ANTIOXIDANT ACTIVITY AND VOLATILE PROFILE OF DAMASK ROSE HYDROSOL (ROSA DAMASCENA MILL.)

ANTIOKSIDATIVNA AKTIVNOST I AROMATSKI PROFIL HIDROLATA OD DAMASKE RUŽE (ROSA DAMASCENA MILL.)

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Abstract: *Rose water is the hydrosol portion of the distillate of rose petals, a by-product of the production of rose oil. The goal of this study was to investigate the impact of petal and leaf mass during distillation on chemical composition of hydrosols. The highest antioxidant activity (94.95 µmol trolox/mL) had hydrosol obtained after distillation of 80 kg of petals. The same hydrosol had the highest contents of beta-citronellol (13.24%), linalool (6.92%) and geraniol (8.41%). Hydrosol obtained after distillation of 60 kg of petals had the highest content of 2-phenylethanol (75.96%). Interestingly the hydrosol obtained after distillation of 60 kg of leafs had different volatile profile with 4,4,8-trimethyltricyclo [6.3.1.0 (1,5)] dodecane-2,9-diol as major volatile (19.15%) followed by linalool (15.99%), alpha-terpineol (12.50%) and beta-patchoulene (9.89%).*

Key words: *Rosa damascena, rose hydrosol volatiles, 2-phenylethanol*

Sažetak: *Ružina vodica predstavlja hidrolat koji nastaje kao nusproizvod proizvodnje eteričnih ulja. Cilj ovog istraživanja je bio utvrditi utjecaj različite mase latica, odnosno lišća tijekom destilacije na kemijski i aromatski sastav hidrolata. Najveću antioksidacijsku aktivnost (94,95 µmol trolox / mL) imao je hidrolat dobiven destilacijom 80 kg latica. Isti hidrolat je imao najveći sadržaj beta-citronelola (13,24 %), linalola (6,92 %) i geraniola (8,41 %). Hidrolat dobiven destilacijom 60 kg latica imao je najveći udio 2-feniletanola (75,96 %). Hidrolat dobiven nakon destilacije 60 kg listova imao različit aromatski profil. Hidrolat lišća imao je najveći sadržaj 4,4,8-trimetiltriciklo [6.3.1.0 (1,5)] dodekan-2,9-diola kao glavne hlapive tvari (19,15 %), a slijedi ga sadržaj linalola (15,99 %), alfa-terpineola (12,50 %) i beta pačulene (9,89 %).*

Ključne riječi: *Damask ruža, hlapive tvari hidrolata ruže, 2-feniletanol*

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1. Introduction

The Damask rose (*Rosa damascena* Mill.) is the most important rose species used to produce rose oil, concrete, absolute and hydrosols, which are valuable and important base materials for the perfume and cosmetic industries [1]. In the world market, Turkey and Bulgaria dominate the production of rose oil, concrete and absolute. Iran and India are major producers of rose water [2]. Beside perfume and cosmetic industries, rose oil and hydrosol have some pharmacological properties. Several studies on the pharmacological properties of the Damask rose [3] have reported that rose's essential oil possesses analgesic, anti-inflammatory, hypnotic, antitussive, and antispasmodic properties [4, 5]. In addition to the essential oil, hydroalcoholic and aqueous extracts, the hydrosol of the Damask rose have shown laxative [6], analgesic [7] and hypoglycemic effects in animal studies [8]. Rose water is the hydrosol portion of the distillate of rose petals, on other words it is a by-product of the production of rose oil. Rose water is also of high value in the food industry and some special foods are prepared using this product [9]. According to some authors, it is also used in biological agriculture against mushrooms, mildew, and insectsandfor fertilizationof soils [10].

Similar to many aromatic and medicinal plants *R. damascena* exhibits antioxidant activity. The antioxidant activity of hydro-alcoholic extract of petals and essential oil of this plant was also evaluated by DPPH for measurement of free radical scavenging activity and by ferric ammonium thiocyanate method for evaluation of lipid peroxidation properties [11]. Furthermore, the results showed a potent antioxidant and lipid peroxidation inhibitory effects comparable to α-tocopherol and suggest that the plant can be considered as a medical source for the treatment and prevention of many free radical disease [12]. Studies on volatile profile of hydrosols showed that the main components of hydrosol in water are phenylethanol (30.8%), citronellol (15.6 %) and geraniol (16.8%) [13, 14].

