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Volatile Profile of Garden Rose (*Rosa hybrida*) Hydrosol and Evaluation of Its Biological Activity In Vitro

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Abstract: Garden rose, *Rosa hybrida*, is primarily used for decoration and has a wide range of growing area, contrary to *R. damascena* that has a limited area of distribution (Turkey and Bulgaria), yet it is extensively used for commercial production of valuable and expensive rose oil. Since the content of essential oil in rose petals is low (0.03–0.04%), its production is quite limited; however, during this process, a significant amount of rose hydrosol is obtained as a secondary product. The aim of this research was to determine the chemical composition of garden rose hydrosols and to evaluate their biological properties. Obtained results show that *R. hybrida* hydrosol containing phenylethyl alcohol, nerol, linalool, and geraniol may be used as an alternative for *R. damascena* hydrosol. However, the total phenolic content was quite low (4.96 µg GAE/mL), which is related to a low level of observed antioxidant activity based on different antioxidant activity assays. Furthermore, *R. hybrida* hydrosol did not exhibit antimicrobial activity against several gram-positive and gram-negative bacteria, as well as yeast and fungi. Anti-inflammatory activity was also low, while no antihyperglycemic activity was detected. With these results in mind, no potential is evident for the therapeutic application of rose hydrosol beyond that found in complimentary medicine such as aromatherapy.

Keywords: hydrolate; rose water; antioxidant activity; antimicrobial activity; antihyperglycemic activity; anti-inflammatory activity

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1. Introduction

Roses have been valued as ornamental and fragrance-bearing plants since ancient times. Medieval civilizations in the Eastern Mediterranean region cultivated, grew, and boiled roses with water to produce aromatic water, used because of its apparent refreshing effects on the mind and spirit [1,2]. This was a harbinger of the modern rose hydrosol. According to Persian folk medicine, Damask rose (*Rosa damascena* Mill.) aromatic water was used for mental refreshing, the improvement of cognitive abilities, its strengthening or stimulating effect on the nerves, prevention or reduction of fatigue, and as a sedative [3,4]. Today, rose hydrosols or rose aromatic water have great commercial importance [5]. They are mainly used for flavoring food (Turkish delight, lokkum), tonic beverage (Jollab), and other drinks, ice creams, yogurt, cake, rice pudding, jam, marmalade, cake, and syrups; it is also sprinkled on many meat dishes [6–13]. It has been traditionally used for skin care and healing-related diseases such as erythema, itchiness, and swelling in the form of soaps, cosmetics, toiletries, and perfumes [6,14].

Rose oil is one of the most valuable and expensive flavor and fragrance products, as well as an aroma-therapeutic agent. Taking into account the increased demand for rose oil on the international market, the climate-related limitations on cultivation area required

for optimum-yield production (Bulgaria, Turkey, India), as well as the fact that it flowers only once per year (May–June), there are attempts to extend the cultivation of essential-oil-bearing roses into different agroclimatic regions, to find other cultivars suitable for essential oil production, or to improve growing management and promote processing [3,7,15]. Apart from *R. damascena*, which is the superior oil-bearing rose globally (with 0.03–0.04% of essential oil content), *R. gallica*, *R. centifolia*, *R. moschata*, *R. rugosa*, *R. bourbonica,* and *R. alba* are also widely used for commercial production of rose oil and rose water, as well as other products such as rose absolute and concrete (Morocco, France, Egypt, China, etc.) [7,16–18]. The hydrosols are produced as by-products during the process of essential oil distillation in quite significant quantities [19]. In some countries (Iran, Tunisia), rose hydrosol is traditionally used in religious ceremonies and as a therapeutic product [6,20,21]. The literature review of the volatile compounds of rose hydrosols, which addresses the commercial samples of *R. damascena*, *R. alba*, *R. brunonii*, *R. canina*, *R. centifolia* and *R. rugose*, is not enough to analyze all difference between these samples. However, there is no data about the volatile composition of the one of the most contemporary roses, *R. hybrida*.

