02	13.09±0.06	3.31±0.00	15.36±0.07	12.47±0.06	nd	6.66±0.02	8.23±0.03	nd
Betulin	nd	nd	nd	5.85±0.02	25.1±0.12	nd	121.22±0.51	12.60	94.87±0.45	45.38±0.22	11.87±0.06	117.17±0.50
Betulonic acid	nd	nd	nd	6.31±0.02	59.49±0.22	nd	82.8±0.41	7.31±0.03	nd	nd	3.87±0.00	30.88±0.14
Oleanolic acid	nd	nd	5.64±0.01	2.52±0.00	9.25±0.04	nd	107.18±0.50	11.45±0.05	256.15±1.00	14.61±0.06	10.26±0.05	69.05±0.32
Ursolic acid	nd	nd	4.34±0.02	nd	22.03±0.12	nd	nd	nd	nd	nd	7.41±0.05	44.46±0.22

¹NA: *N. glauca* Link&Otto, ²NR: *N. rustica* L., ³NT: *N. tabacum* L. (Oriental type, variety "Plodviv 7"), ⁴Mean±SD (n=3), ⁵nd: Not detected, ⁶tr: Trace amount

triterpene basis from previous studies on the same tobacco variety [21], explainable by crop year and other influencing factors. Similar to the observations about the leaf extracts from *N. alata* and *N. rustica*, the four ethanol extracts from *N. tabacum* leaves [Table 2] differed by polyphenol and triterpene concentration. The distribution of the major representatives within the respective group of metabolites in the extracts was as follows: PAs - the highest content in all extracts was that of rosmarinic, sinapic, and chlorogenic acids; flavonoids - the content of luteolin was the highest in all extracts; flavonoid glycosides - all extracts contained high levels of rutin; and triterpenes - the extracts with 70% and 95% ethanol were with the highest concentration of oleanolic acid and betulin.

4. CONCLUSIONS

The comparative analysis of the results about the content of the studied groups of bioactive metabolites (polyphenols and triterpenes) in the leaves and extracts of the three *Nicotiana* species suggests that:

- All *Nicotiana* species represent a plant material containing various metabolites capable of biological activities (PAs, flavonoids, flavonoid glycosides, and triterpenes), which substantiate their consideration as a valuable source for obtaining different phytoproducts.
- Ethanol in a 95% concentration is not a suitable solvent for the leaves of the studied *Nicotiana* species.
- Ethanol in a 30% concentration can be considered for use only in the extraction of *N. rustica* leaves.
- 50% ethanol is the most suitable solvent for the extraction of *N. alata* leaves, while 70% ethanol - for the leaves of *N. tabacum*.
- The high content of polyphenols and triterpenes in the extracts provide grounds for their use in various cosmetic and pharmaceutical products, which could be the subject of future research.

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6. REFERENCES

1. Yadav R, Rathi M, Pednekar A, Rewachandani Y. Detailed review on solanaceae family. *Eur J Pharm Med Res* 2016;3:369-78.
2. Knapp S, Chase MW, Clarkson JJ. Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae). *Taxon* 2004;53:73-82.
3. Jassbi AR, Zare S, Asadollahi M, Schuman MC. Ecological roles and biological activities of specialized metabolites from the genus *Nicotiana*. *Chem Rev* 2017;117:12227-80.
4. Kishore K. Monograph of tobacco (*Nicotiana tabacum*). *Indian J Drugs* 2014;2:5-23.
5. Rodgman A, Perfetti T, editors. *The Chemical Components of Tobacco and Tobacco Smoke*. 2nd ed. Boca Raton, Florida: CRC Press; 2013.

6. Popova V, Ivanova T, Nikolova V, Stoyanova A, Docheva M, Hristeva T, *et al.* Biologically active and volatile compounds in leaves and extracts of *Nicotiana alata* Link and Otto from Bulgaria. *J Pharm Sci Res* 2017;9:2045-51.
7. Winter JC, editor. *Tobacco Use by Native North Americans: Sacred Smoke and Silent Killer*. Norman, USA: University of Oklahoma Press; 2000.
8. Djordjevic MV, Doran KA. Nicotine content and delivery among tobacco products. In: Henningfield JE, London ED, Pogun S, editors. *Nicotine Psychopharmacology. Handbook of Experimental Pharmacology*. Vol. 192. Berlin Heidelberg: Springer-Verlag; 2009. p. 61-82.
9. Kurucu S, Kartal M, Erenmemisoglu A. HPLC analysis of *Nicotiana rustica* L. and chewing tobacco (Maras powder) alkaloids. *FABAD J Pharm Sci* 1998;23:61-4.
10. Saitoh F, Noma M, Kawashima N. The alkaloid contents of sixty *Nicotiana* species. *Phytochemistry* 1985;24:477-80.
11. Sisson VA, Severson RF. Alkaloid composition of the *Nicotiana* species. *Beitr Tabakforsch Int* 1990;14:327-99.
12. Leffingwell J. Basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis D, Nielsen M, editors. *Tobacco: Production, Chemistry and Technology*. London: Blackwell Science; 1999. p. 265-84.
13. Pharmacopoeia Committee, Ministry of Health of the USSR. *State Pharmacopoeia of the USSR*. 11th ed., part 2. Moscow: Medicina; 1990.
14. Marchev A. *Biologically Active Substances from Rare Bulgarian Salvia Species and their in vitro Cultures*. PhD Dissertation. Sofia: Institute of Microbiology; 2014.
15. Marchev A, Georgiev V, Ivanov I, Badjakov I, Pavlov A. Two-phase temporary immersion system for agrobacterium rhizogenes genetic transformation of sage (*Salvia tomentosa* mill.). *Biotechnol Lett* 2011;33:1873-8.
16. Ivanov I, Vrancheva R, Marchev A, Petkova N, Aneva I, Denev P, *et al.* Antioxidant activities and phenolic compounds in Bulgarian *Fumaria* species. *Int J Curr Microbiol Appl Sci* 2014;3:296-306.
17. Popova V, Tasheva S, Ivanova T, Stoyanova A. Technology for obtaining ethanol extracts from three *Nicotiana* species based on their tannin content. *J Multidiscip Eng Sci Technol* 2018;5:9043-8.
18. Regulation (EC) No1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products. *OJ L342*, 22.12.2009. p. 59-209.
19. Popova V. *Obtaining and Analysis of Aromatic Extraction Products for Expanding the Usability of Tobacco (Nicotiana tabacum L.)*. DSc Dissertation. Plovdiv: UFT; 2015.
20. Popova V. Liquid extracts from tobacco (*N. tabacum* L.). *Sci Works UFT* 2014;61:279-83.
21. Popova V, Ivanova T, Stoyanova A, Georgiev V, Hristeva T, Nikolova V, *et al.* Phytochemicals in leaves and extracts of the variety "Plovdiv 7" of Bulgarian oriental tobacco (*Nicotiana tabacum* L.). *Trends Phytochem Res* 2018;2:27-36.

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