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Research Article

Profiling of *Rosa hybrida* cv. Mileva essential oil, evaluation of its bioactivity *in vitro*, chemometric analysis and comparison to other non-commercial roses

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Abstract
Rose essential oil is one of the most valuable and pivotal raw materials in the perfume industry. However, the limited yield of essential oil in rose flowers increased the interest of both breeders and scientists to create and discover species that have potential for multifaceted applications aiming to increase the economic viability of rose cultivation. Consequently, garden roses (*Rosa hybrida*) through ongoing development emerge as possibly profitable for cultivation and processing. This opens possibilities for numerous applications in both food and pharmaceutical industries. The main objective of this study was to identify the essential oil composition of *R. hybrida* cv. Mileva and compare it against essential oil chemical compositions of 32 accessions of other non-commercial (wild and hybrid) roses available literature. The dominant compound detected in *R. hybrida* cv. Mileva essential oil was geranyl acetate (47.9%), followed by nonadecane and heneicosane. It was shown that the essential oil possesses moderate antimicrobial activity, good antioxidant potential and strong anti-inflammatory activity.

Keywords
Antimicrobial activity, Antioxidant potential, Anti-inflammatory activity, GC-MS, Unrooted cluster tree

Introduction

Genus *Rosa* encompasses a large number of species endemic to temperate regions of the Northern Hemisphere. However, only a small number of them have commercial value, and are therefore domesticated and cultivated worldwide[1]. Nowadays, modern roses (created by artificial hybridization) bear great importance for floriculture (as roses are commercially the most important cut flower on the global market), landscape horticulture, agriculture and agrotourism, as well as for various types of related industries[2,3]. Some roses are cultivated for their flowers (dried for tea, fresh for essential oil, absolute and concrete extraction), while others are cultivated for fruit (rosehips)[4,5].

The Damask rose (*Rosa damascena* Mill.) is the most well-known type of rose. It is widely used in traditional medicine, perfumery, and aromatherapy. Due to low essential oil yield, the hydrolate (a valuable by-product of distillation process) commonly known as rose water, is also widely used for flavoring food and beverages as well as for skin care cosmetics[6]. However, growing *R. damascena* requires specific agro-ecological conditions. These conditions can be found in areas known as rose valleys; located in

Bulgaria (Kazanlik valley, between mountains of Stara planina and Sredna gora), Turkey (Burdur and Isparta province), Iran (Layzangan valley of Fars) and India (Kashmir valley)[7,8].

Apart from *R. damascena*, several other species have been subjected to investigations regarding their essential oil content and composition. Depending on the area where a species grows spontaneously or is traditionally cultivated as an ornamental, fruit-bearing, or oil-bearing plant, there are distinct differences between European, Near Eastern, Southwest Asian, Japanese and Chinese varieties[9,10]. Due to the low yield of essential oil, the limited area of distribution of *R. damascena*, as well as the growing demand of the perfumery and cosmetic industry for new naturally sourced fragrances, scientists and breeders are focused on identifying and creating other species within the *Rosa* genus as essential oil bearing species or for multifaceted uses (essential oil, fruit, cut flowers, etc.). Research on the chemical composition of scented roses (*R. hybrida*) has focused on classification of rose flower aromas, changes in volatile profile of rose petals during processing, as well as on its hydrosol composition[6,11,12]. Studies that compare aromas with other *Rosa* sp. are quite rare, as are investigations into their biological activity and potential application.

Hybrid roses typically exhibit distinct flower colors and shapes, vase longevity, characteristic fragrances, resistance to disease, unremitting flowering, winter hardiness, and absence of thorns[13,14]. Furthermore, due to their good adaptability to different environmental conditions, hybrid roses can be found worldwide as ornamental, oil-bearing, or edible plants. There are ongoing efforts to develop new rose hybrids[15].

Numerous preceding studies focused on the chemical composition essential oil of *R. damascena* essential oil, as it is commercially the most important oil-bearing species. However, only few studies investigated essential oil composition of wild[16-33], or hybrid roses[23,24,28,34-36]. As a result, the consequences of hybridization on the volatile profile of roses relative to that of their parent wild species remain understudied. Such research could aid breeders develop hybrids bearing a particular scent, this study focused on isolating the essential oil of *R. hybrida* cv. Mileva and determining its chemical composition. In addition, antioxidant, anti-inflammatory and antimicrobial activities of the oil were examined. Motivated by the fact that there are no comprehensive reviews on the composition of essential oils of rose species other than *R. damascena*, this study also aimed to collect such data from the available literature sources (*R. alba*, *R. banksiae*, *R. brunonii*, *R. canina*, *R. centifolia*, *R. corymbifera*, *R. foetida*, *R. gallica*, *R. moschata*, *R. multiflora*, *R. phoenicia* and hybrids between *R. sertata* × *R. rugosa* and *R. damascena* × *R. gallica*, as well as modern rose cultivars of *R. hybrida*) and probe the existence of chemotypes within the genus *Rosa* by chemometrics.

Material and methods

Plant material and extraction

R. hybrida cv. Mileva™ Frayla® (Voucher No 2-0693, BUNS Herbarium, University of Novi Sad), is a shrub with large pink petals arranged in double flowers, which bloom from May to October. Plants were cultivated under natural day-length conditions on an open field in Temerin (45°23'24.7" N, 19°53'36.0" E). Fully developed flowers were collected in June 2021, in the early morning. Immediately after harvest, fresh flowers were subjected to steam distillation; a detailed description of the equipment and method was given previously[6].

Analysis of R. hybrida essential oil

Chemical composition of rose essential oil after dilution in ethanol (1%) was determined by using analytical gas chromatography/flame ionization detection (GC HP 5890 series II equipped with FID and a column HP-5 MS; 25 m × 0.32 mm ID × 0.52 μm film thickness, He carrier gas; 1.0 mL/min). The injector temperature was 250°C, and the oven temperature was increased from 40 to 260°C at 4°C/min, the transfer line was operated at 300°C. The gas chromatography/ mass spectrometry (GC HP G1800C with

MS detector HP 5971 A) parameters were as follows: ion source temperature, 260°C; electron impact, 70 eV; scan mass range, 40-450 m/z. The percentage of each rose oil compound was computed from GC/FID peak areas, while identification of each compound was done by comparison of experimentally obtained retention indices and mass spectra with those of substance standards deposited in MS libraries (Wiley 11, NIST17, MassFinder 2.3).

In vitro antioxidant activity

The antioxidant activity of *R. hybrida* essential oil was determined spectrophotometrically by using three different assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and reducing power (RP). In summary, for DPPH test rose essential oil was diluted with methanol and centrifuged then mixed with stock solution dissolved in methanol[37]. For ABTS test, rose essential oil was mixed with a stock solution dissolved in acetate buffer[38]. For RP test, the mixture of rose essential oil, distilled water, phosphate buffer and potassium ferricyanide was incubated at 50°C for 20 min and then rapidly cooled, added trichloroacetic acid and then centrifuged. An aliquot, mixed with distilled water and iron(III) chloride[38].