Hybrid roses (*R. hybrida*) as a group are one of the major contemporary roses cultivated as garden plants nowadays. Owing to their complex hybridization history, numerous varieties have appeared, with different flower colors and shapes, as well as various scent notes [22,23]. Due to the mentioned world-wide cultivation and its use in different fields of daily life of people, this study aims to gather more insight into the volatiles of nursery-produced rose hybrids and their potential applications. The main goal of this study was to investigate the quality of the by-product during essential oil distillation of *R. hybrida* flowers grown in Serbia. Furthermore, even though the quality of rose essential oil is regulated by international standards (ISO 9842:2003), there are no boundary values set for rose hydrosols. Therefore, another aim of this investigation was to collect literature data about rose hydrosol quality and compare it with the one obtained from *R. hybrida*.

2. Materials and Methods

2.1. Plant Material

Rosa hybrida cv Mileva™ Frayla® has large soft pink petals arranged in very double flowers with a sweet and strong fragrance and long-term period flowering (from May to October). Voucher specimens were confirmed by Milica Rat, PhD, and deposited at the BUNS Herbarium, University of Novi Sad, under number 2-0693. The plantation was established in 2018 on Pheno Geno Roses Serbia fields; the harvest was performed by hand in June 2021, during the full-flowering stage.

2.2. Hydrosol Extraction

Immediately after harvest, fresh flowers were subjected to hydrodistillation. A description of the procedure for rose hydrodistillation is as follows: approximately 50 kg of fresh rose flowers were placed in a distilling vessel (0.8 m^3), and water was added to soak the petals (approximately half of vessel volume) and heated to boiling. Steam was supplied through a manifold pipe into the bottom of the vessel from a high-pressure boiler and routed upwards through a plumbing system to the vessel, with plant material being extracted. The steam, water vapor, and entrained volatiles exited the tank near the top via a 10 cm diameter pipe and were carried to a water-cooled condenser that was mounted vertically, which acted as a pipe heat exchanger (the distillate flows through a pipe system and is immersed into a cooling fluid (water) in with the re-circulation flow rate of 2.5 m^3/h). After 3 h, the obtained essential oil was decanted, and hydrosol was collected and stored in plastic containers at ambient temperature. Furthermore, liquid–liquid extraction of volatile compounds from *R. hybrida* hydrosol was conducted by dichloromethane, using the Likens–Nickerson apparatus for 2 h.

2.3. Gas Chromatography–Mass Spectrometry

A gas chromatograph equipped with flame ionization (FID), mass selective (MSD) detectors, and non-polar capillary column HP-5MS was used for the analysis of volatile compounds [24]. The injection volume was 1 µL, and the split ratio was 100:1 for all samples, under GC operating conditions: injector temperature 250 °C, MS source temperature 230 °C, and interface temperature 315 °C. The mass spectra were obtained in electron ionization mode at electron energy 70 eV, with a mass scan range of m/z 40–600. Identification of the compounds was conducted according to their linear retention indices (RI_{exp}) and comparison with mass spectral libraries (RI_{lit}). The relative abundance of each detected compound was calculated from GC/FID chromatograms as a percentage area of each peak.

2.4. Determination of pH

The *R. hybrida* hydrosol pH value was measured by WTW Digital pH-meter (±0.005 pH).

2.5. Total Phenolics Content

Determination of total phenolics content of the rose hydrosol was performed by the Folin–Ciocalteu method (slightly modified [25]). The reaction medium consisted of 5 mL H_2O, 0.5 mL 33% Folin–Ciocalteu reagent, and 0.5 mL of hydrosol (water was added in the blank sample). After 3–6 min, 1 mL of 20% Na_2CO_3 was added and incubation at room temperature maintained for 60 min. The content of total phenolics was calculated from a calibration curve formed from absorbances of different concentrations of gallic acid (0.1–11 µg/mL) measured at λ = 756 nm. Results were expressed as µg of gallic acid equivalents in ml of hydrosol (µg GAE/mL).

