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Tobacco resinoid (*Nicotiana tabacum* L.) as an active ingredient of cosmetic gels

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ABSTRACT

Tobacco (*Nicotiana tabacum* L.) is not just an important industrial crop but also a source of phytochemicals with diverse biological and pharmacological activities. The study aimed at the investigation of the chemical composition and the antimicrobial activity of resinoids obtained from three types of tobacco grown in Bulgaria (Virginia, Burley, and Oriental), and the properties of a model cosmetic gel incorporating tobacco resinoid as an active ingredient. The GC-MS composition of the resinoids was presented by aliphatic hydrocarbons (56.54%–68.30%), oxygenated aliphatics (10.41%–20.45%), diterpenes (10.02%–18.63%), and triterpenes (4.55%–6.92%). The tested resinoids were more effective against Gram-positive bacteria (*Bacillus subtilis* ATCC 6633 and *Bacillus cereus*) and yeasts (*Candida albicans* NBIMCC 74) than against Gram-negative bacteria (*Klebsiella pneumoniae*). The resinoid of Oriental tobacco was included as an active ingredient (1%) in a model cosmetic gel formulation. The rheological behavior of the gel characterized it as a non-Newtonian fluid. The inclusion of tobacco resinoid reduced gel viscosity in the applied stress range (1.5 ÷ 72.9 s^{-1}). The outcomes from the study suggest that tobacco resinoid is a suitable ingredient of cosmetic gels, contributing to their organoleptic and functional properties.

INTRODUCTION

Tobacco (*Nicotiana tabacum* L. and to a lesser extent *N. rustica* L.) has been long recognized as one of the most economically important industrial crops and is cultivated in many countries around the world. Parallel to the commercial interest in tobacco as leaf material for different tobacco products, the plant has been undeniably distinguished for its potential to synthesize a plethora of secondary (or specialized) metabolites with various biological or pharmacological activities. In a recent review, Jassbi *et al*. (2017) summarized that "*cultivated tobacco (N. tabacum) is among the most chemically and biologically well-studied species of the plant kingdom, with more than 2500 characterized metabolites, updated by continuous research*". These metabolites, found in high concentrations in tobacco leaves, include many classes of bioactive chemical compounds, such as alkaloids, terpenoids (mono-, sesqui-, di-, and triterpenoids), phenolics (phenolic acids, flavonoids, and coumarins), isoprenoids, and many others (Banožić *et al*., 2019; Rodgman and Perfetti, 2013). The chemistry and pharmacology of tobacco metabolites substantiate a reasonable interest in more innovative or broader use of the plant through extraction and concentration of fractions with potential beneficial effects. On the other hand, tobacco has been historically regarded as a genuine essential oil-bearing (or aromatic) plant and has been used for obtaining aromatic products for the fragrance industry. In fact, as Mookherjee and Wilson (1990) state, "*no natural product in the flavor and fragrance industry can match tobacco for the number of volatile constituents which have been identified*". Traditional (or established) aromatic products obtained from tobacco include tobacco concrete (by extraction with nonpolar solvents, like n-hexane), tobacco resinoid (by extraction with polar solvents, like ethanol), and tobacco absolute (by re-extraction of concrete or resinoid with polar solvents at low temperatures) (Baser and Buchbauer, 2010; Bauer *et al*., 2001), which are used in perfumery or in the casing and top-flavoring of tobacco blends due to their specific olfactory profiles. Additionally,

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these extracts have demonstrated antimicrobial, antioxidant, and pharmacological properties (Docheva *et al*., 2018; Ru *et al*., 2012; Vijayabhaskar *et al*., 2014) and could be considered as active ingredients (natural supplements or antioxidants) in cosmetic preparations. The growing interest in such natural products and the inclusion of various plant extracts and phytochemicals in cosmetic products (Fedorovska *et al*., 2018; Iswandana *et al*., 2018; Khor *et al*., 2018) substantiate comprehensive studies of the composition and properties of extraction products derived from tobacco representing different genotypes and regions. Due to the economic importance of tobacco, the species has developed into numerous commercial types and varieties, each with characteristic properties, composition, and use (Dyulgerski and Docheva, 2017; Korubin-Aleksoska and Ahmad, 2016; Radoukova and Dyulgerski, 2018). In general, three major types of tobacco are produced and used in cigarettes: flue-cured Virginia, Oriental (also known as aromatic or Turkish tobacco, often labeled as the richest in flavor among all tobacco types), and Burley light air-cured. Considering the historically established traditions in the large-scale production of all of the three types in Bulgaria, as well as the availability of the raw material and the respective processing facilities and network, we hypothesized that the leaves from the three types of tobacco grown in the country could be processed to aromatic products with specific composition and character. We further hypothesized that tobacco resinoids would reveal antimicrobial activity and characteristic olfactory profile and could be included in cosmetic preparations (creams, gels, etc.) as an active ingredient.

Therefore, the aim of the present study was to obtain and characterize (composition by gas chromatography-mass spectrometry (GC-MS), antimicrobial activity) the resinoid from the leaves of different tobacco types and the properties of a cosmetic gel containing tobacco resinoid as an active ingredient.