The antioxidant activity was evaluated in 96-well micro-plates by measuring the variation in absorbance at 515 nm after 50 min of reaction for DPPH, at 414 nm after 35 min for ABTS, and at 700 nm after 10 min for RP assay. All tests were performed in triplicate, and the results were expressed as millimoles (mmol) of Trolox equivalent (TE) per 1 g of essential oil.

In vitro anti-inflammatory activity

The anti-inflammatory assay was performed using egg albumin as a source of protein and phosphate-buffered saline. The *R. hybrida* essential oil was incubated in a water bath at 37°C for 15 min and heated at 70°C for 5 min[39]. Diclofenac sodium was used as a reference drug. The minimal concentration of a drug that is required for 50% inhibition of protein denaturation *in vitro* was calculated (IC_{50}).

In vitro antimicrobial activity

The antibacterial efficiency of *R. hybrida* essential oil was tested against six various gram-positive and gram-negative microorganisms: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella* Typhimurium (ATCC 13311), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 19433) and *Listeria monocytogenes* (ATCC 19115). All cultures were preserved in glycerol as cryoprotectant and kept at a temperature of -80°C in the culture collection of the Laboratory for Microbiology, Faculty of Technology Novi Sad, Serbia. Prior to analysis, the cultures were grown on Müller-Hinton Agar (HiMedia, Mumbai, India) at 37°C for a day.

The disc-diffusion method was applied to test the antimicrobial efficiency of the *R. hybrida* essential oil. Summarily, the freshly prepared suspensions of microorganisms were used for inoculation of Müller-Hinton Agar and after the solidification of nutrient media in Petri dishes the 15 µL of tested *R. hybrida* essential oil was applied onto three sterile discs previously applied onto the medium. As a negative control, sterile distilled water was used, while as a positive control, commercially available antibiotic tetracycline (30 µg/mL, Sigma-Aldrich, St. Louid, MO, USA) was chosen.

Data collection and chemometric analysis

Chemical composition of essential oil from flowers of *R. alba, R. banksiae, R. brunonii, R. canina, R. centifolia, R. corymbifera, R. foetida, R. gallica, R. hybrida, R. moschata, R. multiflora, R. phoenicia* and hybrids between *R. sertata* × *R. rugosa* and *R. damascena* × *R. gallica*, as well as different cultivars of garden roses (*R. hybrida*) were collected via PubMed, Scopus, Web of Science and Google Scholar. A total of 20 articles with 32 accessions (published between 1990 and 2021) have been dedicated to the essential oil composition of the aforementioned species. The data from this study and the literature (Table 1) were used for statistical analysis; the Euclidean distances between all the accessions were calculated and the distance matrix was modified by applying an unrooted tree diagram (package "APE" version "R-4.3.1").

Table 1. Percent abundances of major volatiles in different Rosa sp. according to the literature data

No	Species	Citronellol	Dodecane	Eicosane	Eugenol	Ggeraniol	Geranyl acetate	Heneicosane	Heptacosane	Heptadecane	Heptadecene	Hexadecanol	Nerol	Nonadecane	Nonadecene	Pentacosane	Phenylethyl alcohol	Tricosane
1	*R. rugosa* var. *Plena*[20]	60.0	nd	nd	0.3	8.6	nd	nd	nd	nd	nd	nd	2.8	nd	nd	nd	nd	nd
2	*R. centifolia*[21]	22.9	nd	1.5	nd	26.7	1.9	6.3	1.0	0.8	nd	nd	14.3	11.2	nd	1.2	0.6	2.9
3	*R. brunonii*[22]	2.7	nd	4.4	30.1	10.5	1.4	0.3	nd	0.6	nd	nd	nd	3.5	nd	nd	nd	nd
4	*R. banksiae*[19]	nd	41.0	nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.8	nd
5	*R. centifolia*[23]	54.7	nd	nd	nd	2.7	2.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	30.7	nd
6	*R. borboniana*[23]	27.2	nd	nd	nd	1.4	4.2	nd	nd	nd	nd	nd	0.5	nd	nd	nd	40.2	nd
7	*R. hybrida*[23]	12.9	nd	nd	nd	3.0	14.6	nd	nd	nd	nd	nd	1.4	nd	nd	nd	47.2	nd
8	*R. centifolia*[24]	33.6	nd	nd	1.2	19.2	1.9	4.2	nd	3.7	nd	nd	3.8	13.4	nd	nd	3.2	nd
9	*R. sertata* × *R. rugosa*[24]	31.7	nd	nd	0.9	16.0	1.4	7.1	nd	0.2	nd	nd	7.6	16.9	nd	nd	1.3	nd
10	*R. hybrida*[34]	40.4	nd	0.3	5.7	0.7	nd	nd	nd	nd	nd	nd	nd	2.0	nd	nd	nd	nd
11	*R. hybrida*[34]	34.2	nd	0.3	4.9	0.7	nd	nd	nd	nd	nd	nd	nd	8.3	nd	nd	0.1	nd
12	*R. hybrida*[34]	37.3	nd	0.3	8.7	0.8	nd	nd	nd	nd	nd	nd	nd	1.7	nd	nd	0.2	nd
13	*R. hybrida*[34]	39.0	nd	0.2	6.9	0.8	nd	nd	nd	nd	nd	nd	nd	3.9	nd	nd	0.2	nd
14	*R. alba*[16]	13.4	nd	1.9	nd	9.1	nd	17.5	1.7	nd	nd	nd	4.3	15.1	6.8	1.5	nd	4.7
15	*R. canina*[25]	nd	nd	0.6	45.1	nd	nd	4.4	nd	0.4	6.0	nd	nd	6.5	0.4	2.7	13.6	nd
16	*R. gallica*[26]	39.6	nd	1.3	0.1	1.3	0.1	13.2	0.2	0.7	nd	nd	0.1	15.3	0.8	5.4	3.1	7.4
17	*R. moschata*[27]	nd	nd	0.4	38.6	nd	nd	21.6	nd	1.1	nd	nd	nd	5.0	7.7	nd	nd	5.4
18	*R. moschata*[27]	nd	nd	0.2	36.9	nd	nd	20.5	nd	1.3	nd	nd	nd	5.8	10.4	0.9	nd	5.1
19	*R. alba*[28]	30.9	nd	1.2	nd	8.8	0.7	8.1	0.7	nd	0.6	nd	5.0	11.8	5.9	0.6	0.3	1.2
20	*R. damascena* × *R. gallica*[28]	8.1	nd	0.8	nd	23.3	0.6	7.7	0.4	nd	0.7	nd	12.1	18.8	1.8	0.6	1.3	2.2
21	*R. alba*[29]	9.0	nd	1.4	nd	18.3	nd	13.0	nd	nd	nd	nd	7.7	10.8	4.3	nd	nd	3.1
22	*R. foetida*[30]	nd	nd	nd	nd	nd	nd	nd	nd	2.0	16.0	nd	nd	28.0	nd	nd	nd	nd

Table 1 cont.