2.6. Antioxidant Tests

Rose hydrosol antioxidant activity was determined by the method based on the difference in the activity of removing 1,1-diphenyl-2-picrylhydrazyl radical (DPPH radical) between the blank and the working sample [26]. Briefly, hydrosol (0.2 mL) was added to 0.5 mL of 0.04% DPPH ethanol solution and 2.3 mL of methanol, while 0.2 mL of distilled water was added to the blank instead of the sample and, after 30 min, the absorbance was read at λ = 517 nm. DPPH radical scavenging activity is expressed as % relative to the blank.

The superoxide anion ($O_2^{\bullet}-$) scavenging test (NBT-test) was based on a riboflavin-light-NBT system [27]. Briefly, the reaction mixture contained 0.5 mL of phosphate buffer (50 mM, pH 7.6), 0.3 mL riboflavin (50 mM), 0.25 mL PMS (20 mM), and 0.1 mL *p*-Nitro-Blue tetrazolium chloride (NBT, 0.5 mM), prior to the addition of 1 mL of hydrosol. The reaction was started by illuminating the reaction mixture with different concentrations using LED tube lights. After 5–15 min of incubation, the absorbance was measured at 560 nm. Superoxide anion radical scavenging activity (NBT test) was expressed as % inhibition or % relative to the blank.

Hydroxyl radical ($^{\bullet}OH$) scavenging activity of hydrosol was assayed by the method of the deoxyribose degradation assay [28]. Briefly, the reaction mixture contained 2.7 mL phosphate buffer (0.1 M, pH 7), 0.1 mL 2-deoxyribose (0.05 M), 0.1 mL ferric chloride (10 mM), 0.1 mL H_2O_2 (0.015%), and hydrosol (0.1 mL). Correction tubes contained phosphate buffer instead of 2-deoxyribose, while control tubes contained distilled water instead of sample. After incubation on 37 °C, for 60 min, 0.2 mL 0.1 M EDTA and 2 mL TBA reagent solution (20% TCA and 0.5% TBA) were added to all tubes. The mixture was heated for 15 min on a boiling water bath, cooled, and measured at 532 nm. The scavenging activity of the hydroxyl radical (OH test) was expressed as % inhibition or % relative to the blank.

2.7. Antimicrobial Activity

A standard screening method (disc diffusion method) with nine microorganisms (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Typhimurium

ATCC 13311, *Saccharomyces cerevisiae* ATCC 9763, *Candida albicans* ATCC 10231, and *Aspergillus brasiliensis* ATCC 16404) was used for evaluation of antimicrobial properties of rose hydrosol. The preparation of cultures and setup of the used disk diffusion method has been described in detail previously [29]. The obtained results after the appropriate period of incubation were expressed as the diameter of halo zones around disks and interpreted as follows: diameter of halo zone lower than 22 mm—resistant; diameter of halo zone between 22 and 26 mm—intermediary effect and diameter of halo zone above 26 mm—sensitive microorganism.

2.8. Anti-Inflammatory Assay

The in vitro anti-inflammatory activity of the rose hydrosol was tested by protein denaturation bioassay using egg albumin in 96-well micro plates [30]. Briefly, 5 mL of the reaction mixture (0.2 mL of egg albumin + 2.8 mL of phosphate buffered saline + 2 mL of hydrosol in varying concentrations—100, 200, 300, 400, 500 µg/mL) was incubated at 37 °C for 15 min, heated at 70 °C for 5 min, and then cooled. The absorbance was measured at 660 nm, while acetylsalicylic acid (at the same concentrations as hydrosol) was used as a reference drug and used for the determination of absorbance. The results were computed as the inhibitory concentration of extract (mg/mL) that inhibits 50% of protein denaturation (IC_{50}).

2.9. Antihyperglycemic Assay

The in vitro antihyperglycemic activity of the rose hydrosol was tested by α-glucosidase inhibitory potential in 96-well micro plates [30]. Briefly, 0.1 mL of the mixture (2 mmol/L 4-nitrophenyl-α-D-glucopyranoside in 10 mmol/L potassium phosphate buffer + 0.02 mL of the samples, diluted in buffer + 0.1 mL of the enzyme solution) was incubated at 37 °C for 10 min. The absorbance was measured at 405 nm. The increase in absorbance was compared with that of the control (buffer instead of sample solution) to calculate the inhibitory activity. The sample concentration providing 50% inhibition of α-glucosidase enzyme activity (IC50α-GIP) was calculated from the graph of α-GIP (%) against extract concentration.