Although essential oils are widely investigated for their chemical and biological properties, the hydrosols are less studied. For that reason, the goal of the present study was to analyse the impact of petal and leaf mass during distillation on chemical composition and volatile profile of Damask rose hydrosols.

2. Material and methods

2.1. Chemicals

Folin and Ciocalteau phenol reagent obtained from Sigma–Aldrich (Chemie Check GmbH, Steinheim, Germany), sodium carbonate, methanol (HPLC grade) and diethyl ether from Panreac (Barcelona, Spain), 2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonate) and 2,2-diphenyl-1-picrylhydrazyl from Fluka (St. Louis, USA).

2.2. Plant material and hydrosol production

The Damask rose petals and leafs were obtained from producers Jukan Eko Hrana Gračanica, Bosnia and Herzegovina. The hydrosol was obtained after distillation of freshly harvested rose petals (60 kg – H1; 68 kg – H2 and 80 kg – H3) or leafs (60 kg

– HL) for up to 5 hours. The hydrosol was obtained after removing the oils using Clevenger apparatus. For the determination of volatile profile the rose water was extracted with diethyl ether and dried over anhydrous sodium sulphate. The solvent was evaporated to get a concentrated volatile fraction. This fraction was stored at -5 °C until analysis.

2.3. Determination of total polyphenol content

The total phenols content was determined by the modified colorimetric Folin-Ciocalteu method [15]. A 0.2 mL of hydrosol and 1.8 mL of deionizer water were added to a 23 mL test tube. 10 mL of Folin-Ciocalteu reagent (1:10) was added to the solution, and finally 8 mL of 7.5% of sodium carbonate (Na_2CO_3) solution was transferred into the test tubes. The colour was developed in 120 min, and the absorbance was read at 765 nm by spectrophotometer (Jenway 6300, Bibby Scientific, UK). The measurements were performed in triplicates for each sample and the average value was interpolated on a gallic acid calibration curve and expressed as µg of gallic acid per mL of sample equivalents (µg GAE/mL) of sample.

2.4. Antioxidant activity determination

ABTS assay followed the method of Arnao et al. [16] with some modifications. The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured ABTS value was over the linear range of the standard curve. For the DPPH assay 0.2 mL of the hydrosol was diluted with methanol (2 mL), and 1 mL of DPPH solution (0.5 mM) was added. After 15 min the absorbance was measured at 517 nm [17]. The results were expressed as mmol trolox equivalents (TE)/100 mL of sample. Additional dilution was needed if the measured DPPH value was over the linear range of the standard curve.

2.5. Determination of volatile profile by gas chromatography–mass spectrometry

The concentrated diethyl ether extracts of rose water and essential oil samples were subjected to GC-MS analyses on Agilent 5890 B with a mass detector Agilent 5977 A. The capillary column used in this experiment was CP-WAX52CB (Agilent, 60 m × 250 µm × 0.25 µm). Helium (He) 5.0 (purity 99.999%; Messer, Austria) was used as a carrier gas. Working conditions were as follows: injector temperature at 250 °C; MSD interface temperature 250 °C; oven temperature programmed from 40 °C (2 min hold) to 230 °C (5 min hold) at 6 °C/min; carrier gas (He) at a flow rate of 1 mL/min (average velocity 25.502 cm/sec); injection port operated in split less mode. Compounds were identified by comparing their mass spectra with the spectral library (Wiley 9, NIST 0.8) and expressed as peak area. Three replicate measurements were performed for each sample.