MATERIAL AND METHODS

Plant material

The plant material were cured and aged (for 12 months) tobacco leaves, provided by local leaf processing factories, which were further sorted to obtain uniform samples. Three tobacco types were studied, all representing local varieties grown in South Bulgaria tobacco-producing region—flue-cured Virginia (FCV), Burley (BU), and Oriental (OR). The leaves were oven-dried (40°C; 6 hours), ground, and sieved to particle size required by the respective analysis. The moisture content of the leaves before processing was 7.21% ± 0.05% (FCV), 7.93% ± 0.06% (BU), and 7.56% ± 0.05% (OR) and was determined by oven-drying to constant weight at 103°C ± 2°C (The State Pharmacopoeia of the USSR, 1990).

Methods for obtaining of tobacco resinoids

Tobacco resinoids were obtained by batch extraction with 95% ethanol (FILLAB, Bulgaria) under the following conditions: twofold extraction at 70°C for 2.5 and 2 hours, respectively, in a raw material-to-solvent ratio of 1:10. The solvent was then removed by vacuum evaporation at a water bath temperature of 70°C. Resinoid yields were represented on a dry weight basis.

GC-MS analysis of tobacco resinoids

50 µl of tobacco resinoid were mixed with 100 µl pyridine (Sigma-Aldrich) and 100 µl *N*,*O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA, Supelco) and incubated at 70°C for 45 minutes. The sample was then diluted with 150 µl chloroform and injected (1 µl volume) in a system comprised an Agilent 7890A gas chromatograph (Agilent Technologies) and mass selective detector Agilent 5975C (Agilent Technologies). The column was HP-5ms (30 m × 0.32 mm; film thickness 0.25 µm) operated under the following conditions: temperature increase from 40°C (0 minute) to 230°C at 5°C/minute (held at 230°C for 10 minutes); injector and detector temperature 250°C; carrier gas helium at 1 ml/minute constant flow; MSD scan range m/z = 50–550; split mode (5:1).

Antimicrobial activity

Test microorganisms

Twelve microorganisms including Gram-positive bacteria (*B. subtilis* ATCC 6633, *Bacillus cereus*—clinical isolate, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19111, *Enterococcus faecalis*—clinical isolate), Gram-negative bacteria (*Salmonella enteritidis*—clinical isolate, *Klebsiella pneumoniae*—clinical isolate, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 9027), and yeasts (*Candida albicans* NBIMCC 74) from the collection of the Department of Microbiology at the University of Food Technologies, Plovdiv, Bulgaria, were selected for the antimicrobial test.

Culture media

Luria–Bertani glucose agar (LBG-agar) medium (Laboratorios Conda S.A., Madrid, Spain) containing tryptone, yeast extract, NaCl, glucose, and agar was used for cultivation of test microorganisms, as well as for implementation of agar well diffusion assay. For these two purposes, 50 g of LBG-agar medium was dissolved in 1 l of deionized water, the final pH was adjusted to 7.5 and then autoclaved at 121°C for 20 minutes before use.

Antimicrobial assay

The antimicrobial activity of tobacco resinoids was determined by the standard agar well diffusion method in LBG-agar medium (Tumbarski *et al*., 2018). The pathogenic bacteria and yeasts were cultured on LBG-agar medium at 37ºC for 24 hours, while *B. subtilis* ATCC 6633 and *B. cereus*—at 30ºC for 24 hours. The inocula of test microorganisms were prepared by homogenization of a small amount of biomass in 5 mL of sterile 0.5% NaCl and vigorous shaking. The number of viable cells was determined using a bacterial counting chamber Thoma (Poly-Optik, Bad Blankenburg, Germany). Their final concentrations were adjusted to 1.0×10^8 cfu/ml, then inoculated in preliminarily melted and tempered at 45°C–48°C LBG-agar media. The inoculated media were transferred in quantity of 17 mL in sterile Petri plates (d = 90 mm) (Gosselin™, Hazebrouck, France) and allowed to solidify, then six wells (d = 6 mm) per plate were cut.

The samples (tobacco resinoids) and the control (dimethylsulfoxide—DMSO) were pipetted in quantity of 60 µl into the agar wells. The inoculated Petri plates were incubated at 37ºC (for pathogenic bacteria and yeasts) and at 30ºC (for *B. subtilis* ATCC 6633 and *B. cereus*) for 48 hours. The antimicrobial activity was determined by measuring the diameter of the inhibition zones around the wells on the 24th and 48th hour of incubation.

Test microorganisms with inhibition zones of 18 mm or more were considered as sensitive (strong inhibitory effect), moderately sensitive were those with zones from 12 to 18 mm (moderate inhibitory effect), and resistant—with inhibition zones up to 12 mm or completely missing (insignificant or no inhibitory effect) (Tumbarski *et al*., 2017).

Preparation of the cosmetic gel

The preparation of model cosmetic gels included the dispersion of phase A acrylamide/sodium acrylate copolymer in water and glycerol at ambient temperature and constant stirring to complete dissolution and then the addition of preservative to obtain a clear gel. Phase B (tolu balsam dissolved in a solubilizer) and phase C (ethanol 95% with the active ingredient dissolved) components were sequentially added. The composition of cosmetic gels is summarized in Table 3.