2.10. Statistical Analysis

Statistical analysis of volatile compounds in different samples of rose hydrosols from literature and their visualization with an unrooted tree diagram was conducted using cluster analysis (R"APE" package—Analysis of Phylogenetics and Evolution), while calculation of the distance matrix was conducted using the Euclidean method [31].

3. Results

3.1. Chemical Composition of Rose hydrosol

Volatile Composition of *R. hybrida* Hydrosol

The *R. hybrida* hydrosol is a colorless clear liquid, with a pleasant rose scent. There were 44 volatile compounds detected in the hydrosol by using GC–MS (Table 1), among which the dominant were phenylethyl alcohol (23.5%), nerol (17.2%), linalool (13.2%), and geraniol (8.3%). A representative gas chromatogram of *R. hybrida* hydrosol is presented in Figure 1. Fragrance is determined by phenylethyl alcohol, which possesses a warm odor, mild-bland rose floral impression, shaded with slight honey-green tones [32], as well as nerol and geraniol, which have floral and fruity odors [33], while linalool odor is described as floral, citric, fresh, and sweet [34]. All these compounds are highly valuable, and are some of the most widely used in cosmetic formulations and in the fragrance industry, from personal care, household, and laundry products to food and tobacco [35].

Table 1. Volatile compounds from *R. hybrida* hydrosol.

No.	R.T.	Compound	Class	RI_{exp}	%
1	4.136	(Z)-3-Hexenol	O	854	0.5
2	4.189	NI-1	-	857	0.2
3	4.334	*n*-Hexanol	O	866	1.1
4	7.347	6-Methyl-5-hepten-2-one	O	987	4.3
5	7.499	Dehydro-1,8-cineole	O	991	1.3
6	7.569	6-Methyl-5-hepten-2-ol	O	993	0.4
7	8.873	1,8-Cineole	OMT	1031	0.4
8	10.427	*cis*-Linalool oxide (furanoid)	OMT	1074	0.2
9	11.052	*trans*-Linalool oxide (furanoid)	OMT	1091	0.1
10	11.561	Linalool	OMT	1106	13.2
11	11.773	*cis*-Thujone	OMT	1108	0.2
12	11.957	*cis*-Rose oxide	OMT	1113	0.2
13	12.264	Phenylethyl alcohol	O	1124	32.5
14	12.621	*trans*-Rose oxide	OMT	1126	0.1
15	13.020	*cis*-*p*-Mentha-2,8-dien-1-ol	OMT	1139	0.1
16	13.391	Camphor	OMT	1146	0.2
17	13.815	Nerol oxide	OMT	1157	0.1
18	14.407	*p*-Mentha-1,5-dien-8-ol	OMT	1171	1.5
19	14.834	Terpinen-4-ol	OMT	1180	0.3
20	15.455	α-Terpineol	OMT	1194	4.2
21	15.487	NI-2	-	1195	1.8
22	16.018	Isopiperitenol	OMT	1205	0.1
23	17.146	Nerol	OMT	1231	17.2
24	17.647	Neral	OMT	1243	2.0
25	17.791	Carvone	OMT	1246	0.2
26	18.023	NI-3	-	1250	0.1
27	18.312	Geraniol	OMT	1258	8.3
28	18.821	Orcinol dimethyl ether	O	1270	0.6
29	18.977	Geranial	OMT	1273	3.2
30	19.635	NI-4	-	1287	0.2
31	20.135	Thymol	OMT	1302	0.7
32	20.575	Carvacrol	OMT	1311	0.1
33	22.910	Eugenol	O	1362	0.6
34	23.379	(E)-3,7-Dimethyl-2,6-octadienoic acid	O	1372	1.5
35	24.943	Methyl eugenol	O	1408	0.6
36	26.315	Dihydro-β-ionone	O	1441	tr
37	26.834	Dihydro-β-ionol	O	1453	0.8
38	32.677	Viridiflorol	OST	1596	0.1
39	32.744	Cubeban-11-ol	OST	1598	0.1
40	33.108	NI-5	-	1608	0.1
41	33.343	Humulene epoxide II	OST	1613	0.2