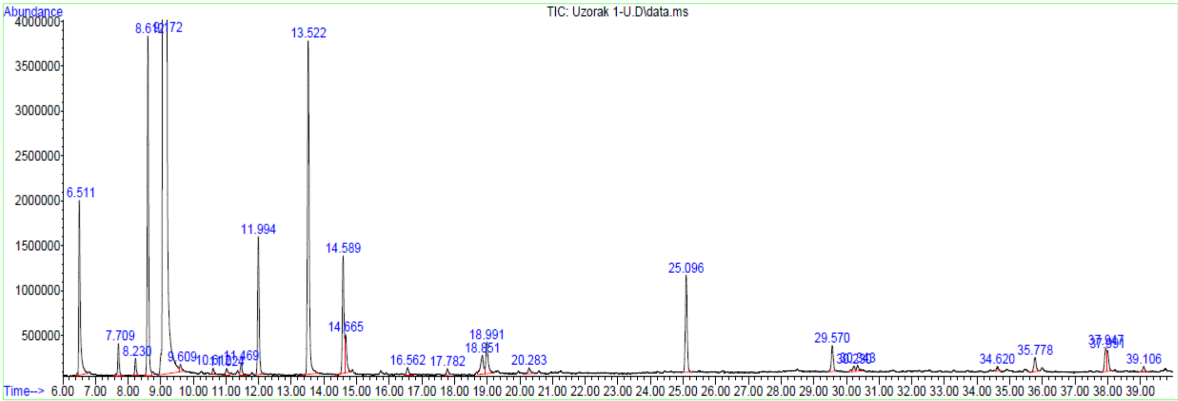

Figure 1. Example of GC–MS chromatogram of rose hydrosol

3. Results and discussion

In this study, we explored antioxidant activity and volatile profile of *R. damascena* Mill. hydrosols, produced through water-steam distillations of rose petals and leafs harvested from the fields of the family farm Jukan Eko Hrana, Gračanica, Bosnia and Herzegovina.

The total polyphenol content (TPC) and antioxidant activity (AA) are presented in Table 1. The highest TPC (73.73 µg GAE/mL) showed leaf hydrosol (HL). Comparing hydrosols produced from the rose petals, the highest TPC (53.54 µg GAE/mL) had hydrosol produced from 80 kg of rose petals that is higher than those reported by Ulusoy et al. [18] for *Rosa damascena* Mill. hydrosol 5.2 ± 0.3 µg GAE/mL, respectively. Regarding the AA of petal hydrosols measured by DPPH and ABTS method, the highest AA had hydrosol produced from 80 kg of rose petals (94.95; 136.45 µmol trolox/mL, respectively). These results are in accordance with those reported by Aazza [19]. Aazza [19] reported antioxidant activity for seven different hydrosols ranging from 1.1 – 313.4 µmol trolox/mL.

Table 1. Total polyphenol content and antioxidant activity of rose hydrosols

Sample	**TPC (µg GAE/mL)**	**DPPH (µmol trolox/mL)**	**ABTS (µmol trolox/ mL)**
H1	25.30 ± 0.64	61.56 ± 1.61	98.50 ± 1.27
H2	30.61 ± 0.37	88.42 ± 1.09	123.79 ± 1.55
H3	53.54 ± 0.33	94.95 ± 1.54	136.45 ± 1.19
HL	73.73 ± 0.71	93.14 ± 1.44	129.22 ± 1.46

TPC-total polyphenol content; hydrosol obtained after distillation of freshly harvested rose petals: 60 kg – H1; 68 kg – H2 and 80 kg – H3 or 60 kg of leafs – HL.

The chromatographic profiles of both *Rosa damascena* Mill. petals and leaf hydrosols are shown in Table 2. Seventy-five compounds were identified describing total volatile profile of petals and leaf hydrosols.

The main volatile compound in hydrosols obtained after distillation of petals was 2-phenylethanol and it ranges from 58.16 to 75.96%. The 2-phenylethanol have good solubility in water and passes almost completely into the aqueous phase during steam distillation, but is completely lost in process of preparing rose oil. Consequently, rose water contains a high quantity of 2-phenylethanol [20]. The main compounds beside 2-phenylethanol were beta-citronellol, linalool and geraniol. Hydrosol obtained after distillation of 80 kg of petals had the highest contents of beta-citronellol (13.24%), linalool (6.92%) and geraniol (8.41%). The contents of citronellol and geraniol obtained in this research were lower than those reported by Ulusoy et al. [18] for *Rosa damascena* Mill. Hydrosol, 29.44 and 30.74%, respectively. Geraniol and citronellol are the main compounds of rose oils and hydrosols although the chemical composition of the final product is influenced by the origin of the plant, agro-meteorological conditions and technology used for production [21]. Furthermore, the rose aroma quality depends on citronellol and geraniol content; moreover, it depends on the citronellol/geraniol ratio. It is considered that ratio between 1.2 and 1.3 is a characteristic of the high quality rose oils and hydrosols [22]. In our study this ratio was achieved after distillation of 68 kg of petals. To our knowledge this is first time that rose leaves hydrosol was investigated in respect to the volatile profile. The hydrosol obtained after distillation of 60 kg of leafs had different volatile profile with 4,4,8-trimethyltricyclo [6.3.1.0 (1,5)] dodecane-2,9-diol as major volatile (19.15%) followed by linalool (15.99%), alpha-terpineol (12.50%) and beta-patchoulene (9.89%). Further investigation on antioxidant activity of named compound should be conducted in order to establish the impact of each compound on the total antioxidant activity.