No	Species	Citronellol	Dodecane	Eicosane	Eugenol	Ggeraniol	Geranyl acetate	Heneicosane	Heptacosane	Heptadecane	Heptadecene	Hexadecanol	Nerol	Nonadecane	Nonadecene	Pentacosane	Phenylethyl alcohol	Tricosane
23	*R. foetida*[31]	nd	nd	nd	nd	nd	nd	16.7	nd	4.4	nd	18.6	nd	25.8	nd	nd	nd	nd
24	*R. moschata*[32]	0.9	nd	3.8	2.2	1.1	nd	21.1	nd	2.7	2.4	nd	nd	12.7	34.8	0.2	1.9	1.6
25	*R. brunonii*[17]	2.7	nd	0.6	23.9	19.2	0.2	7.7	1.1	0.7	nd	3.9	nd	6.4	nd	0.9	0.9	3.2
26	*R. hybrida*[35]	nd	nd	10.8	nd	nd	nd	1.6	nd	26.5	nd	nd	nd	38.5	11.6	nd	nd	nd
27	*R. corymbifera*[33]	0.2	nd	0.3	0.2	0.6	nd	5.6	14.8	0.1	nd	nd	nd	0.9	nd	8.6	1.7	10.6
28	*R. phoenica*[33]	nd	nd	1.6	nd	nd	nd	12.3	17.1	0.2	nd	nd	nd	3.6	1.3	nd	5.1	12.9
29	*R. hybrida*[36]	nd	nd	34.7	nd	nd	nd	nd	nd	nd	nd	36.5	nd	nd	nd	15.7	nd	nd
30	*R. banksiae*[18]	nd	4.8	nd	nd	nd	nd	8.0	0.4	nd	16.7	12.1	nd	11.6	nd	1.6	nd	2.7
31	*R. multiflora*[18]	nd	8.0	nd	nd	nd	nd	5.2	0.9	nd	4.9	5.4	nd	5.8	nd	2.7	nd	3.5
32	*R. multiflora*[18]	nd	2.8	nd	nd	nd	nd	1.0	2.4	nd	0.3	nd	nd	0.2	nd	2.1	nd	2.4
33	*R. hybrida**	1.2	nd	nd	nd	0.7	47.9	3.5	nd	0.4	nd	nd	nd	8.5	0.9	1.0	nd	nd
	Average	**15.2**	**1.7**	**2.0**	**6.2**	**5.3**	**2.3**	**6.3**	**1.2**	**1.4**	**1.4**	**2.3**	**1.8**	**8.8**	**2.6**	**1.4**	**4.8**	**2.1**

*This study; nd - not detected

Results and discussion

Volatile profile of R. hybrida cv. Mileva essential oil

The main compound in *R. hybrida* essential oil was geranyl acetate (47.9%), followed by nonadecane (8.5%), β-caryophyllene (4.4%), heneicosane (3.5%), neryl acetate (2.2%), τ-muurolene (2.1%), β-bisabolene (2.0%), citronellol (1.2%), β-selinene (1.3%), and α-selinene (1.2%) among 81 compounds, 37 of which were identified (Fig. 1, Supplementary Table 1). Some of the identified compounds are listed in Table 1 (under the reference TS).

R. hybrida cv. Mileva essential oil possesses a pleasant floral, sweet, fresh, and rose-type aromatic notes. These are likely attributed to terpene alcohols (citronellol and geraniol), and their acetate derivatives (geranyl and neryl acetate) [24,40,41], which are the dominant compounds (51.3% of the essential oil). Sesquiterpenes (*β*-caryophyllene, *τ*-muurolene, *β*-bisabolene, α- and β-selinene) are an important group of fragrance ingredients (17.4% of the essential oil) in essential oil. These compounds are characterized by sweet, woody-spicy, clove-like, balsamic aromatic tones[42]. Despite their presence in low concentrations, they contribute to a specific richness of smell[43]. Aliphatic hydrocarbons, such as nonadecane and heneicosane (17.8% of the essential oil), possess fixative properties that can influence the longevity of the fragrance emitted by other volatiles in the essential oil and contribute to an overall long-lasting scent[44,45]. Chemical analysis conducted on different genotypes of oil-bearing roses (*R. damascena*, *R. alba* and *R. damascena* × *R. gallica*) showed presence of a substantial number of components (60). The main components were citronellol and geraniol (ratio of which significantly influences aroma quality), followed by nonadecane and nerol. Different quantitative composition leads to differences in chromatographic profiles[28]. The main compounds in *R. hybrida* essential oil from Saudi Arabia were citronellol (34.2-40.4%) in fresh plant material, while in dry/stored plant material its content significantly decreased (2.3-4.8%), and content of some other compounds increased (α-pinene, eugenol, methyl eugenol, myrcene, rose oxide, β-gurjunene and nonadecane)[34]. In addition to location and storage conditions, essential oil composition depends on geographic origin, growing conditions and extraction method[23,24,35,36].

Antioxidant and anti-inflammatory activity of R. hybrida cv. Mileva essential oil

The obtained results of the antioxidant and anti-inflammatory activities of *R. hybrida* essential oil are summarized in Table 2. Evaluation of antioxidant activity has shown that the strongest activity is detected via ABTS assay, reaching 454 mM TE/g. Furthermore, the reducing power was 30 mM TE/g, while the DPPH assay was 9.5 mM TE/g. In addition, the sample has demonstrated strong anti-inflammatory activity reaching 52.2 ± 0.8 % for the concentration of 10 mg/mL of essential oil.

R. hybrida essential oil showed significant antioxidative activity; however, the obtained values were comparable to those of many other commercial essential oils[46]. For example, *R. alba* essential oil is also known to exhibit good DPPH activity (similar to ascorbic acid and BHT), as well as a good ability to inhibit lipid peroxidation of liposomal suspension induced by Fe^{2+} [29]. However, the DPPH antioxidant activity of *R. damascena* essential oil, as well as its chemical composition, greatly depends on the extraction method[47]. Investigations have shown that the DPPH radical scavenging activity of *R. damascena* essential oil was 42.07%. Irradiation significantly increased the degree of scavenging activity[48].

It is interesting to note that the essential oil of *R. hybrida* cv. Mileva has shown rather promising results in the protein denaturation assay which models anti-inflammatory activity. Previously, the *R. damascena* essential oil and hydro-alcoholic extract were found to relieve alimentary inflammatory conditions using an acetic acid-induced ulcerative colitis model in rats[49]. Conversely, on a different occasion, *R. damascena* essential oil did not exhibit anti-inflammatory activity on carrageenan-induced paw oedema in an animal model[50].