Table 1. *Cont.*

No.	R.T.	Compound	Class	RI_{exp}	%
42	34.249	γ-Eudesmol	OST	1636	0.2
43	34.981	β-Eudesmol	OST	1655	0.1
44	35.121	α-Eudesmol	OST	1658	0.1
		Oxygenated Monoterpenes (OMT)			52.6
		Oxygenated Sesquiterpenes (OST)			0.8
		Other (O)			44.2
		Not identified (NI)			2.4
		Total			100.0

R.T. (min)—retention time; RI_{exp}—retention index determined experimentally by C8–C36 *n*-alkanes on a HP-5MS non-polar capillary column; tr—trace (less than 0.05%).

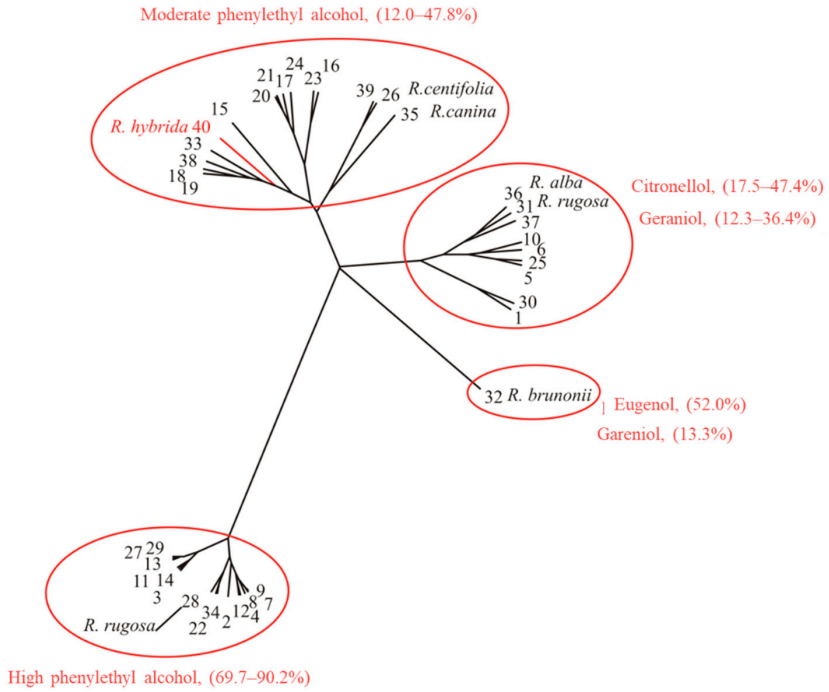

Figure 1. The unrooted cluster tree for different *Rosa* sp. hydrosols samples (according to Table 2).

Table 2. Volatile compounds (represented in percentages, %) of different rose hydrosols according to literature.

No.	Reference	Phenyl Ethyl Alcohol	Citronellol	Geraniol	Eugenol	Nerol	Dibuthyl Phthalate *	Linalool	Curzerene	Methyl Eugenol	Nonadecane
1	[6]	1.7	47.4	22.6	1.5	0.0	0.0	5.3	0.0	1.9	10.8
2	[36]	69.7	7.2	7.0	0.4	4.2	0.0	2.9	0.0	0.4	0.9
3	[36]	81.6	1.8	0.9	0.7	0.2	0.0	3.3	0.0	0.8	0.6
4	[36]	73.9	2.3	1.2	0.8	0.6	0.0	1.5	0.0	0.9	1.2
5	[36]	21.5	22.7	12.3	0.3	11.6	0.0	2.4	0.0	0.1	0.5

