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Bulgarian contribution to the investigation of natural aromatic products: a brief retrospective review

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Bulgaria is one of the countries with established traditions in the production and processing of aromatic (essential oil-bearing) and medicinal plants, as well as in the investigation of the respective plant-derived products. These natural aromatic products (such as essential oils, concretes, absolutes, and other extraction concentrates) combine valuable olfactory profiles and beneficial biological and pharmacological activities, which substantiate their use in perfumery, cosmetics, aromatherapy, medicine, food, and many other areas. Therefore, this work presents a brief retrospective review (1900 - 2019) of the achievements of Bulgarian researchers in the study of the chemical composition, the antimicrobial, antioxidant and other activities of traditional and contemporary natural aromatic products, obtained from different - indigenous or uncommon to the country - medicinal and aromatic plants. The review does not claim to be exhaustive in terms of Bulgarian research achievements in the indicated timespan, neither has the objective to cover world research on aromatic and medicinal plants.

Keywords: medicinal and aromatic plants, natural aromatic products, biological activity, Bulgaria

INTRODUCTION

Bulgaria has the prerequisites – abundant flora, favorable climate, and socio-economical potential – to produce valuable, high quality aromatic products derived from aromatic, medicinal and edible plants. Indeed, such production has been a fact for more than 350 years. The country is recognized worldwide for the aromatic products obtained from plants like Damask rose (*Rosa damascena* Mill.), lavender (*Lavandula angustifolia* Mill.), peppermint (*Mentha piperita* L.), bigroot geranium (*Geranium macrorrhizum* L.), thyme (*Thymus vulgaris* L.), chamomile (*Matricaria chamomilla* L.), *etc*. [1].

It should be outlined that one and the same plant material can be processed by different methods, thus obtaining aromatic products, which have substantially different chemical composition, olfactory and other properties, and subsequently – different use potential. Processing of rose flowers by distillation, for example, yields rose oil and rose water, by traditional solvent extraction – rose concrete and rose absolute, and these four natural aromatic products are diametrically different in their composition and properties. Common (traditional, standardized) aromatic products are obtained from essential oil-bearing plants by well-established techniques, and they generally include: essential oils – obtained by hydro- or steam distillation or cold pressing of citrus rinds; concretes – by extraction with nonpolar organic solvents, which are subsequently removed; absolutes – by re-extraction of concrete with polar solvents, and some others (resinoids, oleoresins, tinctures). Thus, the qualitative and quantitative composition of the aromatic products, and respectively – their physical, chemical, and biological properties, depend greatly on the isolation procedure. These aromatic products are mixtures of many substances originating from the metabolism of terpenes, phenylpropanoids, amino and fatty acids, and other phytochemicals [1].

This work presents a brief review of the achievements of Bulgarian authors (1900 – 2019) in the investigation of the biological properties of natural aromatic products and their application in cosmetics. The review highlights the leading research trends of the respective time period, with a focus on the following aspects of the characteristics of traditional and new aromatic products:

i) chemical composition and identification of fragrance compounds;

ii) antimicrobial and antioxidant activity;

iii) potential application in different cosmetic products.

The authors of this brief review would like to emphasize that its objective has not been to cover the extensive international research on the products obtained from the plants referenced herein or to neglect the numerous publications of authors around the world, but rather to present the less popular research of Bulgarian scientists to the wider

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international audience. We are truly convinced that despite the relatively small market share of the aromatic products produced in Bulgaria on a worldwide basis, there has been an undoubted contribution of Bulgarian scientists to the development of aromatic plants research not only in the past, as is the case with the famous Bulgarian rose, but also in present days. This brief review, too, does not claim to be exhaustive or to provide historical coverage of all achievements of Bulgarian researchers on natural aromatic products investigation in the indicated timespan; due to the limited volume of the article, many authors have probably remained unmentioned, for which we sincerely apologize.

1900 – 1940. The period of launching the first studies on certain indices of Bulgarian essential oils

- The oleaginous (oil-yielding) rose (*R. damascena*) has been introduced in Bulgaria as early as the 14th century, while the industrial processing of rose sets its beginning in the middle of 17th century. The emblematic production and the reputation of the Bulgarian rose essential oil awarded the country the brand "Bulgaria – the land of the oleaginous rose".
- Lavender (*L. angustifolia*) was introduced in 1903 and its cultivation for industrial purposes began soon after that, with the first few tonnes of lavender oil being produced in 1925 year.
- The first investigations about the physical and chemical properties of rose oils [2-7] and of peppermint and hyssop oils [8] are conducted.