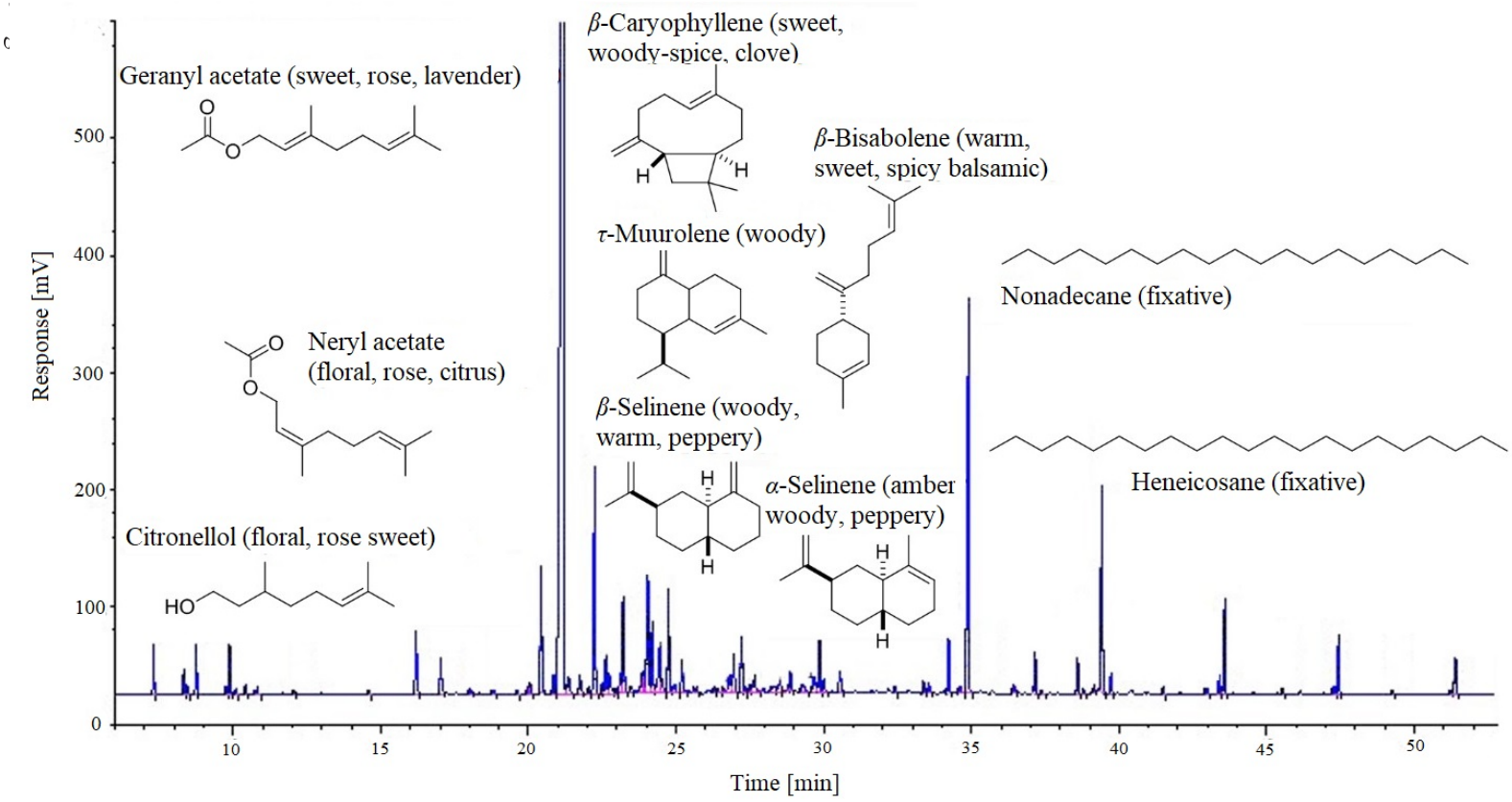

Figure 1. A total ion chromatogram of *R. hybrida* cv. Mileva essential oil

Table 2. Antioxidant (DPPH, ABTS and RP) and anti-inflammatory (AIA) activity of the *R. hybrida* cv. Mileva essential oil and standards

Analyses	Essential oil	Standard
DPPH	9.5±0.5	0.14 ±0.01[a]
ABTS	454±5	1.06 ±0.04[a]
RP	30±1.1	0.12 ±0.02[a]
AIA	52.2±0.8	1.14 ±0.03[b]

Mean value of three replicates (± standard deviation) for biological assays: DPPH- DPPH assay (μmol TE 100 g^{-1}); ABTS- $ABTS^{\bullet+}$ method (μmol TE 100 mL^{-1} essential oil); RP- reducing power (μmol TE 100 mL^{-1} essential oil); AIA- anti-inflammatory activity (% of inhibition); IC_{50} values of used standard compounds in the bioactivity assays: a- Trolox; b- Diclofenac sodium

Antimicrobial activity of R. hybrida cv. Mileva essential oil

According to the antimicrobial profile of *R. hybrida* essential oil presented in Table 3, it can be concluded that the antimicrobial activity was noticed against all tested microorganisms. The highest antimicrobial activity was recorded against *S. aureus*, while the lowest activity was noticed against *E. faecalis*. The inhibition zone for gram-negative microorganisms was between 12 and 15 mm, while the diameter of the halo zone varied from 8.3 to 18 mm with gram-positive microorganisms.

The essential oil exhibited notable antimicrobial activity against all tested micro-organisms. Comparing the obtained values to the reported antimicrobial activity of geranyl acetate, it can be concluded the oil possesses a somewhat greater activity than its main component[51]. In addition, geranyl acetate was found to have no effect on *P. aeruginosa*; this highlights the role of minor constituents in the antimicrobial activity of the oil. Essential oils of other rose species are also known to exert a broad spectrum of antimicrobial activities against fungi and bacteria[52]. *R. damascena* essential oil, rich in citronellol and geraniol, was found to express strong antibacterial activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, *S. aureus*, *C. violaceum* and *E. carotovora*[53]. Furthermore, *R. damascena* essential oil exhibited the antimicrobial activity against *Proteus vulgaris* and *Klebsiella pneumoniae*, while *Enterococcus faecalis*, *S. typhimurium* and *P. aeruginosa* were less sensitive[54]. These studies indicated that, due to its properties, rose essential oil could be used as a natural preservative additive in the food industry, as antimicrobial agent for treating infectious diseases, as well as an antibacterial agent for the disinfection of various surfaces[53,54]. It should be pointed out that not all *Rosa* sp. essential oils possess significant antimicrobial properties; *R. damascena*, *R. gallica*, *R. damascena* × *R.gallica*, *R. centifolia*, *R. alba*, *R. banksiae*, and *R. polyantha* showed little to no antimicrobial activity[45,55,56].