The appearance, color, and odor of the gels were assessed organoleptically by a 7-member panel, and gel pH was measured by a laboratory pH-meter (Bante 920, China).

Rheological behavior of the cosmetic gel

The rheological properties of the formulated cosmetic gel were studied on a Rheotest-2 (Medingen, Germany) rotating viscosimeter, at a temperature of 30°C and velocity gradient (D) ranging from 1.5 to 72.9 s^{-1} (Tzaneva *et al*., 2017).

RESULTS AND DISCUSSIONS

In this study, the resinoid content (yield) was 23.07% ± 0.18% for FCV, 8.54% ± 0.06% for BU, and 23.51±% for OR tobacco. These results clearly differentiated between the resinoid-yielding potential of bright and dark tobaccos and were in compliance with previous findings that flue-cured Virginia and sun-cured Oriental tobaccos yielded 2–3 times more resinoid than Burley light air-cured tobacco (Georgiev and Stoyanova, 2006; Popova *et al*., 2015). Data confirmed that cured tobacco leaf is a high-yielding plant material for obtaining extraction aromatic products for the cosmetic industry. On a resinoid yield basis, there were no substantial numerical differences between current results and previous data for other varieties of the respective tobacco type (Popova *et al*., 2015; 2018).

All resinoids represented dark brown semi-solid masses and had intensive specific tobacco odor. The olfactory evaluation clearly differentiated the resinoids on a tobacco type basis, and the one of OR tobacco expectedly was described as the richest and most complicated in terms of fragrance notes and undertones.

The GC-MS chemical composition of tobacco resinoids is listed in Table 1.

In each of the three tobacco resinoids, 33 constituents were identified, representing, respectively, 95.54%, 95.23%, and 95.32% of the total composition of BU, OR, and FCV tobacco resinoids.

In the resinoid of BU tobacco, 20 of the constituents were in concentrations over 1%, and the main (above 3%) components were sclareol (14.42%), n-tritriacontane (10.84%), n-triacontane (6.89%), n-octacosane (6.32%), n-dotriacontane (5.15%), n-nonacosane (5.03%), n-hexatriacontane (5.01), n-pentatriacontane (4.58%), squalene (4.55%), n-octatriacontane (4.17%), trimethylsilyl octanoate (3.45%), and tetracosane (3.07%).

In the resinoid of OR tobacco, 20 of the constituents were in concentrations over 1%, as well, and 10 of them were identified as main components (above 3%): n-octacosane (20.16%), n-tritriacontane (12.89%), squalene (6.23%), n-hentriacontane (5.29%), n-hexacosane (5.20%), n-heptatriacontane (5.12%), sclareol (4.90%), n-tetratriacontane (4.75%), n-pentatriacontane (3.66%), and n-triacontane (3.21%).

Nineteen components exceeding 1% were identified in the resinoid of FCV tobacco, and the most abundant (above 3%) were n-tritriacontane (20.99%), trimethylsilyl octanoate (7.49%), squalene (6.92%), sclareol (5.64%), n-hexacosane (5.78%), n-hexatriacontane (4.88%), n-heptatriacontane (4.57%), trimethylsilyl stearate (4.19%), n-tetratriacontane (4.16%), n-triacontane (3.56%), and n-octacosane (3.51%).

There were numerical differences in the individual composition of the resinoids from the three tobacco types, which correlated to the differentiation established by the olfactory evaluation. The qualitative and quantitative differences in the composition of tobacco resinoids determined in the current study and in previous reports were most probably related to the different tobacco types and varieties, the climatic conditions of the respective locality where the plants were grown, as well as to the plant parts processed and extracted (Cvetanovska *et al*., 2018; Popova *et al*., 2015; 2018).

The chemical composition of BU, OR, and FCV tobacco resinoids included individual representatives of various classes of chemicals, and their distribution, given as percent of the total sum of the identified compounds (equaled to 100%), is presented in Figure 1. In this study, the dominant group of chemical constituents in the resinoids were the aliphatic hydrocarbons, followed by oxygenated aliphatic compounds, diterpenes, and triterpenes. The high share of aliphatic hydrocarbons was responsible for the appearance and color of the three resinoids (dark brown semi-solid masses), while the presence of aroma-active components such as oxygenated aliphatics and diterpenes contributed to the nuancing in resinoids' odor.

The results from the antimicrobial assay presented in Table 2 demonstrate that the three resinoids were more effective against Gram-positive bacteria and yeasts than against Gram-negative bacteria. The screening for antimicrobial activity showed the insignificant inhibitory effect of all the resinoids on *B. subtilis* ATCC 6633 and *B. cereus* in both tested concentrations of 25 and 50 mg/ml. The tobacco resinoids of BU and OR exhibited insignificant antimicrobial activity against *L. monocytogenes* ATCC 19111 only in concentrations of 50 mg/ml. The resinoid of OR tobacco showed insignificant antimicrobial effect on *E. faecalis* in both tested concentrations. The rest of the Gram-positive bacteria tested, *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 25923, remained unaffected.