Table 2. Volatile profile of *Rosa damascena* Mill. hydrosols.

Compounds	**RT (min)**	**%**			
		H1	**H2**	**H3**	**HL**
Heptanal	3.48	0.09	0.02	0.03	n.d.
Ethanone	3.69	n.d.	n.d.	n.d.	0.27
Benzaldehyde	4.68	n.d.	0.01	0.04	2.57
5 Methyl Furfural	4.71	n.d.	n.d.	0.05	n.d.
2H-Pyran	4.89	n.d.	n.d.	n.d.	0.33
6-Methyl-5-Hepten-2-One	5.22	0.07	0.05	0.06	n.d.
2,4-Hexadiene	5.37	n.d.	0.05	0.06	0.18
3-Hexen-1-Ol, Acetate	5.73	n.d.	n.d.	n.d.	0.19
1,8-Cineole	6.45	n.d.	n.d.	n.d.	0.10
Benzenemethanol	6.51	2.53	2.51	1.76	0.14
Benzeneacetaldehyde	6.82	n.d.	0.06	0.14	n.d.
Octyl Formate	7.62	n.d.	n.d.	n.d.	0.29
Linalool Oxide	7.71	0.44	0.36	0.33	4.16
Cis-Linaloloxide	8.23	0.24	0.23	0.20	2.90

Compounds	RT (min)	% H1	H2	H3	HL
(5-Ethyl-Cyclopent-1-Enyl)-Methanol	8.39	n.d.	n.d.	n.d.	0.28
Linalool	8.63	4.53	5.65	7.00	15.99
2-phenylethanol	9.22	75.96	71.61	58.16	1.45
Camphor	10.22	n.d.	n.d.	n.d.	0.24
Neroloxide	10.62	0.09	n.d.	0.11	n.d.
Borneol L	11.02	0.13	0.07	0.09	0.35
P-Mentha-1,5-Dien-8-Ol	11.08	n.d.	n.d.	0.12	n.d.
Epoxylinalol	11.15	n.d.	n.d.	n.d.	0.75
2-Dodecene	11.26	n.d.	n.d.	n.d.	0.11
Bicyclo[2.2.1] Heptan-2-Ol, 1,5,5-Trimethyl	11.36	n.d.	0.08	0.10	1.28
4-Methyl-1-(1-Methylethyl)	11.47	0.20	0.12	0.20	1.82
Silane	11.80	n.d.	n.d.	0.04	0.36
Alpha terpineol	12.00	2.09	3.01	3.77	12.50
Benzoic Acid	12.14	n.d.	n.d.	n.d.	0.33
Beta-Citronellol	13.55	5.51	5.98	13.24	3.83
1,5-Dimethylcyclohexene-5-Carboxaldehyde	13.74	n.d.	n.d.	0.20	n.d.
Z-Citral	14.03	n.d.	n.d.	0.09	n.d.
Geraniol	14.62	1.82	5.19	8.41	2.01
Benzene	14.66	0.63	n.d.	n.d.	n.d.
3,7-Dimethyl-2,6-Octadienal	15.25	n.d.	n.d.	0.12	n.d.
6a-Methano-1H,4H-Pentalen-1-One	15.74	n.d.	0.09	0.24	0.20
1-Hexen-3-Yne, 2-Methyl	16.55	0.14	0.13	0.25	0.68
Cyclohexanemethanol	17.79	0.11	0.06	0.06	n.d.
Citronellyl Propionate	18.70	n.d.	n.d.	0.06	n.d.
Geranic Acid	18.85	0.50	n.d.	0.33	0.55
1,7-Octanediol, 3,7-Dimethyl	19.00	0.67	1.39	1.99	n.d.
Geranyl Propionate	19.28	n.d.	0.13	0.26	n.d.
Methamidophos	19.34	n.d.	n.d.	n.d.	0.24
Geranyl Acetate	19.97	n.d.	0.18	0.12	n.d.
Nerol	20.29	0.25	0.32	0.43	0.33
1,3-Pentadiene, 2,4-Dimethyl	20.96	n.d.	0.05	0.06	n.d.
N-Dimethylamino-Methyldithiocarbamate	21.54	n.d.	n.d.	n.d.	0.23
Hydroxydihydroedulan	22.00	n.d.	n.d.	n.d.	0.16
(-)-beta-Selinene	23.67	n.d.	n.d.	n.d.	0.19
1H-Inden-1-One	24.98	n.d.	n.d.	n.d.	0.16
Phenol, 2,6-Bis(1,1-Dimethylethyl)-4-Methyl	25.10	1.67	1.01	0.87	2.17
4-Acetyl-1-Methyl-1-Cyclohexene	29.50	n.d.	n.d.	n.d.	0.86
Gamma-Selinene	29.58	0.49	0.39	0.12	n.d.
Alpha-Selinene	30.23	0.10	0.08	n.d.	n.d.
1,5-Dimethyl-8-(1'-Methylethenyl)Bicyclo[4.4.0]Dec-4-En-3-One	30.35	0.13	0.11	n.d.	n.d.
Longifolenaldehyde	31.05	n.d.	n.d.	n.d.	0.32
1-Ethyl[9](2,4)Pyrazolophane	31.40	n.d.	n.d.	n.d.	0.84
1,4-Dimethyltricyclo[5.3.0.0(4.10)]Decan-8-One	31.88	n.d.	n.d.	n.d.	0.50
(-)-Caryophyllene Oxide	33.30	n.d.	n.d.	n.d.	1.17