Chemometric analysis of R. hybrida cv. Mileva essential oil and comparison to other non-commercial roses

To date, a comprehensive review of *Rosa* sp. essential oil composition has not been done,

Table 3. The inhibition zone (in mm) of the *R. hybrida* cv. Mileva essential oil and controls (Tetracycline 30 μg/mL and sterile distilled water)

Bacterial strain	Essential oil	Tetracycline 30 μg/mL	Sterile distilled water
E. coli	12.0±0.0	27.0±0.0	0.0±0.0
P. aeruginosa	12.3±0.6	27.0±0.0	0.0±0.0
S. Typhimurium	15.0±0.0	29.3±0.6	0.0±0.0
S. aureus	18.0±0.0	28.0±0.0	0.0±0.0
E. faecalis	8.3±0.6	27.0±0.0	0.0±0.0
L. monocytogenes	14±0.0	26.3±0.6	0.0±0.0

Results are expressed as mean for three repetition ± standard deviation

therefore relevant data were collected from available literature (Table 1). Subsequently, an unrooted cluster tree (Fig. 2) was constructed in order to classify species, varieties and cultivars in possible chemotypes according to the main constituents of their essential oils.

For this purpose, 33 accessions concerning volatile profiles of different *Rosa* sp. (excluding *R. damascena*) available in the literature were analyzed. The results are summarized in Table 1, where 17 volatile compounds present on average with more than 1.0% are listed (references are sorted according to the publication year, from the oldest (1990) to the newest). On average, the most abundant compound was citronellol (15.2%), followed by nonadecane (8.8%), heneicosane (6.3%), eugenol (6.2%), and geraniol (5.3%).

Floral fragrances differ between rose species and varieties[57]. In this study, it was shown that species within the *Rosa* genus can be categorized into chemotypes according to their volatile profile. A comprehensive review of relevant literature together with results obtained in this study on the volatiles of *R. hybrida*, supports the presence of eight different chemotypes. For example, *R. hybrida*, cv. Mileva (from this study) and cv. Black Prince seem to have a unique and specific aroma profile, which could not be classified in the common chemotypes, while *R. hybrida* accessions from Pakistan, Saudi Arabia and Indonesia were all grouped into singular chemotypes (phenylethyl alcohol, citronellol, and aliphatic hydrocarbons, respectively)[23,34-36].

According to the unrooted cluster tree (Fig. 2), there are several possible chemotypes of *Rosa* sp. essential oils: (1) with dominant citronellol, the most abundant chemotype in this study (10 accessions); (2) with dominant

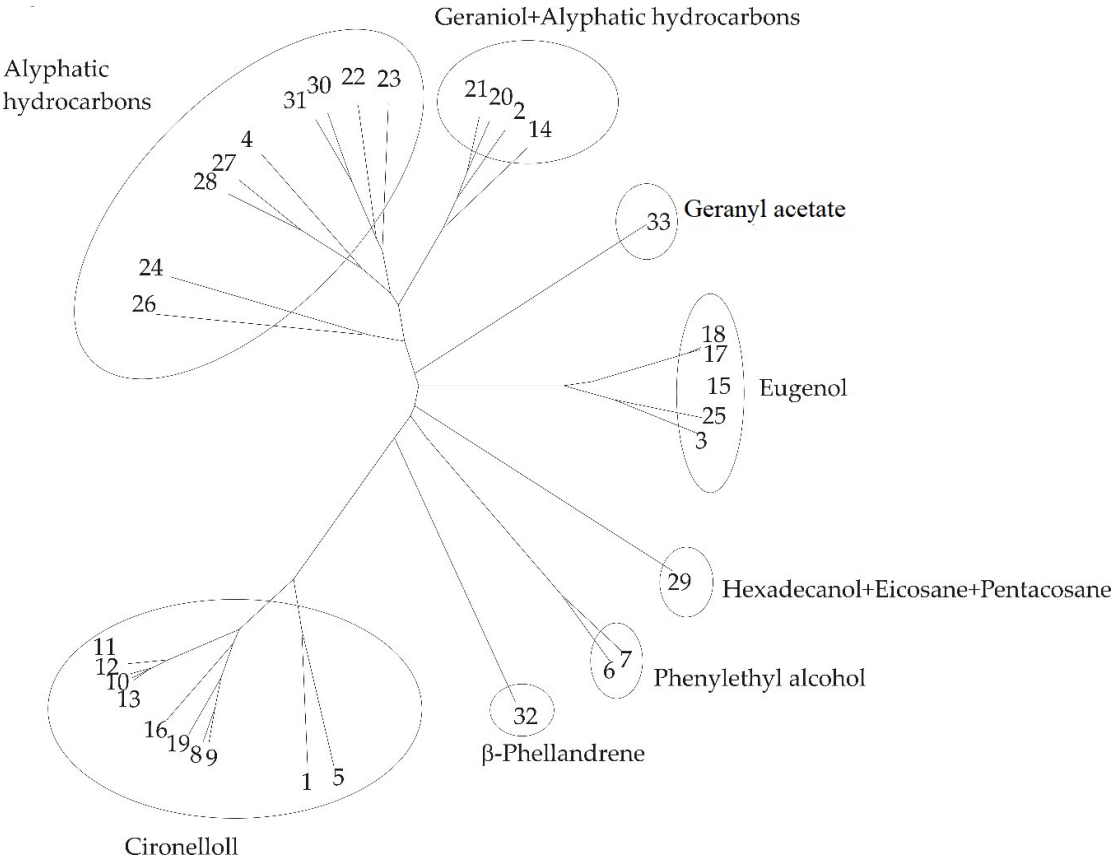

Figure 2. The unrooted tree of different *Rosa* sp. accessions according to literature (listed in Table 1) and comparison with results from this study

aliphatic hydrocarbons (heptacosane, tricosane, nonadecane, and nonadecene); (3) a combination of aliphatic hydrocarbons and monoterpenes (geraniol and citronellol), (4) with dominant eugenol; and (5) unspecific class (contains β-phellandrene, phenylethyl alcohol, hexadecanol (cetyl alcohol) and geranyl acetate).

Such heterogeneity of volatile profiles of *R. hybrida* is not surprising. Previous scent analysis of ten cultivars of *R. hybrida* showed the presence of hundreds of volatile compounds[58]. Some cultivars such as Papa Meilland, Alister Stella Grey, and Hacienda were shown to emit a typical rose scent, mostly composed of monoterpene alcohols (geraniol, geranial, nerol), phenylethyl alcohol. In other cultivars such as Pariser Charme, Old Blush and Mutabilis, geraniol was the predominant volatile compound.

Genotypes naturally play a significant role in defining the volatile profiles of *Rosa* sp[59]. This is evidenced by the absence of fragrance in many commercially relevant rose hybrids. Caution must be exercised by the breeder if the goal is to produce a hybrid. Unfortunately, the genetic markers that regulate the rose volatiles biosynthesis remain only partially elucidated. While the biosynthetic pathways and enzymes involved in the production of fatty acid derivatives (e.g., (E)-2-hexenal), C13 norisoprenoids (e.g., ionones and ionols) and phenylethyl alcohol are well-described, less is known about the enzymes which regulate the production of monoterpenes and their esters in roses[59-61]. As terpenes significantly to the aromas of many rose hybrids (*R. hybrida* cv. Mileva included), rose hybrid breeding might greatly benefit from further research in this area. It is nevertheless important to point out that the results of one of the most meticulous research efforts into the rose genome suggested that the pathways leading to pigments and volatiles are co-regulated, so the breeder has to be aware that traits such as petal color might affect the volatile profile, and that it may not be possible to obtain hybrids of a particular pigmentation-scent combination[60]. When analyzing and comparing the contents of essential oils of particular roses, it should however be noted that the composition of the essential oils does not solely depend on genotypes; harvest time and postharvest processing (distillation method), as well as abiotic factors can also influence the composition of *Rosa* sp. volatiles[23,62-65].