The three tobacco resinoids in the applied concentrations of 25 and 50 mg/ml exhibited insignificant antimicrobial activity only against *K. pneumoniae*. The rest of the Gram-negative bacteria—*S. enteritidis*, *E. coli* ATCC 25922, *P. vulgaris* ATCC 6380 and *P. aeruginosa* ATCC 9027, remained unaffected. The inhibitory effect of the extracts in both concentrations on yeasts

Table 1. Chemical composition of tobacco resinoids.

No	Compound	RI[a]	Content (%)		
			Burley	Oriental	Virginia
1.	Trimethylsilyl octanoate	1,258	3.45 ± 0.03	1.74 ± 0.01	7.49 ± 0.06
2.	Trimethylsilyl nonanoate	1,355	0.44 ± 0.00	0.52 ± 0.00	0.58 ± 0.00
3.	Nicotine	1,367	0.23 ± 0.00	0.27 ± 0.00	0.30 ± 0.00
4.	Megastigmatrienone 1	1,559	0.57 ± 0.00	0.66 ± 0.00	0.74 ± 0.00
5.	Megastigmatrienone 2	1,582	0.62 ± 0.00	0.73 ± 0.00	0.81 ± 0.00
6.	Megastigmatrienone 3	1,629	0.73 ± 0.01	0.86 ± 0.01	0.95 ± 0.01
7.	Megastigmatrienone 4	1,656	0.42 ± 0.00	0.48 ± 0.00	0.53 ± 0.00
8.	Trimethylsilyl myristate	1,841	1.26 ± 0.01	1.74 ± 0.01	1.93 ± 0.01
9.	Sclareol oxide	1,876	0.64 ± 0.00	0.75 ± 0.00	0.83 ± 0.00
10.	Trimethylsilyl palmitate	2,039	1.84 ± 0.02	2.16 ± 0.02	2.40 ± 0.02
11.	Manool	2,056	0.91 ± 0.01	1.06 ± 0.01	1.18 ± 0.01
12.	Phytol	2,163	1.12 ± 0.01	1.31 ± 0.01	1.45 ± 0.01
13.	α-Linolenic acid	2,219	0.64 ± 0.00	0.75 ± 0.00	0.83 ± 0.00
14.	Sclareol	2,222	14.42 ± 0.32	4.90 ± 0.03	5.64 ± 0.05
15.	3-α-acetoxy-Manool	2,236	0.87 ± 0.01	1.02 ± 0.01	1.14 ± 0.01
16.	3-α-hydroxy-Manool	2,286	0.67 ± 0.00	0.98 ± 0.01	0.87 ± 0.01
17.	Trimethylsilyl stearate	2,340	1.99 ± 0.02	0.77 ± 0.00	4.19 ± 0.04
18.	n-Tetracosane	2,400	3.07 ± 0.02	2.02 ± 0.02	0.69 ± 0.00
19.	n-Pentacosane	2,500	1.46 ± 0.02	1.35 ± 0.01	0.39 ± 0.00
20.	n-Hexacosane	2,600	2.14 ± 0.02	5.20 ± 0.05	5.78 ± 0.05
21.	n-Heptacosane	2,700	2.02 ± 0.02	1.47 ± 0.01	1.63 ± 0.01
22.	n-Octacosane	2,800	6.32 ± 0.05	20.16 ± 0.18	3.51 ± 0.02
23.	Squalene	2,812	4.55 ± 0.05	6.23 ± 0.03	6.92 ± 0.06
24.	n-Nonacosane	2,900	5.03 ± 0.04	0.74 ± 0.01	0.82 ± 0.00
25.	n-Triacontane	3,000	6.89 ± 0.04	3.21 ± 0.03	3.56 ± 0.02
26.	n-Hentriacontane	3,100	1.68 ± 0.01	5.29 ± 0.05	4.88 ± 0.05
27.	n-Dotriacontane	3,200	5.15 ± 0.05	0.30 ± 0.00	0.67 ± 0.00
28.	n-Tritriacontane	3,300	10.84 ± 0.09	12.89 ± 0.11	20.99 ± 0.22
29.	n-Tetratriacontane	3,400	0.97 ± 0.01	4.75 ± 0.05	4.16 ± 0.03
30.	n-Pentatriacontane	3,500	4.58 ± 0.03	3.66 ± 0.03	2.96 ± 0.03
31.	n-Hexatriacontane	3,600	5.01 ± 0.04	1.86 ± 0.01	1.51 ± 0.01
32.	n-Heptatriacontane	3,700	0.84 ± 0.00	5.12 ± 0.04	4.57 ± 0.03
33.	n-Octatriacontane	3,800	4.17 ± 0.03	0.28 ± 0.00	0.42 ± 0.00
	Total identified compounds (%)	-	95.54	95.23	95.32
	Aliphatic hydrocarbons		60.17	68.30	56.54
	Oxygenated aliphatic compounds		11.96	10.41	20.45
	Diterpenes		18.63	10.02	11.11
	Triterpenes		4.55	6.23	6.92
	Alkaloids		0.23	0.27	0.30

[a]RI—retention (Kovat's) index.