1940 – 1970. The beginning of systematic research of Bulgarian essential oils

The period marks the beginning of systematic research focused on different aspects of the process of obtaining the traditional essential oils and on understanding their biological activities. A series of studies have been published, which reveal:

- The impact of different technological factors on the yield and the quality of Bulgarian rose oil [9-13] and lavender oil [14];
- The chemical composition of the aromatic products from rose [15-31], lavender [32, 33], pine [34-37], tobacco [38-45].
- The first data from pharmacological and clinical studies about the therapeutical properties of rose oil [46, 47].
- The antimicrobial activities of rose oil [48-57], of thyme, pelargonium, ajowan, and coriander oils [56-58], and of pine oil [59].
- Based on their wide variety of bioactivities (antibacterial, antimycotic, anti-inflammatory, antioxidant, and other), infusions and essential oils from medicinal and aromatic plants have been used in different cosmetic products - unguents [55] and hair-care products [59, 60]. Rose products (water, oil and concrete) have been used in a number of formulations for oral hygiene and stomatology [53].

1970 – 1990. Continued studies on traditional and new aromatic plants; beginning of studies on waste recovery

During this period, continue the studies on the technology, the chemical composition and the biological activities of the traditional rose and lavender essential oils, as well as those of new aromatic plants. The investigations on the use potential of plant waste begin.

- The influence of different technological factors on the yield and quality of different essential oils has been followed by a number of researchers: rose essential oil [61-63], lavender oil [64].
- The chemical composition of the traditional aromatic products has been investigated by different authors – products from rose [65-75], from lavender [76].
- The antimicrobial activities of rose oil and its major compound geraniol [77-79], of lavender, savory, peppermint, basil, and dill oils [80-83] have been established.
- Investigations of the waste remaining after the distillation and extraction of rose oil [84-89] and from rose and lavender waste after extraction and distillation [90] have been conducted.
- The use of extracts and essential oils as functional ingredients in different cosmetic products has been investigated – skin-care creams [91-95] and products for oral hygiene [96-98].

1990 – 2019. A period of disturbance and transition (1990-2000) and a new period (2000-2019) of marked revival in the research of traditional and novel aromatic products

The period between 1990 and 2000 reflected the post-communist disturbance and transition processes in the political, social and economic life in Bulgaria. For more than a decade after the political changes in the country, there has been a period of relative stagnation in the clinical studies of the traditional for Bulgaria aromatic products. The new period of marked revival in the research of traditional and novel aromatic products and their biological activities paralleled the beginning of the 21st century.

- The chemical composition and antimicrobial activities of essential oils obtained from a wide range of aromatic and medicinal plants, either traditional or nontraditional for Bulgaria, have been identified in numerous studies [99-127].

- In the years after 2000, many Bulgarian authors and their collaborating foreign partners have revealed the antimicrobial activity of the individual main components of different aromatic products against various microorganisms. It is determined that Bulgarian essential oils (rose, lavender, peppermint, basil, sage, oregano, *etc.*), demonstrate high antimicrobial activity against various pathogenic and spoilage microorganisms, belonging to the groups of Gram-possitive and Gram-negative bacteria, dimorph yeasts and fungi. It is of great importance that Bulgarian essential oils from these essential oil-bearing plants achieve antimicrobial activity against fluconazole resistant strains of *Candida albicans* and non-albicans Candida strains and Methicillin-resistant *Staphylococcus* strains. The obtained results expand the possibilities for application of Bulgarian essential oils not only as flavouring or perfuming agents in food industry and cosmetics, but even as preparations with wide pharmacological importance [128-149].

- Along with these studies, the antioxidant activity of various traditional or non-traditional for Bulgaria aromatic products, as well as that of their main individual components, has been investigated [150-155].

- A distinct trend in the period has been the continuation of investigations on the chemical composition and the biological activities of different waste materials, with the aim of their valorization and prospective use; for example, those on rose waste [156-174].