Conclusions

This study investigated the chemical composition, antioxidant and anti-inflammatory properties of the essential oil of *R. hybrida* cv. Mileva, a promising new cultivar. The essential oil of the essential oil contains a total of 37 identified compounds, with geranyl acetate as the dominant, followed by nonadecane, β-caryophyllene, heneicosane, neryl acetate, τ-muurolene, β-bisabolene, citronellol, β- and α-selinene. The essential oil of this rose hybrid demonstrated significant antioxidant (45400 mmol TE/100 g in the ABTS assay) and anti-inflammatory (52.2% at 10 mg/mL in the egg albumin denaturation assay) activities. By employing chemometrics, the contents of the oil of the hybrid rose were compared to those of previously examined species in the Rosa family. During this approach, several rose chemotypes were defined and compared. The new classification based on volatile composition could be valuable for advancing processes of rose breeding and selection. This is especially beneficial for those aimed at producing edible and oil-bearing species, with aromas suitable for food and beverages, aromatherapy, cosmetics, perfumes, and household products. Having found the Mileva™ Frayla® hybrid essential oil belongs to the pleasant-smelling geranyl acetate chemotype, potential aroma-related applications of this hybrid warrant further exploration.

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Supplementary data

Table S1 is given in supplementary file.

References

1. **Khosh-Khui, M. (2014).** Biotechnology of scented roses: a review. Int. J. Hortic. Sci. Technol. 1: 1-20.
2. **Rajendran, P., Ajithkumar, K., Smitha, R. (2017).** Local rose races of the Western Ghats- a potential gene pool for rose breeding. Acta. Hortic. 1165: 63-72.
3. **Zlesak, D.C., Nelson, R., Harp, D., Villarreal, B., Howell, N., Griffin, J., Hammond, G., George, S. (2017).** Performance of landscape roses grown with minimal input in the north-central, central, and south-central United States. Hort. Technology. 27: 718-730.
4. **Ilyasoglu, H. (2014).** Characterization of rosehip (*Rosa canina* L.) seed and seed oil. Int. J. Food Prop. 17: 1591-1598.
5. **Igual, M., García-Herrera, P., Cámara, R.M., Martínez-Monzó, J., García-Segovia, P., Cámara, M. (2022).** Bioactive compounds in rosehip (*Rosa canina*) powder with encapsulating agents. Molecules. 27: 4737.
6. **Božanić Tanjga, B., Lončar, B., Aćimović, M., Kiprovski, B., Šovljanski, O., Tomić, A., Travičić, V., Cvetković, M., Raičević, V., Zeremski, T. (2022).** Volatile profile of garden rose (*Rosa hybrida*) hydrosol and evaluation of its biological activity *in vitro*. Horticulturae. 8: 895.
7. **Rusanov, K., Kovacheva, N., Stefanova, K., Atanassov, A., Atanassov, I. (2009).** *Rosa damascena* - genetic resources and capacity building for molecular breeding. Biotechnol Biotechnol Eq. 23: 1436-1439.
8. **Mosleh, G., Badr, P., Azadi, A., Sardari, F.A., Iraji, A., Khademian, S., Abolhassanzadeh, Z., Mohagheghzadeh, A. (2021).** Quality control of *Rosa* × *damascena* flowers from Layzangan of Fars province in Iran. J. Med. Plants. 20: 48-58.
9. **Cui, W.H., Du, X.Y., Zhong, M.C., Fang, W., Suo, Z.Q., Wang, D., Dong, X., Jiang, X.D., Hu, J.Y. (2022).** Complex and reticulate origin of edible roses (Rosa, Rosaceae) in China. Hortic. Res. 9: uhab051.
10. **Alom, S., Ali, F., Bezbaruah, R., Kakoti, B.B. (2021).** *Rosa alba* Linn.: a comprehensive review on plant profile, phytochemistry, traditional and pharmacological uses. World J. Pharm. Res. 10: 798-811.
11. **Feng, Y., Cheng, X., Lu, Y., Wang, H., Chen, D., Luo, C., Liu, H., Gao, S., Lei, T., Huang, C., Yu, X. (2022).** Gas chromatography-mass spectrometry analysis of floral fragrance-related compounds in scented rose (*Rosa hybrida*) varieties and a subsequent evaluation on the basis of the analytical hierarchy process. Plant Physiol. Biochem. 185: 368-377.
12. **Bianchi, G., Nuzzi, M., Avitabile Leva, A., Rizzolo A. (2007).** Development of a headspace-solid phase micro extraction method to monitor changes in volatile profile of rose (*Rosa hybrida*, cv David Austin) petals during processing. J. Chromatogr. A. 1150: 190-197.
13. **Feng, D., Jian, H., Zhang, H., Qiu, X., Wang, Z., Du, W., Xie, L., Wang, Q., Zhou, N., Wang, H., Tang, K., Yan, H. (2022).** Comparison of volatile compounds between wild and cultivated roses. Hort. Sci. 57: 657-663.
14. **Tešanović, M., Bonić, Ž., Bošković, J. (2018).** Origin of cultivated roses and approaches used to study important rose traits. J. Agron. Technol. Eng. Manag. 1: 45-50.
15. **Božanić Tanjga, B., Ljubojević, M., Đukić, A., Vukosavljev, M., Ilić, O., Narandžić, T. (2022).** Selection of garden roses to improve the ecosystem services they provide. Horticulturae. 2: 883.
16. **Nedkov, N., Dobreva, A., Kovacheva, N., Bardarov, V., Velcheva, A. (2009).** Bulgarian rose oil of white oil-bearing rose. Bulg. J. Agric. Sci. 15: 318-322.
17. **Verma, R.S., Padalia, R.C., Chauhan, A. (2016).** Chemical composition of essential oil and rose-water extract of Hymalayan musk rose (*Rosa brunonii* Lindl.) from