C. albicans NBIMCC 74 was also insignificant. The solvent DMSO, which was used as a control, did not exhibit antimicrobial activity.

As discussed above, the main constituents of the resinoids in this study were aliphatic hydrocarbons, which are associated with lower or no antimicrobial activities (Baser and Buchbauer, 2010). Therefore, the inhibition action of tobacco resinoids on microbial growth in this study was probably due to the synergistic or antagonistic effect of oxygenated aliphatic compounds with the diterpenes (Baser and Buchbauer, 2010; Cutler *et al*., 1977; Kroumova *et al*., 2016; Nugroho and Verpoorte, 2002; Seo *et al*., 2012; Wahlberg and Enzell, 1987).

The aromatic products obtained from tobacco leaves or flowers by extraction, such as concrete, resinoid, absolute, as well as tobacco leaf and flower oils, are not included by Regulation No 1223/2009 (the Cosmetics Regulation) in the list of banned (Annex II) or restricted (Annex III) cosmetic ingredients, and are not regarded as ingredients containing allergens. According to the provisions of the Regulation, such allergens must not exceed

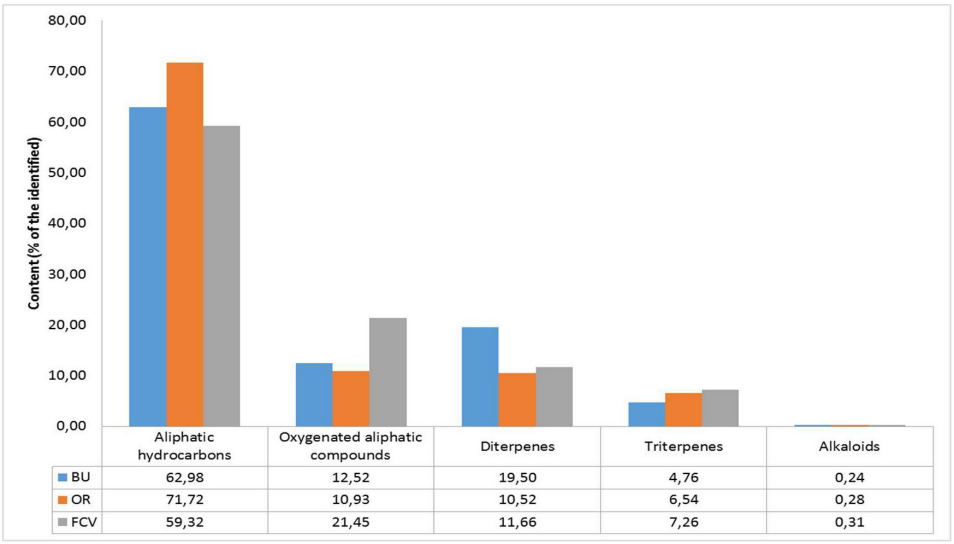

Figure 1. Tobacco resinoid composition by classes of compounds (% of the identified).

Table 2. Antimicrobial activity of tobacco resinoids.

Test microorganism	Inhibition zone (mm)					
	BU[a]		FCV		OR	
	25 mg/ml	50 mg/ml	25 mg/ml	50 mg/ml	25 mg/ml	50 mg/ml
Gram-positive bacteria						
B. subtilis ATCC 6633	9 ± 0.01	11 ± 0.03	9 ± 0.01	9 ± 0.01	11 ± 0.02	12 ± 0.03
B. cereus	9 ± 0.00	10 ± 0.01	9 ± 0.01	9 ± 0.01	10 ± 0.02	13 ± 0.04
S. epidermidis ATCC 12228	-[b]	-	-	-	-	-
S. aureus ATCC 25923	-	-	-	-	-	-
L. monocytogenes ATCC 19111	-	8 ± 0.00	-	-	-	8 ± 0.00
E. faecalis	-	-	-	-	8 ± 0.00	9 ± 0.01
Gram-negative bacteria						
S. enteritidis	-	-	-	-	-	-
K. pneumoniae	10 ± 0.01	11 ± 0.02	10 ± 0.01	10 ± 0.01	10 ± 0.02	11 ± 0.02
E. coli ATCC 25922	-	-	-	-	-	-
P. vulgaris ATCC 6380	-	-	-	-	-	-
P. aeruginosa ATCC 9027	-	-	-	-	-	-
Yeasts						
C. albicans NBIMCC 74	9 ± 0.01	10 ± 0.02	10 ± 0.02	11 ± 0.02	10 ± 0.01	11 ± 0.02

[a]BU—Burley tobacco resinoid; FCV—flue-cured Virginia tobacco resinoid; OR—Oriental tobacco resinoid.
[b]No inhibition zone detected.

Table 3. Formulation of a cosmetic gel containing tobacco resinoid.