Table 2. *Cont.*

No.	Reference	Phenyl Ethyl Alcohol	Citronellol	Geraniol	Eugenol	Nerol	Dibuthyl Phthalate *	Linalool	Curzerene	Methyl Eugenol	Nonadecane
6	[37]	23.7	29.4	30.7	0.0	16.1	0.0	0.0	0.0	0.0	0.0
7	[38]	76.0	3.5	6.6	0.0	1.5	0.0	0.0	0.0	0.0	1.0
8	[38]	80.7	3.1	4.2	1.6	1.4	0.0	0.3	0.0	0.0	2.0
9	[38]	76.7	3.8	7.9	0.1	1.9	0.0	0.0	0.0	0.0	1.5
10	[39]	17.2	36.7	21.5	5.0	10.7	0.0	1.4	0.0	4.4	0.0
11	[40]	82.3	5.7	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0
12	[40]	77.4	4.3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	1.3
13	[40]	89.4	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	[40]	83.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4
15	[21]	12.0	5.9	11.7	17.8	0.0	0.0	9.0	0.0	0.0	2.4
16	[21]	20.8	3.4	4.0	2.5	0.0	18.8	1.5	5.4	0.0	0.0
17	[21]	47.8	5.9	2.3	3.6	0.0	11.4	0.0	3.9	1.0	0.0
18	[21]	33.4	10.3	24.0	3.1	0.0	6.8	4.1	2.0	0.7	0.0
19	[21]	39.5	8.6	12.6	2.3	0.0	8.8	2.0	2.7	0.0	0.0
20	[21]	33.8	5.6	0.0	0.0	0.0	10.1	0.5	7.3	1.3	0.0
21	[21]	37.7	8.2	0.0	2.9	0.0	18.0	1.4	3.0	1.1	0.0
22	[21]	73.3	8.4	0.0	3.6	0.0	4.0	1.9	1.2	1.5	0.0
23	[21]	26.8	6.5	0.0	0.0	0.0	9.9	0.7	4.6	0.0	0.0
24	[21]	34.9	2.7	0.0	0.0	0.0	13.9	0.0	11.0	0.0	0.0
25	[41]	25.0	20.9	21.2	2.1	10.8	0.0	1.9	0.0	2.7	0.0
26	[41]	45.6	24.6	11.3	3.1	3.8	0.0	2.0	0.0	2.4	0.0
27	[42]	90.2	4.5	0.6	1.5	0.0	0.0	0.0	0.0	1.0	0.0
28	[42]	78.7	13.5	tr	5.7	0.0	0.0	0.1	0.0	1.0	0.0
29	[42]	87.9	2.4	1.5	1.7	0.0	0.0	2.3	0.0	0.7	0.0
30	[43]	2.0	40.1	16.0	2.3	0.0	0.0	3.2	0.0	0.0	0.0
31	[44]	14.3	17.5	30.6	0.1	7.5	0.0	0.0	0.0	0.1	0.0
32	[45]	9.4	0.6	13.3	52.0	1.2	0.0	1.7	0.0	0.0	2.8
33	[46]	35.6	8.3	27.9	6.2	12.7	0.0	0.0	0.0	1.2	0.0
34	[3]	77.0	12.7	2.5	5.1	0.0	0.0	0.0	0.0	2.8	0.0
35	[3]	46.9	8.3	2.9	28.8	0.0	0.0	1.0	0.0	0.6	0.0
36	[47]	6.0	28.7	36.4	0.1	6.1	0.0	3.6	0.0	0.1	0.3
37	[47]	5.0	28.7	16.4	2.7	10.8	0.0	2.1	0.0	1.8	2.1
38	[48]	36.0	19.2	13.2	6.0	5.8	0.0	8.4	0.0	3.7	0.0
39	[49]	45.4	34.1	12.2	2.2	0.1	0.0	2.1	0.0	1.3	0.0
40	This study	32.5	0.0	8.3	0.6	17.2	0.0	13.2	0.0	0.6	0.0
	AVERAGE	46.3	12.5	9.8	4.2	3.1	2.5	2.0	1.0	0.8	0.7

* contaminants (dibutyl phthalate was probably transferred into the samples from the storage containers).