Kumaon region of western Himalaya. J. Essent. Oil Res. 28: 332-338.
18. **Elhawary, E.A., Mostafa, N.M., Labib, R.M., Singab, A.N. (2021).** Metabolomic profiles of essential oils from selected rosa varieties and their antimicrobial activities. Plants. 10: 1721.
19. **Yu, A.N., Wang, X.P., Yang, X.H. (2007).** Chemical composition of the essential oils of flowers of *Rosa banksiae* from China. Chem. Nat. Compd. 43: 728-729.
20. **Ueyama, Y., Hashimoto, S., Nii, H., Furukawa, K. (1990).** The essential oil from the flowers of *Rosa rugosa* Thunb. var. *plena* Regel. Flavour Fragr. J. 5: 219-222.
21. **Gora, J., Lis, A., Kalemba, D. (1995).** Chemical composition of the essential oil of *Rosa centifolia* L. petals. J. Essent. Oil Res. 7: 89-90.
22. **Kaul, V.K., Gujral, R.K., Singh, B. (1999).** Volatile constituents of the essential oil of flowers of *Rosa brunonii* Lindl. Flavour Fragr. J. 14: 9-11.
23. **Younis, A., Khan, M.A., Khan, A.A., Riaz, A., Pervez, M.A. (2007).** Effect of different extraction methods on yield and quality of essential oil from four *Rosa* species. Floriculture Ornamental Biotech. 1: 73-76.
24. **Gochev, V., Wlcek, K., Buchbauer, G., Stoyanova, A., Dobreva, A., Schmidt, E., Jirovetz, L. (2008).** Comparative evaluation of antimicrobial activity and composition of rose oils from various geographic origins, in particular Bulgarian rose oil. Nat. Prod. Commun. 3: 1063-1068.
25. **Hosni, K., Kerkenni, A., Medfei, W., Brahim, N.B., Sebei, H. (2010).** Volatile oil constituents of *Rosa canina* L.: quality as affected by the distillation method. Org. Chem. Int. 2010: 621967.
26. **Shamspur, T., Mostafavi, A. (2010).** Chemical composition of the volatile oil of *Rosa kazanlik* and *Rosa gallica* from Kermen province Iran. J. Essent. Oil-Bear. Pl. 13: 78-84.
27. **Honarvar, M., Javidnia, K., Khosh-Khui, M. (2011).** Essential oil composition of fresh and dried flowers of *Rosa moschata* from Iran. Chem. Nat. Compd. 47: 826-828.
28. **Dobreva, A., Velcheva, A., Bardarov, V., Bardarov, K. (2013).** Chemical composition of different genotypes oil-bearing roses. Bulg. J. Agric. Sci. 19: 1213-1218.
29. **Mileva, M.M., Kusovski, V.K., Krastev, D.S., Dobreva, A.M., Galabov, A.S. (2014).** Chemical composition, in vitro antiradical and antimicrobial activities of Bulgarian *Rosa alba* L. essential oil against some oral pathogenes. Int. J. Curr. Microbiol. App. Sci. 3: 11-20.
30. **Asgarpanah, J., Ziarati, P., Safialdinardebily, M. (2014).** The volatile oil composition of *Rosa foetida* Herrm. flowers growing wild in Kurdistan province (Iran). J. Essent. Oil-Bear Pl. 17: 169-172.
31. **Akhoondi, R., Mirjalili, M.H., Hadian, J. (2015).** Quantitative and qualitative variations in the essential oil of *Rosa foetida* Herrm. (Rosaceae) flowers as affected by different drying methods. J. Essent. Oil Res. 27: 421-427.
32. **Karami, A., Jandoust, S. (2016).** Essential oils of Persian musk rose (*Rosa moschata* Herrm.) as influenced by dring and harvest time. Nat. Volatiles Essent. Oils. 3: 9-14.
33. **Zgheib, R., Najam, W., Azzi-Achkouty, S., Sadaka, C., Ouaini, N., Beyrouthy, M.E. (2020).** Essential oil chemical composition of *Rosa corymbifera* Borkh., *Rosa phoenica* Boiss. and *Rosa damascena* Mill. from Lebanon. J. Essent. Oil-Bear Pl. 23: 1161-1172.
34. **Bazaid, S.A. (2009).** Chemical composition of *Rosa hybrida* essential oil as a function of location and storage in Saudi Arabia. Am-Eurasian J. Sustain. Agric. 3: 24-28.
35. **Oktavianawati, I., Letisya, N., Citra, P., Utari, D.P., Winata, I.N.A., Handayani, W., Nugraha, A.S. (2019).** Essential oil composition of rose flowers from Karangpring village Jember district extracted by distillation and enfleurage. J. Ilmu Dasar. 20: 67-74.
36. **Araujo, N.A.F., Brandao, R.M., Barguil, B.M., Cardoso, M.G., Pasqual, M., Rezende, R.A.L.S., Pereira, M.M.A.,**

Buttros, V.H.T., Doria, J. (2020). Plant growth-promoting bacteria improve growth and modify essential oil in rose (*Rosa hybrida* L.) cv Black Prince. Front. Sustain. Food Syst. 4: 606827.

37. **Oliveira, A.P., Silva, L.R., Ferreres, F., Guedes de Pinho, P., Valentão, P., Silva, B.M., Pereira J.A., Andrade, P.B. (2010).** Chemical assessment and *in vitro* antioxidant capacity of *Ficus carica* latex. J. Agric. Food Chem. 58: 3393-3398.
38. **Tumbas Šaponjac, V., Gironés-Vilaplana, A., Djilas, S., Mena, P., Ćetković, G., Moreno, D.A., Čanadanović-Brunet, J., Vulić J., Stajčić S., Krunić, M. (2014).** Anthocyanin profiles and biological properties of caneberry (*Rubus* spp.) press residues. J. Sci. Food Agric. 94: 2393-2400.
39. **Ullah, A., Zaman, S., Juhara F., Akter, L., Tareq S.M., Masum, E.H., Bhattacharjee, R. (2014).** Evaluation of antinociceptive, *in-vivo* and *in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. BMC Complement. Altern. Med. 14: 346.
40. **Di Nicolantonio, L., Gigliobianco, M.R., Peregrina, D.V., Angeloni, S., Ilorini, L., Di Martino, P., Censi, R. (2022).** Impact of the interactions between fragrances and cosmetic bases on the fragrance olfactory performance: a tentative to correlate SPME-GC/MS analysis with that of an experienced perfumer. Cosmetics. 9: 70.
41. **Kumar Bora, P., Borah G., Kalita D., Proteem Saikia, S., Haldar, S. (2023).** Mushroom-mediated reductive bioconversion of aldehyde-rich essential oils for aroma alteration: a rose-like floral bioflavor from citronella oil. J. Agric. Food Chem. 71: 1690-1700.
42. **Bledsoe, J.O. (2000).** Terpenoids. Ed: Kroschwitz, J.I., Howe-Grant M. In book: Kirk-Othmer Encyclopedia of Chemical Technology. Wiley, New York.
43. **Froissard, D., Rapior, S., Bessière, J.M., Fruchier, A., Buatois, B., Fons, F. (2014).** Volatile organic compounds of six french Dryopteris species: natural odorous and bioactive resources. Nat. Prod. Commun. 9: 137-140.
44. **Berechet, M.D., Calinescu, I., Stelescu, M.D., Manaila, E., Craciun, G., Purcareanu, B., Mihaiescu, D.E., Rosca, S., Fudulu, A., Niculescu-Aron, I.G., Miha, R. (2015).** Composition of the essential oil of *Rosa damascena* Mill. cultivated in Romania. Rev. Chim. 66: 1986-1991.
45. **Fu, Y., Zhang, Y., Zeng, S., Luo, L., Xi, H., Li, P., Wang, D., Liao, T., Chen, J., Sun, S., Xie, J. (2022).** The effect of long-chain alkanes on flavour release and olfactory characteristics of rose essential oil. Flavour Fragr. J. 37: 72-80.
46. **Wang, H., Yih, K., Yang, C., Huang, K. (2017).** Anti-oxidant activity and major chemical component analyses of twenty-six commercially available essential oils. J. Food Drug Anal. 25: 881-889.
47. **Patrascu, M., Radoiu, M. (2016).** Rose essential oil extraction from fresh petals using synergetic microwave and ultrasound energy: chemical composition and antioxidant activity assessment. J. Chem. Chem. Eng. 10: 136-142.
48. **Karamalakova, Y., Nikolova, G., Kovacheva, N., Zheleva, A., Gadjeva, V. (2019).** Study of the radical-scavenging activities and radioprotective properties of Bulgarian essential rose oil from *Rosa damascena* Mill. Bulg. Chem. Commun. 51: 101-107.
49. **Latifi, G., Ghannadi, A., Minaiyan, M. (2015).** Anti-inflammatory effect of volatile oil and hydroalcoholic extract of *Rosa damascena* Mill. on acetic acid-induced colitis in rats. Res. Pharm. Sci. 10: 514-522.
50. **Hajhashemi, V., Ghannadi, A., Hajiloo, M. (2010).** Analgesic and anti-inflammatory effects of *Rosa damascena* hydroalcoholic extract and its essential oil in animal models. Iran. J. Pharm. Res. 9: 163-168.
51. **Jirovetz, L., Eller, G., Buchbauer, G., Schmidt, E., Denkova, Z., Stoyanova, A.S., Nikolova, R., Geissler, M. (2006).** Chemical composition, antimicrobial activities and odor descriptions of some essential oils with

