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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM NARCISSUS (*NARCISSUS POETICUS* L.) AND ABSOLUTE FROM FOUR ROSE (*ROSA DAMASCENA* MILL.) CULTIVARS

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Abstract. The 28 components of *N. poeticus* essential oil and 37 of *R. damascena* Mill. absolutes of Moldovan origin were identified by GC-MS analysis. The major component of *N. poeticus* essential oil was *γ*-terpineol (52.62%). In addition to previously described terpene constituents: (*Z*)-*β*-ocimene (6.81%), eucalyptol (5.48%), (*E*)-*β*-ocimene (2.78%), *β*-caryophyllene (0.88%), *β*-myrcene (0.41%) - several compounds not previously described in *Narcissus* oil were identified, including lilac alcohols B and D (0.53 and 0.42%, respectively), lilac aldehydes A and C (0.43% and 0.78%, respectively), etc. The chemical constituents of *R. damascena* absolutes belong to several classes. The main constituent, as expected, is phenylethyl alcohol, the content of which varies from 59.85% to 78.17%. The terpene fraction is represented by several compounds like *β*-cytronellol (0.79–6.53%), nerol (5.89%), elemol (0.37%) and *α*-eudesmol (0.32%). The *in vitro* assessment of the essential oil from *N. poeticus* and *R. damascena* absolutes against four bacterial strains and two fungal species showed high antibacterial and antifungal activity, with effective concentrations ranging from 150 to 300 μg/mL for *N. poeticus* oil and from 300 to 600 μg/mL or *R. damascena* absolutes.

Keywords: *Narcissus poeticus* L. essential oil, *Rosa damascena* Mill. absolute, GC-MS analysis, antimicrobial assessment.

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Introduction

The genus *Narcissus* (fam. Amaryllidaceae) comprises about 80 species, with Narcissus (*Narcissus poeticus* L.) being the first species formally described by Linnaeus Carl [1]. Native to the Middle East or the Mediterranean region, *N. poeticus* is currently distributed across most continents [2]. Aside from its ornamental value, the species has pharmaceutical and perfumery importance due to its specific chemical composition and the products that are mentioned below.

Like other species of this genus, *N. poeticus* is a rich source of alkaloids used in the treatment of oncological and Alzheimer's diseases [3–5]. In addition, extracts from this species have been reported to exhibit high antifungal activity, which is attributed to their rich content of phenolic compounds [6].

The species is also of considerable importance to the perfumery industry. The absolute and essential oil obtained from the concretes of *Narcissus* spp. are mandatory ingredients of a wide range of classic and luxury perfumes [7]. However, the essential oil content of *N. poeticus* is quite low, consequently, the absolute is most commonly used. Therefore, most studies on the chemical composition refer to the analysis of volatile oil of *N. poeticus* obtained by *n*-hexane or supercritical fluid extractions [1,8,9]. The high market value of *N. poeticus* absolute can be attributed to the relatively low quantities of the products obtained from the plants. According to the limited literature available, the concrete is obtained with a yield of approximately 0.2%, while the absolute content in the concrete can reach up to 37% [10,11]. Following a thorough bibliographic study, no references were found describing the chemical composition of the volatile oil of *N. poeticus* obtained by hydrodistillation of the concrete.

The Damask rose (*Rosa damascena* Mill.) is the most widely cultivated among the few *Rosa* species grown on a large scale, including in Europe and Asia. This predominance is primarily due to the high-quality of its derived products, such as essential oil, rose water, concrete and absolute, which are key raw materials in the production of luxury cosmetics and perfumery products [12].

Extracts and products obtained from *R. damascena* have additional applications, particularly in medicine, where they are used in the treatment of various conditions and diseases. Additionally, the buds, petals and essential oil are used in the food industry for production of teas, jams, and both soft and alcoholic drinks. In general, the essential oil content in freshly harvested petals is low and varies depending on the cultivar and geographical origin. For this reason and in the absence of effective natural or synthetic substitutes, rose essential oil is among the most expensive essential oils on the market [13].

The chemical composition of rose essential oil strongly depends on pedoclimatic conditions and varies across different geographical areas; however, it is generally characterized by a high content of phenylethyl alcohol, a compound typical of all cultivated *Rosa* species and cultivars [14].