Compounds	RT (min)	% H1	H2	H3	HL
Dimethyl Dimethylphosphoramidate	33.70	n.d.	n.d.	n.d.	0.51
Benzene, 1,1'-(1,2-Ethynediyl)Bis	34.52	n.d.	n.d.	n.d.	0.14
Guanosine, N,N-Dimethyl	34.63	0.08	0.09	0.07	1.62
Lup-20(29)-Ene-3,6-Dione	34.93	n.d.	n.d.	n.d.	1.78
Bicyclo[5.2.0]Nonane	35.06	n.d.	n.d.	n.d.	0.45
5-Ethylnona-2,3-Dien-2-One	35.48	n.d.	n.d.	n.d.	1.98
beta-Patchoulene	35.77	0.30	0.15	0.15	9.89
Caryophylla-3,8(13)-Dien-5.Beta.-Ol	36.03	n.d.	n.d.	n.d.	1.71
5,7,7-Trimethylbicyclo[3.3.0]Oct-8-En-2-One	36.41	n.d.	n.d.	n.d.	0.45
Alpha.-Cedrol	37.68	n.d.	n.d.	n.d.	0.34
4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]Dodecane-2,9-Diol	37.95	0.44	0.24	0.42	19.15
1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester	37.99	0.33	0.34	n.d.	n.d.
Nonadecane	39.11	0.10	n.d.	n.d.	n.d.
(2E,4E)-N-Isopropyl-6-Methyl-2,4-Heptadienamide	40.92	n.d.	n.d.	n.d.	0.18
Hexanedioic Acid	59.96	0.35	0.25	0.25	0.78

Hydrosol obtained after distillation of freshly harvested rose petals: 60 kg – H1; 68 kg – H2 and 80 kg – H3 or 60 kg of leafs – HL; RT – retention time in minutes.
n.d. – not detected

4. Conclusions

This research studied the chemical composition of hydrosols obtained after distillation of rose petal and leaves. The amount of petals during distillation significantly influenced the chemical compositions of studied hydrosols. The total phenol content was the highest in the hydrosol obtained after distillation of 60 kg of rose leaves, while antioxidant activate in hydrosol obtained after distillation of 80 kg of rose petals. The volatile profile was determined using GC/MS. The main constituents of hydrosols obtained after distillation of rose petals were 2-phenylethanol, geraniol and beta-citronellol.

The percentages of the main compounds in petals hydrosols differed strongly between the studied samples. The hydrosol obtained after distillation of 60 kg of leafs had different volatile profile with 4,4,8-trimethyltricyclo [6.3.1.0 (1,5)] dodecane-2,9-diol as major volatile followed by linalool, alpha-terpineol and beta-patchoulene.

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