3.2. Chemical Diversity of Volatile Compounds Rose hydrosols

According to the literature review, there are 17 papers (39 samples) dealing with volatile compounds of rose hydrosols, among which are the commercial samples *R. damascena*, *R. alba*, *R. brunonii*, *R. canina*, *R. centifolia,* and *R. rugosa*. However, this is the first time that the volatile composition of *R. hybrida* is determined. The ten volatile compounds—most represented on average for all these samples are given in Table 2—and their cluster analysis are shown in Figure 1.

According to the unrooted cluster tree, there are four main chemotypes: (1) high phenylethyl alcohol (69.7–90.2%), (2) moderate phenylethyl alcohol (12.0–47.8%), (3) citronellol + geraniol (17.5–47.4% and 12.3–36.4%, respectively), and (4) eugenol + geraniol (52.0% and 13.3%, respectively). In addition, investigations conducted in Latvia with seven cultivars of winter-hardy *R. rugosa* show that the main volatile compounds in hydrosol were phenylethyl alcohol (28.6–79.9%), citronellol (28.0–57.0%), and nerol (up to 39.0%), depending on variety, while linalool was not detected [50].

High phenylethyl alcohol chemotype includes samples of *R. damascena* from India [36,38,40] and Iran [3,21], as well as *R. damascena* and *R. rugosa* from China [42]. The high content of phenylethyl alcohol is mainly linked with residue rose water, which remains after cohobation (repeated distillation of rose hydrosol) [46]. This process is used in commercial production of rose oil, known as *rose otto*, which is a mixture of essential oil and re-extracted volatile oil from hydrosol [51].

The moderate phenylethyl alcohol chemotype includes samples of *R. centifolia* [41] and *R. canina* [3], as well as in the *R. hybrida* sample from Serbia investigated in this study. *R. damascena* hydrosols from Turkey [46,48,49] and Iran [21] also belong to this chemotype.

The citronellol + geraniol chemotype could also be divided into three subgroups: the first subgroup includes samples with high content of citronellol, more than 40% [6,43]. The second subgroup contains citronellol, geraniol. and nerol; it is noted in *R. damascena* and *R. alba* from Bulgaria [47], as well as *R. rugosa* from Poland [44]. The third subgroup is a combination with phenylethyl alcohol, which is noted in four samples [36,37,39,41]. The last chemotype, a combination of eugenol and geraniol, is noted only in the *R. brunonii* hydrosol sample [52].

3.3. pH Value

The *R. hybrida* hydrosol is slightly acidic, pH = 6.07 (Table 3). A study conducted in France with commercial hydrosols of *R. damascena* and *R. centifolia* showed that the pH value ranged from 4.0 to 7.1 [41], while for *R. damascena* hydrosol from Morocco it was 6.91 [53].

Table 3. pH value, total phenolic content, and biological activities of *R. hybrida* hydrosol.

Activity	Method	Result
pH		6.07
total phenolics	Folin–Ciocalteu method	4.96 μg GAE/mL
antioxidant	DPPH	24.66%
	NBT	nd
	•OH scavenging assay	12.07%
antimicrobial	disc diffusion method (*A. brasiliensis*, *B. cereus*, *C. albicans*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *S. cerevisiae*, *S. typhimurium*, *S. aureus*)	nd
anti-inflammatory	protein denaturation bioassay using egg albumin	IC_{50} = 3.28 mg/L
antihyperglycemic	α-glucosidase inhibitory potential	nd

nd—not detected.

3.4. Total Phenolic Compounds

The total phenolic content (TPC) in *R. hybrida* hydrosol was 4.96 µg GAE/mL (Table 3). Similar results (5.2 µg GAE/mL) were obtained from Turkish *R. damascena* hydrosol [37]. Significantly higher values of TPC are noted in *R. damascena*, from 32.52 µg GAE/mL [47] to 57.02 µg GAE/mL [53], while hydrosol of *R. alba* contains 72.72 µg GAE/mL [47].