- Essential oils and extracted aromatic products from traditional and new medicinal and aromatic plants have been incorporated in different skin-care products; creams [148] and lotions [126].

- A new step in the development of aromatic products in Bulgaria has been the introduction of low-temperature extraction with liquefied gases. The main achievements of Bulgarian researchers in the field are discussed in more detail below.

Low-temperature extraction with liquefied gases

In many countries nowadays, essential oil bearing plants are processed by extraction with liquefied gases (CO_2, air, freons, and others). The produced extracts are considered harmless, and therefore they can be widely used in food and flavour industry, cosmetics and medicine. The use of liquefied gases overcomes some of the major drawbacks of installations working with volatile polar and nonpolar solvents [175-177].

There are currently six extracting installations working with liquified gases in Bulgaria. Three of them are operating with supercritical CO_2, situated respectively in the town of Dimitrovgrad, in the village of Mirkovo, Sofia region, and in the Bulgarian Academy of Sciences, Sofia. The other three installations (two industrial - in the town of Pavel Banya, Kazanlik region and in the city of Plovdiv, and one laboratory - at the University of Food Technologies in Plovdiv) are operating with 1,1,1,2-tetrafluoroethane (known also as hydrofluorocarbon-134a, HFC-134a, and freon 134a). These installations have been used in the last decades for processing different essential oil bearing plants – rose, lavender, peppermint, chamomile, sage, hyssop, juniper, and many others.

Extraction with CO_2

- In the last 15 years the installation in the town of Dimitrovgrad, Bulgaria has been set to obtain extracts from different essential oil bearing and medicinal plants, representing both industrially processed plant materials (e.g. fennel, coriander) and non-traditional for the country, experimentally processed ones (e.g. linden, hop). The specified plant materials have been processed for the purpose of providing an alternative of the respective imported CO_2 extracts in different fields of application, e.g. as ingredients in cosmetic preparations, in foods and drinks, and others. A summary of the essential oil bearing and medicinal plants processed on the installation is presented in a chronological order in Table 1, as well as data about the yield and the chemical composition (main components, above 3%) of the extracts.

- The research carried out by Bulgarian scientists confirms that CO_2 is a suitable extractant for processing plant materials that are rich in thermolabile components and therefore are not processed through high-temperature distillation, like linden.

- All of the obtained CO_2 extracts revealed antimicrobial activity against different test-microrganisms [184], and according to the data, their antimicrobial activity was similar to that of the respective essential oils.

- To the best of our knowledge, no data have been published about the inclusion of the obtained CO_2 extracts in cosmetic or other products, since they were considered economically not competitive to the imported ones.

Table 1. Yield and composition of extracts obtained with CO_2

Plants	Main components (above 3%), %	Yield, %	Ref.
Coriander	linalool[1] (71.6), α-pinene (6.3)	0.8	[178]
Fennel	anetole (72.3), fenchone (11.3)	1.4	[182]
Hop	*α*-acids (36.1), *β*-acids (19.5)	9.7	[179]
Ginger	*α*-zingiberene (36.9), *β*-phellandrene (15.3), *β*-bisabolene (8.8), (*E,E*)-*α*-farnesene (7.0), *α*-curcumene (6.6), camphene (3.2)	3.7	[180]
Black pepper	*β*-caryophyllene (43.9), limonene (17.1), *δ*-3-carene (11.8), *α*-phellandrene (4.4), *allo*-aromadendrene (3.3), myrcene (3.1)	2.2	[180]
Linden	benzaldehyde (14.2), phenylethyl alcohol (9.8), 2-methylpropanal (7.1), n-hexanal (4.8), *trans*-pentenal (4.3), ethylalcohol (3.9), nonanal (3.2)	1.0	[181]

[1]Allergic fragrance in accordance with [183]

Extraction with tetrafluoroethane (TFE)

- To the best of our knowledge, the research group from Bulgaria has been among the pioneers, who started to use and systematically studied 1,1,1,2-tetrafluoroethane as a low-temperature extractant of aromatic and medicinal plants.