Certain authorities, including the International Organization for Standardization, have issued the quality standard for rose oil obtained by steam distillation, which specifies the content of the main components as follows: citronellol (20–34%), nerol (5–12%), geraniol (15–22%) and paraffins C_{17} (1.0–2.5%), C_{19} (8–15%), and C_{21} (3.0–5.5%) [15].

The absolute is obtained through ethanol maceration of the rose concrete, a solid product that results from non-polar solvent extraction of fresh flowers, followed by complete solvent distillation [14]. Only a few studies have investigated the chemical composition of rose absolute. One of the most relevant papers reports the analysis of absolutes from Bulgaria, Egypt and Morocco using gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame-ionization detection (GC-FID) [16]. The authors identified over 130 constituents, the majority being mono- and sesquiterpenes, along with phenylethyl alcohol (18.35–27.75%).

Regionally, the cultivars of *R. damascena* remain poorly studied. A team of Moldovan researchers conducted a comparative study on the concrete content in the petals and its yield from three local cultivars - Raduga, Lani and Ukraina [17]. More recently, Ukrainian researchers reported the chemical composition of the essential oil obtained from the petals of the Veselka (Rainbow) cultivar, produced *via* the *in vitro* micropropagation method, and its antimicrobial activity [18]. At present, no data have been published on the chemical composition of rose absolute of Moldovan origin.

For this reason, and in line with the purposes of this study, the chemical composition of essential oil from the *N. poeticus* and *R. damascena* absolutes was evaluated using GC-MS techniques. Additionally, the antimicrobial activity of the essential oil and the absolutes was assessed against several fungal species, as well as non-pathogenic and phytopathogenic bacterial strains. It should be noted that such studies were conducted for the first time for the species *N. poeticus* and *R. damascena* cultivars of Moldovan origin.

Experimental

Materials

The plants of *N. poeticus* and *R. damascena* (Krimskaya cultivar) were harvested during the flowering stage from the fields located near the town of Căușeni, Pervomaisc village (46°42'04" N 29°05'21" E) in April 2020. Plants of three other cultivars of *R. damascena* (Raduga, Lani, and Ucraina) were harvested during the flowering stage in May 2024 from the experimental fields of the Institute of Genetics, Physiology, and Plant Protection, located near the town of Chisinau (46°97′10.3″ N 28°89′16.4″ E).

All the solvents and reagents were of analytical grade. Anhydrous sodium sulphate, 96% ethanol and petroleum ether (PE) were purchased from Sigma-Aldrich.

Narcissus essential oil hydrodistillation

N. poeticus essential oil was obtained by hydrodistillation of the concrete using a Neo-Clevenger type extractor for 1.5 hours. The obtained oil was dried with anhydrous sodium sulphate, filtered and subjected to chromatographic analysis.

Rose concrete preparation and absolute extraction

The samples of freshly collected petals of four *R. damascena* cultivars (Lani, Raduga, Ukraina-in laboratory and Krimskaya-industrially) were extracted three times (1 h each), with light petroleum ether (PE) in 1:5, 1:4 and 1:3 ratios.

The combined ether extracts were distilled under reduced pressure and the crud concretes were obtained.

The *R. damascena* absolutes were obtained by two extractions of the concrete with ethanol under stirring for 6 and 3 hours, respectively, using ratios of 1:10 and 1:5. Each organic extract was kept at 4°C for wax sedimentation and filtered several times. Then, the combined alcohol extracts were distilled under reduced pressure and the obtained absolutes were stored at low temperature until chromatographic analyses.

GC-MS analysis

Analyses were carried out on an Agilent Technologies 7890A system with 5975C Mass-Selective Detector (GC-MSD) equipped with split-splitless injector (split, 250°C, split ratio 1:50, 1 mL) and HP-5 ms capillary calibrated column (30 m × 0.25 mm × 0.25 mm). The carrier gas used was helium 1.1 mL/min. Oven: 70°C for 2 min, 5°C/min to 200°C, 20/min to 300°C for 5 min. MSD in scan 30–300 amu for 15 min, 30–450 amu, solvent delay 3 min 40 sec.