characteristic floral-rosy scent and their principal aroma compounds. Recent Res. Devel. Agron. Hortic. 2: 1-12.

52. **Shohayeb, M., Abdel-Hameed, E.S.S., Bazaid, S.A., Maghrabi, I. (2014).** Antibacterial and antifungal activity of *Rosa damascena* Mill. essential oil, different extracts of rose petals. Glob. J. Pharmacol. 8: 1-7.
53. **Ulusoy, S., Bosgelmez-Tinaz, G., Secilmis-Canbay, H. (2009).** Tocopherol, carotene, phenolic contents and antibacterial properties of rose essential oil, hydrosol and absolute. Curr. Microbiol. 59: 554-558.
54. **Mahboubi, M. (2015).** *Rosa damascena* as holy ancient herb with novel applications. J. Tradit. Complement. Med. 6: 10-16.
55. **Shabani, F., Omidvar, S., Kaboudi, P.S., Pasha, H., Khafri, S., Najafzadehvarzi, H., Amiri, F.N., Faramarzi, M., Bouzari, Z. (2022).** The effects of *Rosa foetida* extract along with self-care education on primary dysmenorrhea: study protocol for a randomized clinical trial. Trials. 23: 637.
56. **Saghafi, F., Mirzaie, F., Gorji, E., Nabimeybodi, R., Fattahi, M., Mahmoodian, H., Zareshahi, R. (2021).** Antibacterial and anti-Trichomonas vaginalis effects of *Rosa damascena* Mill petal oil (a Persian medicine product), aqueous and hydroalcoholic extracts. BMC Complement. Med. Ther. 21: 265.
57. **Kim, H.J., Kim, K., Kim, N.S., Lee, D.S. (2000).** Determination of floral fragrances of *Rosa hybrida* using solid-phase trapping-solvent extraction and gass chromatography-mass spectometry. J. Chromatogr. A. 902: 389-404.
58. **Magnard, J.L., Roccia, A., Caissard, J.C., Vergne, P., Sun, P., Hecquet, R., Dubois, A., Oyant, L.H.S., Jullien, F., Nicolè, F., Raymond, O., Huguet, S., Baltenweck, R., Meyer, S., Claudel, P., Jeauffre, J., Rohmer, M., Foucher, F., Hugueney, P., Bendahmane, M., Baudino, S. (2015).** Biosynthesis of monoterpene scent compounds in roses. Science. 394: 81-83.
59. **Bendahmane, M., Dubois, A., Raymond, O., Le Bris, M. (2013).** Genetics and genomics of flower initiation and development in roses. J. Exp. Bot. 64: 847-857.
60. **Raymond, O., Gouzy, J., Just, J., Badouin, H., Verdenaud, M., Lemainque, A., Vergne, P., Moja, S., Choisne, N., Pont, C., Carrčre, S., Caissard, J.C., Couloux, A., Cottret, L., Aury, J.M., Szécsi, J., Latrasse, D., Madoui, M.A., François, L., Fu, X., Yang, S.H., Dubois, A., Piola, F., Larrieu, A., Perez, M., Labadie, K., Perrier, L., Govetto, B., Labrousse, Y., Villand, P., Bardoux, C., Boltz, V., Lopez-Roques, C., Heitzler, P., Vernoux, T., Vandenbussche, M., Quesneville, H., Boualem, A., Bendahmane, A., Liu, C., Le Bris, M., Salse, J., Baudino, S., Benhamed, M., Wincker, P., Bendahmane, M. (2018).** The Rosa genome provides new insights into the domestication of modern roses. Nat Genet. 50: 772-777.
61. **Roccia, A., Hibrand-Saint Oyant, L., Cavel, E., Caissard, J.C., Machenaud, J., Thouroude, T., Jeauffre, J., Bony, A., Dubois, A., Vergne, P., Szécsi, J., Foucher, F., Bendahmane, M., Baudino, S. (2019).** Biosynthesis of 2-phenylethanol in rose petals is linked to the expression of one allele of RhPAAS. Plant Physiol. 179: 1064-1079.
62. **Cho, Y.S., Cho, I.H., Park, H.J., Chun, H.K. (2006).** Analysis of fragrance volatiles of Korean Rosa hybrida using gas chromatography-mass spectrometry. Agric Chem Biotechnol. 49: 180-185.
63. **Yang, L., Ren, J., Wang, Y., Hu, Q. (2014).** Diurnal fluctuation of volatile compounds emitted from four seasons rose (*Rosa damascena* Mill.) cultivated in Beijing. J. Appl. Bot. Food Qual. 87: 9-15.
64. **Hirata, H., Ohnishi, T., Watanabe, N. (2016).** Biosynthesis of floral scent 2-phenylethanol in rose flowers. Biosci. Biotechnol. Biochem. 80: 1865-1873.
65. **Todorova, M., Dobreva, A., Petkova, N., Grozeva, N., Gerdzhikova, M., Veleva, P. (2022).** Organic vs conventional farming of oil-bearing rose: effect on essential oil and antioxidant activity. BioRisk. 17: 271-285.