- For the last 15 years, more than 25 different essential oil bearing and medicinal plants have been processed in the laboratory installation situated at the University of Food Technologies in Plovdiv, Bulgaria. The set of processed plant materials includes species traditional for the country [185-187], as well as non-typical for the essential oil industry aromatic plants and spices [126, 180, 188-208]. The former group has been analysed mainly with the view of comparison and providing of alternatives to the established products, and the latter group – with the purpose of diversifying the range of available aromatic products.

- Data about the yield, chemical composition (components in concentration higher than 3%) and antioxidant (DDPH radical scavenging) activity of some of the obtained extracts are listed chronologically in Table 2.

Table 2. Yield, composition and antioxidant activity of extracts with 1,1,1,2-tetrafluoroethane

Plants	Main components (above 3%), %	Yield, %	IC_{50}, mg/mL[1]	Ref.
Black pepper	*β*-caryophyllene (56.8), limonene[2] (18.9), terpinolene (3.9), *p*-cymene (3.1)	1.6	-[3]	[180]
Ginger	*α*-zingiberene (42.1), *β*-phellandrene (14.2), *β*-bisabolene (11.3), *cis*-menth-2-en-1-ol (9.8), *α*-curcumene (8.5), (E,E)-*α*-farnesene (5.9)	1.1	-	[180]
Anise	anethole (92.9-93.4)	2.7	8.3	[188]
Linden	phenylethyl alcohol (24.7), phenylethyl benzoate (5.7), nonadecane (10.8), heneicosane (9.1), tricosane (8.0), phytol (6.0), citronellol[2] (3.2)	0.1	-	[189]
Hyacinth	benzyl benzoate[2] (23.0), cinnamyl alcohol[2] (12.8), benzyl acetate (6.4), *p*-cumin-aldehyde (5.4), *p*-cymene (5.0), *α*-terpinene (4.7)	0.2	-	[190]
Lilac	*from violet lilac*: lilac alcohol (13.6), 1,2,4-trimethoxy benzene (9.0), squalene (8.0), tricosane	1.2	-	[191]

	(7.3), methyl eugenol (6.6), geranil geraniol (5.7), elemicin (4.4), lilac alcohol D (4.2), palmitic acid (3.7), 8-hydroxy linalool (3.5)			
	from white lilac: lilac alcohol (8.6), 1,2,4-trimethoxy benzene (10.1), squalene (6.9), tricosane (6.1), geranil geraniol (4.0), lilac alcohol D (7.0), palmitic acid (4.8), *β*-pinene (3.8)	1.0	-	[191]
Corian-der	linalool[2] (71.4), *γ*-terpinene (7.8), *α*-pinene (5.9), geranyl acetate (4.0)	60-80[4]	17.7	[192]
Cinna-mon	cinnamal[2] (77.3), coumarin[2] (4.3)	50-60[4]	0.4	[193]
Fennel	anetole (68.3), fenchone (17.7)	25-30[4]	6.0	[194]
Carda-mom	terpinyl acetate (36.8), 1,8-cineole (29.2), linalyl acetate (5.2), sabinene (3.9), linalool[2] (3.1)	30-35[4]	63.3	[195]
Cumin	*γ*-terpinene (23.5), *p*-cymene (22.2), cumin aldehyde (18.8), *β*-pinene (15.3), *p*-mentha-1,4-dien-7-al (5.9), linalool[2] (4.3), *α*-pinene (3.1)	3.8-4.0	6.4	[154]
Pimento	methyl eugenol (55.9), myrcene (14.7), eugenol[2] (9.6), *β*-caryophyllene (4.4)	100-130[4]	< 0.1	[197]
Clove	eugenol[2] (69.7), eugenyl acetate (13.4), *β*-caryophyllene (9.3)	8-12[4]	<0.1	[198]
Lilium	1-hexacosanol (21.1), 1-octacosanol (20.4), n-dotriacontane (6.6), n-triacontane (5.9), n-nonanal (5.5), n-nonadecane (5.0), n-pentacosane (4.7), n-tricosane (4.5), n-heptacosane (4.1)	0.1	-	[196]
Dill (herba)	D-carvon (53.1), limonene[2] (37.1)	0.7	1.2	[199]
Sage	1,8-cineole (25.2), *β*-caryophyllene (7.5), *cis*-thujone (6.7), *α*-humulene (6.1), *trans*-thujone (5.4), *β*-pinene (5.4), camphor (4.8), *allo*-aroma-dendrene (4.6), borneol (3.7), *α*-pinene (3.6), bornyl acetate (3.6)	100-150[4]	-	[200]
Sumac	limonene[2] (23.7), *α*-pinene (15.7), caryophyllene oxide (10.7), 2-hexenal (4.0), *p*-cymene-8-ol (3.6), farnesyl	0.2	-	[201]