Antimicrobial activity assessment

The evaluation of the antimicrobial activity of the (5%) hydroalcoholic solution of *N. poeticus* essential oil and *R. damascena* absolutes was performed in triplicate against non-pathogenic Gram-positive and Gram-negative strains of *Bacillus subtilis* NCNM-BB-01 (ATCC 33608) and *Pseudomonas fluorescens* NCNM-PFB-01 (ATCC 173325), respectively, as well as against phytopathogenic strains of *Erwinia carotovora* NCNM 177 BE-03 (ATCC 15713), *Xanthomonas campestris* NCNM BX-01 (ATCC 17553), and yeast-fungal strains of *Candida albicans* NCNM Y-25 (ATCC 14053) and *Saccharomyces cerevisiae* NCNM Y-20 (ATCC 4117).

For antimicrobial activity testing, the successive double-dilution method was used [19,20]. Test solutions were serially diluted in broth media (peptone for bacteria, Sabouraud for fungi), inoculated with standardized microbial suspensions, and incubated at 35°C for 24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration without visible growth, while minimum bactericidal/fungicidal concentrations (MBC/MFC) were determined by culturing on agar plates. The concentration of preparation that completely inhibited microbialcolony growth was considered to be its minimal bactericidal and fungicidal concentrations.

Results and discussion

GC–MS identification of essential oil absolutes constituents

The concrete of *N. poeticus* was obtained industrially with a 0.25% yield by extracting the flowers with *n*-hexane and subsequent concentration of the extract under reduced pressure until dry.

As previously mentioned, *N. poeticus* essential oil was obtained in the laboratory with a 20% yield by hydrodistillation of concrete in a Neo-Clevenger type extractor for 1.5 hours. The oil did not contain waxes, and appeared as a colourless liquid with a yellow tint, exhibiting a strong pungent, green, woody smell complemented with a deep floral scent. The chemical composition of the oil was determined by the GC-MS method, with 28 constituents identified, the retention times and contents of which are represented in Table 1.

Table 1

Phytochemical composition of *N. poeticus* L. essential oil of Moldovan origin.

No.	*RT* (min)*	*Component*	*%*	*No.*	*RT* (min)*	*Component*	*%*
1	4.08	(*E*)-3-Hexen-1-ol	0.54	***15***	10.38	Lilac aldehyde A	0.43
2	4.23	*n*-Hexanal	1.48	***16***	10.62	Lilac aldehyde C	0.78
3	4.78	*n*-Heptanal	1.23	***17***	11.08	α-Terpineol	1.33
4	6.57	β-Myrcene	0.41	***18***	11.79	γ-Terpineol	52.62
5	6.72	*n*-Decane	0.15	***19***	12.02	Lilac alcohol D	0.42
6	6.90	(*Z*)-3-Hexenyl acetate	2.92	***20***	12.29	Lilac alcohol B	0.53
7	7.04	*n*-Hexyl acetate	0.63	***21***	12.53	β-Cyclocitral	0.24
8	7.37	*p*-Methyl anisole	0.24	***22***	13.36	Linalyl acetate	0.49
9	7.57	Eucalyptol	5.48	***23***	17.74	β-Caryophyllene	0.88
10	7.64	(*E*)-β-Ocimene	2.78	***24***	19.41	β-Ionone	0.52
11	7.90	(*Z*)-β-Ocimene	6.81	***25***	19.53	Pentadecane	0.33
12	8.49	Octanol	0.28	***26***	25.75	Benzyl benzoate	10.32
13	9.23	Geranyl benzoate	3.16	***27***	30.63	Heneicosane	0.27
14	9.33	Nonanal	3.99	***28***	32.03	Heptacosane	0.16
		Total (%)			**99.42**		

**RT - retention time.*

Of the total identified components (99.42%), the major fraction is represented by terpene compounds (76.36%), especially monoterpenes (hydrocarbons - 10.0%; oxygenated - 65.48%), followed by aliphatic compounds: hydrocarbons (0.91%), alcohols (0.82%), aldehydes (6.70%) and esters (13.87%). Sesquiterpenes account for only (0.88%) and are represented by hydrocarbon *β*-caryophyllene.

The major constituents of the essential oil are monoterpene hydrocarbon *γ*-terpineol (52.62%) and *α*-terpineol 1.33%, also identified by authors [1] in a low amount in CO_2 supercritical extract from *N. poeticus* flowers (1.08–3.42%). According to [1] *α*-terpineol is a constituent responsible for anise, mint and lilac notes in the aroma of essential oil. It is followed by two other monoterpene hydrocarbons (*E*)-*β*-ocimene (2.78%) and (*Z*)-*β*-ocimene (6.81%), as well as bicyclic monoterpene ether eucalyptol (5.48%), also reported by [10] after headspace solid-phase microextraction (HS-SPME) and GC-MS analysis of *N. poeticus* mono- and double flowers cultivars in relative contents of 2.32%, 34.86% and 8.85%, respectively. Other terpenes are represented by *β*-myrcene (0.41%), previously reported [1] and unreported *β*-caryophyllene (0.88%).