Phase	Ingredient INCI[a]	Properties	Content (% w/v)	
			Model gel	Control
A	Aqua	solvent	ad 100	ad 100
	Glycerine	moisturizer	3.00	3.00
	Acrylamide/sodium acrylate copolymer	consistency giving factor	1.00	1.00
	2-Bromo-2-nitropropane-1,3-diol	preservative	0.10	0.10
B	PEG-40 Hydrogenated Castor Oil	solubilizer	2.00	2.00
	Tolu Balsam	aroma compound	0.20	0.20
C	Ethanol, 96%	solubilizer	5.00	5.00
	N. tabacum L. Leaf Extract[b]	active ingredient	1.00	-

[a]The names are given according to the INCI (Regulation No 1223/2009).
[b]The extract was resinoid of OR tobacco.

the permissible concentration of 0.01% in shower gels and rinse-off products or 0.001% in body oils, massage oils, and creams (Sarkic and Stappen, 2018). Tobacco leaf extracts [International Nomenclature of Cosmetic Ingredients (INCI) name: *N. tabacum* Leaf Extract] are assigned with perfuming and skin conditioning functions (Cosmetic ingredient database, CosIng).

As seen from the results in Table 1, tobacco resinoids contained a number of compounds in significant concentrations that have been considered as beneficial for cosmetic formulations. For example, squalene, a triterpene associated mostly to shark liver oil, but also present in reasonable quantities in many plant sources (e.g., olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil) was found to be a major constituent in the studied tobacco resinoids—4.55% in BU, 6.23% in OR, and 6.92% in FCV resinoid, respectively. Squalene is widely used in topically applied cosmetic products due to its advantages for the skin, exercised through different mechanisms, such as chemoprevention, anti-oxidation, skin hydration, emolliating (moisturizing), and emulsifying properties, etc. (Huang *et al*., 2009). The labdane diterpene derivative sclareol, representing another major constituent of the tobacco resinoids in the study (4.90%, 5.64 %, and 14.42%, respectively), is widely used as a fragrance in cosmetics and perfumery and as flavoring in food, and is identified with a variety of biological activities, such as antimicrobial, antifungal, anti-inflammatory, and immunomodulatory (Tsai *et al*., 2018).

Based on the motives stated above, the resinoid obtained by ethanol extraction from OR tobacco leaf was included as an active ingredient in the formulation of a model cosmetic gel (Table 3), selected on the grounds of its better olfactory profile and higher antimicrobial activity compared to the other tobacco resinoids in the study.

For the sake of comparison, a control gel formulation was developed, without the inclusion of the active ingredient (tobacco resinoid). The cosmetic gel containing tobacco extract (Fig. 2) was a clear, viscous gel, light brown in color, with typical tobacco, warm, sweet odor, balanced to the balsamic, citrus, and floral notes perceived. The pH of the control formulation was 6.12 and that of the cosmetic gel containing tobacco resinoid—5.92; that slight reduction could be related to the acidic nature of the active ingredient (Jufri *et al*., 2018).

The objective characterization of the properties of the formulated cosmetic gel was conducted by following its rheological behavior and viscosity. The rheological properties of cosmetic products, such as gels, creams, lotions, etc., depend strongly on their composition alongside many other factors. The rheograms of the analyzed cosmetic gels, the one with the active ingredient and the control, taken for a velocity gradient (D) range from 1.5 to 72.9 s^{-1} at a temperature of 30°C are presented in Figure 3. The profile of the curves revealed that the analytical sample, like the control, in rheological behavior represented non-Newtonian fluids.

Viscosity is considered one of the main rheological properties of gels. The change of gel viscosity (η) in response to velocity gradient (D) variation in the range between 1.5 and 72.9 s^{-1} is shown in Figure 4.

The depicted graphical dependencies revealed that the increase in velocity gradient provoked a decrease in gel viscosity, which further confirmed the non-Newtonian rheological behavior of both samples. Data showed that the viscosity of the control cosmetic gel remained comparatively higher than that of the gel formulation containing the tobacco resinoid within the entire stress range. The reduction of gel viscosity was obviously related to the presence of the active ingredient, representing a concentrated ethanol extract with a considerable share of aliphatic hydrocarbons, which varied the gel-forming capacity of the copolymer in the formulation. As seen from the graphic, the absolute differences between the viscosity of the control gel (η_c) and the active ingredient containing gel (η_g) were bigger in the

Figure 2. Appearance of the formulated cosmetic gels: left—formulation without tobacco resinoid (control), right—formulation containing tobacco resinoid (1%).

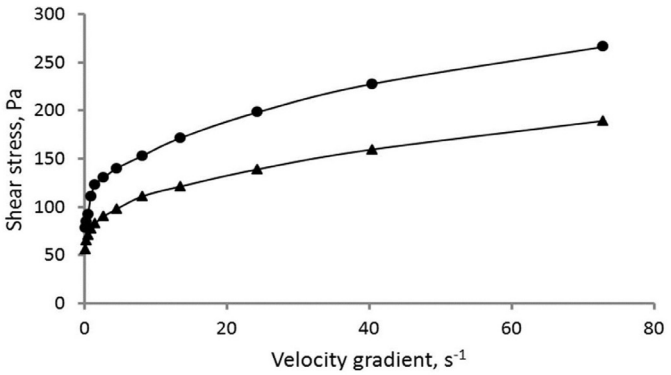

Figure 3. Rheograms of model cosmetic gels: ● – control sample; ▲ – sample containing *N. tabacum* resinoid.