	acetone (3.4), β-pinene (3.2)			
Marigold	α-bisabolon oxide (8.3), β-farnesene (8.1), α-bisabolon oxide A (7.3), α-pinene (6.8), γ-cadinene (5.5), *p*-cymene (5.4), γ-terpinene (5.1), δ-cadinene (5.1)	2.7-3.0	10.3	[202]
Tobacco	*from Burley tobacco*: nicotine (72.9), phytol acetate (7.8)	0.5	-	[126, 203]
	from Virginia flue-cured tobacco: nicotine (60.9), phytol acetate (20.8)	0.3	-	[126, 203]
	from Oriental tobacco: phytol acetate (9.0), acetic acid (8.7), nicotine (6.2), norambreinolide (5.7)	0.7	-	[126]
Pepper-mint	menthone (30.5), menthol (21.1), menthyl acetate (9.9), *iso*-menthone (4.4), *cis*-sabinene hydrate (3.8), 1,8-cineole (3.4), pulegone (3.0)	50-70[4]	0.4	[187]
Savory	thymol (73.9)	0.8-1.0	0.6	[204]
Basil	estragol (47.0), linalool[2] (10.6), methyl eugenol (8.9), 1,8-cineole (3.7), β-bisabolene (3.0)	350-400[4]	0.3	[205]
Thyme	geraniol[2] (20.5), thymol (14.9), carvacrol (10.3), geranyl acetate (7.4), linalool[2] (6.6), germacrene D (5.3), *p*-cymene (5.3), β-caryophyllene (3.4)	100[4]	0.2	[206]
Juniper	α-pinene (32.0), myrcene (21.1), germacrene D (8.3), sabinene (5.2), limonene[2] (4.0).	60-75[4]	43.2	[207]
Oregano	carvacrol (70.1), *p*-cymene (11.8), p-cymene-2,5-dione (3.8)	60-80[4]	-	[208]
Rose	phenylethyl alcohol (59.1), citronellol[2] (12.3)	1.2-1.5	-	[185]
Lavender	linalool[2] (32.5), linalyl acetate (23.0), borneol (5.1), *cis*-linalol oxide (4.5), (E)-β-farnesene (4.1), lavandulol (4.2), β-caryophyllene (3.3)	1.2	-	[186]

[1]DPPH radical scavenging activity; [2]Allergic fragrance in accordance with [183]; [3]Not determined; [4]Yield is given as kg raw/kg extract.

In order to evaluate the quality of the new products, a comparative analysis regarding selected representatives of traditional and non-traditional for Bulgaria plants has been carried out against current ISO standards that set up the variation limits of main or characteristic components of the produced essential oils. As a result of this comparison, it is established that the extracts are in accordance with the standards, except for the following specific features:

i) The TFE extract from lavender (clonal lavender, Bulgaria) [188] is with higher content of linalool (32.5%) and lavandulol (4.2%), and lower – of linalyl acetate (23.0%) than ISO 3515:2002 lavender oil (22.0-34.0% linalool, min. 0.3% lavandulol, and 30.0-42.0% linalyl acetate, respectively);

ii) The TFE extract obtained from peppermint [187] is with higher content of menthone (30.5%) and menthyl acetate (9.9%), and lower content of menthol (21.1%) than ISO 856:2006 peppermint oil (13.0-28.0% menthone; 2.0-8.0% menthyl acetate, and 32.0-49.0% menthol, respectively);

iii) The TFE extract from sage [200] is with lower content of α-thujone (5.7%) and camphene (0.8%), and higher – of 1,8-cineole (25.2%) and bornyl acetate (3.6%) than ISO 9909:1997 sage oil (18.4% α-thujone, 1.5-7.0% camphene, 5.5-13% 1,8-cineole, and max. 2.5% bornyl acetate, respectively);

iv) According to the qualitative and quantitative content of the major constituents the produced TFE rose extract [185] is significantly different from the essential oil according to ISO 9842:2003, but is almost equal to the rose absolute [1];

v) The TFE extracts obtained from some typical spice species, such as anise [188], cardamom [195], coriander [192], *etc.* match up the major constituents pointed out as characteristic of the respective standardized oils, ISO 3475:2000, ISO 4733:2004, and ISO 3516:1997.