It should be mentioned that the proportion of esters is relatively high (13.87%). To this category belong reported benzyl benzoate (10.32%) responsible for balsamic, herbal, almond, cheese, cherry, floral, pineapple, strawberry and sweet odours [1], geranyl benzoate (3.16%), previously unreported *n*-hexyl acetate (0.63%) and linalyl acetate (0.49%).

The aliphatic alcohols and aldehydes are represented by reported heptanal (1.23%) and nonanal (3.99%), which are responsible for a wide range of notes in the aroma and (*E*)-3-hexen-1-ol (0.54%) [1], along with those reported for the first-time octanol (0.28%) and *n*-hexanal (1.48%). The fraction of higher alkanes is represented by pentadecane (0.33), heneicosane (0.27%) and heptacosane (0.16%).

Surprisingly, lilac alcohols B (0.53%) and D (0.42%), along with lilac aldehydes A (0.43%) and C (0.78%) were identified for the first time in the composition of *N. poeticus* essential oil. These compounds are considered to be the principal olfactory molecules of lilac flowers [21].

The concrete of *R. damascena* was obtained from the freshly collected petals of four cultivars: Lani, Raduga, Ukraina (in laboratory) and Krimskaya (industrially), by extracting them with light petroleum ether according to procedure described above (experimental section). As a result, concretes were obtained with yields ranging in the order Lani (0.26%) ≈ Krimskaya (0.28%) < Ukraina (0.32%) < Raduga (0.36%). The data obtained are in line with those previously reported by Moldovan researchers, which were as follows: Lani (0.28%) > Ukraina (0.32%) > Raduga (0.40%) [17].

Next, the absolutes of *R. damascena* were extracted from concretes according to procedure described above (experimental section) with yields ranging in the order Krimskaya (42.8%) < Raduga (54.1%) < Ukraina (55.95%) < Lani (72.3%). The *R. damascena* absolute is a viscous liquid, golden yellow to dark brown in colour. It has an intense floral, sweet, deep, and persistent aroma with "green" notes due to non-volatile compounds.

The chemical composition of the *R. damascena* absolute was determined by the GC-MS method, with 37 constituents identified, the retention times and contents of which are represented in Table 2.

According to GC-MS analysis of the absolute samples, they are dominated by phenylethyl alcohol in a range of 59.85% to 78.17%, in accordance with the EU standard [15]. The order is as follows: Raduga (59.85%) < Ucraina (70.93%) < Krimskaya (71.49%) < Lani (78.17%), respectively.

From the total of identified components (97.07–99.09%), the major fraction is represented by aliphatic compounds: saturated and unsaturated hydrocarbons, which on average constitute 7.63–24.95%, esters (2.24–6.09%), other alcohols (0.16–6.2%), epoxides (0.99–1.84%) and aldehydes (0.1–0.35%). Here, the compounds with the highest content can be mentioned *n*-nonadecane (9.89%, Ukraina), (*Z*)-9-tricosene (1.73%, Raduga), oxalyl acid dodecyl-ethyl tridecyl ester (3.2%, Raduga), benzyl alcohol (6.09%, Lani) and phenyl acetaldehyde (0.35%, Lani).

The terpene fraction includes oxygenated monoterpenes in the order Raduga (0.79%) < Lani (1.22%) < Ukraina (1.25%) < Krimskaya (12.42%), and it is represented by *β*-citronellol (0.79–6.53%) and nerol (5.89%, Krimskaya), respectively. The fraction of oxygenated sesquiterpenes (0.93%) was detected only in the absolute obtained from the Krimskaya cultivar, which consists of *β*-eudesmol (0.24%) and *α*-eudesmol (0.32%). Surprisingly, in three of the absolute samples, the triterpene compound lupeol was also identified, with a content of Lani (0.17%) = Ukraina (0.17%) < Raduga (0.51%).

Table 2

Phytochemical composition of absolutes from four *R. damascena* Mill. cultivars.