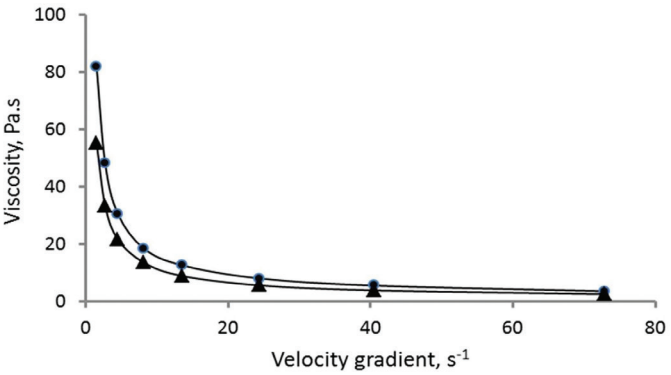

Figure 4. Viscosity of model cosmetic gels (η) versus the velocity gradient (D): ●—control sample; ▲—sample containing N. tabacum resinoid.

Table 4. Absolute difference between the viscosities of the control (η_c) and *N. tabacum* resinoid containing (η_g) cosmetic gels as a function of the velocity gradient (*D*).

D (s^{-1})	1.5	2.7	4.5	8.1	13.5	24.3	40.5	72.9
$\eta_c - \eta_g$ (Pa.s)	26.6	14.8	8.9	4.9	3.8	2.4	1.7	1.1

range of the lower values of the velocity gradient (1.5÷24.3 s^{-1}) compared with those registered at the higher D values (Table 4).

CONCLUSION

The study revealed the chemical composition (by GC-MS) and the antimicrobial activity of a concentrated extraction aromatic product, i.e., tobacco resinoid, obtained from the leaves of three tobacco types (*N. tabacum*) grown in Bulgaria—flue-cured Virginia, Burley, and Oriental. Tobacco resinoids contained individual compounds (major and minor) belonging to different chemical classes (aliphatic hydrocarbons, oxygenated aliphatics, diterpenes, and triterpenes). The resinoids exhibited a weak antimicrobial effect on the tested microorganisms, still being relatively more effective against Gram-positive bacteria and yeasts than against Gram-negative bacteria. The resinoid of Oriental tobacco was included in a model cosmetic gel formulation, characterized in terms of rheological and sensory properties. The rheological behavior of the gel was that of a non-Newtonian fluid and the inclusion of tobacco resinoid reduced gel viscosity in the applied stress range (1.5÷72.9 s^{-1}). From the evaluation procedure, it can be concluded that tobacco resinoid, a product not restricted by the Cosmetics Regulation, is a suitable ingredient of cosmetic gels, contributing to their organoleptic and functional properties.

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CONFLICT OF INTEREST

All authors state that there is no conflict of interest.

REFERENCES

Banožić M, Banjari I, Jakovljević M, Šubarić D, Tomas S, Babić J, Jokić S. Optimization of ultrasound-assisted extraction of some bioactive compounds from tobacco waste. Molecules, 2019; 24(8):1611.

Baser KHC, Buchbauer G. Handbook of essential oils: science, technology, and applications. CRC Press, Boca Raton, FL, 2010.

Bauer K, Garbe D, Surburg H. Common fragrance and flavor materials. Preparation, properties and uses. 4th edition, Wiley-VCH, Weinheim, New York, 2001.

Cosmetic ingredient database (CosIng) of the European Commission. Available via https://ec.europa.eu/growth/sectors/cosmetics/cosing_en (Accessed 30 April 2019).

Cutler HG, Reid WW, Delétang J. Plant growth inhibiting properties of diterpenes from tobacco. Plant Cell Physiol, 1977; 18:711–4.

Cvetanovska L, Cvetanovska A, Krstikj M. Production of essential oil and concret in various types of tobacco (*Nicotiana tabacum* L.). Eur J Pharm Med Res, 2018; 5(3):57–60.

Docheva MH, Popova VT, Ivanova TA, Nikolova VV, Hristeva TH, Nikolov NN. Polyphenol content and antioxidant activity of aqueous/methanol extracts from different tobacco species (*Nicotiana*). Bulg Chem Comm, 2018; 50(4):553–9.

Dyulgerski Y, Docheva M. Production characteristics and chemical indicators of perspective lines of Burley tobacco. Tutun/Tobacco, 2017; 67(1–6):41–7.

Fedorovska MI, Polovko NP, Fedenko SM, Antymys OV. Study of vasodilating and regenerative effect of the gel with nettle juice intended for Telogen effluvium treatment. J Appl Pharm Sci, 2018; 8(1):93–7.

Georgiev E, Stoyanova A. A guide for the specialist in aromatic industry. UFT Academic Publishing House, Plovdiv, Bulgaria, 2006.

Huang ZR, Lin YK, Fang JY. Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules, 2009; 14(1):540–54.