Thus, it can be summarized that 1,1,1,2-tetrafluoroethane extraction has been found appropriate for a set of essential oil bearing and medicinal plant materials, which either:

i) Contain thermolabile components and are not processed by distillation, like lilac, hyacinth, and linden;

ii) Require low pH of distillation water, like tobacco, or

iii) Represent fruits containing both essential and glyceride oil, for example – black pepper, coriander, cumin, anise, fennel, and dill.

- Some of the new aromatic products – CO_2 and TFE extracts, comprise representatives of the list of potentially allergenic substances defined by the Cosmetics Regulation No 1223:2009 [183] (Table 1 and Table 2). Although being main ingredients of the extracts, their concentration does not exceed the range specified by the respective ISO standards.

- The comparative analysis of data justifies the conclusion that the extracts obtained with CO_2 and TFE from the same plant material (as in the case of coriander, fennel, ginger, black pepper, and linden) possess different, characteristic chemical composition, which could be attributed to the selectivity of the solvent applied [175-177].

- The analysis of the references cited above reveals that the antimicrobial activity of respective TFE extracts has been determined, against different pathogenic and spoilage bacteria and yeasts, from clinical and food isolates and reference strains. All studied extracts demonstrated antimicrobial activity against the tested microorganisms, although lower in comparison with the common preservatives and antioxidants used in cosmetics. Extracts characterized with the highest content of thymol (thyme and savory) and of eugenol (clove and pimento), identified as the major constituents responsible for the antimicrobial activity of the extracts, have demonstrated the highest activity. The antimicrobial activity of the extracts was similar to that of the respective essential oils, as established by the Bulgarian authors cited earlier, as well as by the authors referenced in [209].

- Approximately half of the obtained TFE extracts have been analysed with respect to their antioxidant properties (mainly by determining the DPPH radical-scavenging activity). The extracts from clove, pimento, thyme, basil, peppermint, cinnamon, and savory have demonstrated higher antioxidant activity than the rest of the extracts, which is attributed to the higher concentration of eugenol [109, 198], thymol [153, 204], cinnamal [110, 193], menthol [142, 187], and other active components [115, 197, 205, 206].

- The produced TFE extracts from black pepper, cardamom, cumin, and coriander have been successfully included as antioxidants in different meat products [210-212] and as preservatives in food emulsions [213].

- The produced TFE extracts from lavender have been successfully included as antimicrobial agents in cosmetic emulsions of the type oil/water [188].

Therefore, it can be summarized that – given the pronounced antimicrobial and antioxidant properties of the extracts – they can be incorporated in various cosmetic preparations (creams, gels, *etc.*) as successful substitutes of chemical preservatives and antioxidants, or as substitutes of perfume in natural and biocosmetic products. As these extracts do not yield to the respective essential oils by biological activity (antimicrobial, antioxidant, *etc.*), they can easily be considered as promising bioactive components for incorporation in cosmetic products with anti-aging, anti-inflammatory, and other beneficial effects.

CONCLUSIONS

The review of the scientific publications by Bulgarian authors in the period 1900-2019 demonstrates that there have been established historic and sound traditions in the obtaining of aromatic products from a plethora of indigenous and uncommon to the country essential oil bearing plants. The same is true for the study of their chemical composition and biological activity with a view to their application in cosmetic and other products.

For the last 15 years, there has been a clear trend of expanding the range of available plant-derived ingredients by introducing innovative methods for obtaining aromatic products worldwide, and Bulgarian scientists have contributed to that trend.

The scientific achievements in the study of CO_2, tetrafluoroethane and other extracts today are far from being comprehensive, but undoubtedly open doors to their industrial obtaining and to the production of innovative bio and natural cosmetics in Bulgaria.

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