3.5. Biological Activity of Rose hydrosol

Rose hydrosol contains minute amounts of diluted water-soluble essential oil compounds (below 0.1%). The biological activities of hydrosols are consequently low [39], but organoleptic properties remain pronounced [41]. The pH value, total phenolic content, and biological activities of *R. hybrida* hydrosol investigated in this study are shown in Table 3.

3.6. Antioxidant Activity

According to all tests, the antioxidant activity of *R. hybrida* hydrosol was low or even absent (Table 3). The free radical scavenging activity according to the DPPH test was 24.66%, hydroxyl radical showed 12.07%, while superoxide anion activity was not detected. Similarly, results obtained in Poland by the FRAP (ferric reducing antioxidant power) method revealed that *R. damascena* hydrosol had low antioxidant activity (below 20%), while *R. centifolia* exhibited even lower activity (below 5%) [54]. A study conducted in Morocco showed that the antioxidant capacity of *R. damascena* hydrosol according to the DPPH test was 47.07%, while according to FRAP it was 100.71 AAE µg/mL [53]. Furthermore, *R. damascena* and *R. alba* hydrosols from Bulgaria had higher TPC values (32.52 and 72.72 µg GAE/mL, respectively) and demonstrated low capacity to inhibit lipid peroxidation (approximately 20%) as well as to neutralize hydroxyl radical and superoxide anion (under 40%) [47].

3.7. Antimicrobial Activity

The results obtained in this study indicate that rose hydrosol does not possess antimicrobial properties against the tested panel of microorganisms (Table 3). These results are consistent with the findings of other authors [37,55]. There is only one report which claims that rose water has a great potential of reducing the number of *Candida albicans* and methicillin-resistant *Staphylococcus aureus* (MRSA), followed by attenuation of neutrophil stimulation using modified testing methods. One of the newer research efforts on the *Rose damascena* hydrosol was also related to its in vivo antibacterial performance after hand-rubbing [49]; the results obtained in the mentioned study show that rose hydrosol has no impact on skin flora, confirming the results presented in this study. The low activity of rose hydrolate can be related to chemical composition, i.e., the low percentages of well-known antimicrobial plant biomolecules. However, the antimicrobial potency of rose essential oil and different extracts has been confirmed against a broad spectrum of microorganisms in many studies so far [37,56,57]. Nevertheless, the presence of an antimicrobial effect of essential oils and extracts does not guarantee that rose hydrosol has to have such an effect, due to qualitatively and quantitatively different chemical compositions.

3.8. Anti-Inflammatory Activity

The anti-inflammatory activity of *R. hybrida* hydrosol, according to protein denaturation bioassay using egg albumin, was found to have an IC50 value of 3.28 mg/L (Table 3). Rose water from Bulgaria (containing phenylethyl alcohol 43.7%, citronellol 18.2%, and geraniol 14.8%) suppressed neutrophil activation induced by TNF-α, with an IC50 of 3–15% [14].

3.9. Antihyperglycemic Activity

In vitro evidence of *R. hybrida* hydrosol antihyperglycemic activity according to the α-glucosidase inhibitory method was not found (Table 3). However, a methanolic extract of *R. damascena* was previously found to exhibit antihyperglycemic properties in diabetic and

normal rats, and it is useful in insulin resistant therapy because of the increased insulin sensitivity in animals [57].

4. Conclusions

Rosa hybrida cv Mileva™ Frayla® hydrosol contains phenylethyl alcohol, nerol, linalool, and geraniol as the main volatile compounds. According to the unrooted cluster tree, it could be classified as a moderate phenylethyl alcohol chemotype, together with many other commercial samples of rose water from Iran and Turkey. Because of its pleasant odor, rose hydrosol is widely used in food flavoring and as fragrance in perfumery and the cosmetic industries. However, antioxidant, antimicrobial, anti-inflammatory, and antihyperglycemic effects were found to be either low or absent, which is attributable to the low content of volatile and phenolic compounds detected in the hydrosol.

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