Iswandana R, Chalista W, Sitepu ES. Formulation and penetration enhancement activity of sticks containing caffeine. J Appl Pharm Sci, 2018; 8(1):43–9.

Jassbi AR, Zare S, Asadollahi M, Schuman M. Ecological roles and biological activities of specialized metabolites from the genus *Nicotiana.* Chem Rev, 2017; 117(19):12227–80.

Jufri M, Rachmadiva, Gozan M, Suyono EA. Formulation, stability test and *in vitro* penetration test of emulgel from tobacco leaves extract. J Young Pharm, 2018; 10(2)Suppl:s69–72.

Khor P-Y, Seow L-J, Fatin H. *In vitro* evaluation of photoprotective potential of the different solvent extracts of *Graptophyllum pictum* leaves. J Appl Pharm Sci, 2018; 8(1):147–51.

Korubin-Aleksoska A, Ahmad MA. Basmak – a new type of tobacco in the Balkans. IOSR J Agric Vet Sci, 2016; 9(8):12–7.

Kroumova A, Artiouchine I, Wagner G. Use of several natural products from selected *Nicotiana* species to prevent black shank disease in tobacco. Beitr Tabakforsch Int, 2016; 27(3):113–25.

Mookherjee B, Wilson R. Tobacco constituents—their importance in flavor and fragrance chemistry. Perfum Flavor, 1990; 15:27–49.

Nugroho L, Verpoorte R. Secondary metabolism in tobacco. Plant Cell Tissue Organ Cult, 2002; 68:105–25.

Popova V, Gochev V, Girova T, Iliev I, Ivanova T, Stoyanova A. Extraction products from tobacco—aroma and bioactive compounds and activities. Curr Bioact Compd, 2015; 11(1): 31–7.

Popova V, Ivanova T, Stoyanova A, Georgiev V, Hristeva T, Nikolova V, Docheva M, Nikolov N, Damianova S. Phytochemicals in leaves and extracts of the variety "Plovdiv 7" of Bulgarian oriental tobacco (*Nicotiana tabacum* L.). Trends Phytochem Res, 2018; 2(1):27–36.

Radoukova T, Dyulgerski Y. Biological indicators of Bulgarian and introduced Burley tobacco varieties. Bulg J Agric Sci, 2018; 24(6):1059–64.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council, of 30 November 2009, on Cosmetic Products. 2009. Available via https://ec.europa.eu/health/sites/health/files/endocrine_disruptors/docs/cosmetic_1223_2009_regulation_en.pdf (Accessed 30 April 2019).

Rodgman A, Perfetti T (eds.). The chemical components of tobacco and tobacco smoke. 2nd edition, CRC Press, Boca Raton, FL, 2013.

Ru Q-M, Wang L-J, Li W-M, Wang J-L, Ding Y-T. In vitro antioxidant properties of flavonoids and polysaccharides extract from tobacco (*Nicotiana tabacum* L.) leaves. Molecules, 2012; 17:11281–91.

Sarkic A, Stappen I. Essential oils and their single compounds in cosmetics—a critical review. Cosmetics, 2018; 5(11):1–21.

Seo S, Gomi K, Kaku H, Abe H, Seto H, Nakatsu S, Neya M, Kobayashi M, Nakaho K, Ichinose Y, Mitsuhara I, Ohashi Y. Identification of natural diterpenes that inhibit bacterial wilt disease in tobacco, tomato and arabidopsis. Plant Cell Physiol, 2012; 53(8):1432–44.

The State Pharmacopoeia of the USSR. 11th edition, Medicina, Moscow, Russia, 1990.

Tsai S-W, Hsieh M-C, Li S, Lin S-C, Wang S-P, Lehman CW, Lien CZ, Lin C-C. Therapeutic potential of sclareol in experimental models of rheumatoid arthritis. Int J Mol Sci, 2018; 19:1351.

Tumbarski Y, Deseva I, Mihaylova D, Stoyanova M, Krastev L, Nikolova R, Yanakieva V, Ivanov I. Isolation, characterization and amino acid composition of a bacteriocin produced by *Bacillus methylotrophicus* strain BM47. Food Technol Biotech, 2018; 56(4):546–52.

Tumbarski Y, Lincheva V, Petkova N, Nikolova R, Vrancheva R, Ivanov I. Antimicrobial activity of extract from aerial parts of potentilla (*Potentilla reptans* L.). Industrial Technologies, 2017; 4(1):37–43.

Tzaneva D, Djivoderova M, Petkova N, Denev P, Hadzhikinov D, Stoyanova A. Rheological properties of the cosmetic gel including carboxymethyl chitosan. J Pharm Sci Res, 2017; 9(8):1383–7.

Vijayabhaskar K, Sammaiah G, Ramadevi V, Kumar GP, Chaitanyaprasad K, Bharath G. Development and estimation for formulations of microbial bio transformed extract of *Nicotiana tabaccum* roots (Leguminosae) for hair growth potential. Int J Pharm Chem Biol Sci, 2014; 4(3):686–90.

Wahlberg I, Enzell CR. Tobacco isoprenoids. Nat Prod Rep, 1987; 4:237–76.

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