No.	*RT* (min)*	*Component*	Lani (%)	Raduga (%)	Ukraina (%)	Krimskaya (%)
1	7.45	Benzyl alcohol	6.09	trace	0.25	2.72
2	7.70	Phenyl acetaldehyde	0.35	0.22	0.18	0.10
3	9.45	Phenylethyl alcohol	78.17	59.85	70.93	71.49
4	11.50	Dodecane	-	0.39	-	-
5	12.40	β-Citronellol	1.22	0.79	1.25	6.53
6	13.09	Nerol	-	-	-	5.89
7	14.17	Tridecane	-	1.07	trace	-
8	16.77	*n*-Tetradecane	-	1.00	trace	-
9	18.30	2,6,10-Trimethyltetradecane	-	0.39	trace	-
10	19.25	Pentadecane	-	1.18	0.15	-
11	20.67	Elemol	-	-	-	0.37
12	21.59	Hexadecane	-	1.12	trace	-
13	23.03	β-Eudesmol	-	-	-	0.24
14	23.11	α-Eudesmol	-	-	-	0.32
15	23.84	Heptadecane	-	1.69	0.67	0.10
16	25.97	Octadecane	trace	0.96	0.15	-
17	27.48	9-Nonadecene	trace	0.51	0.64	0.19
18	27.99	*n*-Nonadecane	2.19	8.01	9.89	2.16
19	29.49	Eicosane	0.22	0.90	0.53	0.19
20	30.39	10-Heneicosene	0.25	0.34	0.13	0.28
21	30.48	Heneicosane	1.78	3.43	3.50	1.62
22	31.26	Heptacosane	0.12	0.24	0.10	0.10
23	31.48	(*Z*)-5-Octadecenyl-1-ol acetate	trace	0.19	0.10	0.16
24	31.86	(*Z*)-9-Tricosene	1.14	1.73	0.30	1.04
25	31.90	Tricosane	1.10	1.64	0.96	0.93
26	32.65	(*Z*)-3-Octadecenyl-1-ol acetate	trace	0.22	0.12	0.22
27	32.92	(*Z*)-12-Pentacosene	0.93	0.74	1.15	0.92
28	33.61	Lupenone	trace	0.86	0.19	trace
29	33.83	Pentatriacontene	0.18	trace	trace	0.10
30	33.88	1-Heptacosanol	0.57	2.26	0.51	0.61
31	34.01	Lupeol	0.17	0.51	0.17	trace
32	34.67	Tetracosa-2,6,14,18,22-pentaene-10,11-diol-2,6,10,15,19,23-hexamethyl	0.11	0.16	trace	trace
33	34.89	Hexacosyl acetate	0.30	-	0.25	0.40
34	34.93	(*Z*)-Octadecanoic acid (2-phenyl)-1,3-dioxane-4-yl) methyl ester	0.30	0.63	0.26	0.62
35	35.06	Oxalyl acid dodecyl-ethyl tridecyl ester	0.82	3.20	2.52	0.47
36	35.15	Tricyclo(20.8.0.0(7,16))triacontane, 1(22),7(16)-diepoxy-	1.16	0.99	1.84	trace
37	36.58	Oxalic acid, dodecyl-2-phenylethyl ester	0.94	1.85	2.10	0.37
Total (%)			**98.11**	**97.07**	**99.09**	**98.14**

**RT - retention time.*

Antimicrobial activity assessment

The *in vitro* antimicrobial evaluation of the Narcissus essential oil and Rose absolute samples was performed against four bacterial strains and two fungal species.

The antibacterial assessments of a (5%) ethanolic solution of essential oil (NEO) from *N. poeticus* of Moldovan origin showed higher activity against both non-pathogenic Gram-positive and Gram-negative bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*), as well as phytopathogenic Gram-negative bacteria (*Erwinia carotovora* and *Xanthomonas campestris*) at a concentration of 150 µg/mL. The antifungal properties of *N. poeticus* oil against *Candida albicans* and *Saccharomyces cerevisiae* strains are relatively weak and manifested at a concentration of 300 µg/mL (Table 3). So, in this case we can talk about a certain selectivity of narcissus essential oil toward bacteria.

Table 3

The antimicrobial activity of *N. poeticus* L. essential oil.

Sample	*Bacillus subtilis*	*Pseudomonas Fluorescens*	*Erwinia Carotovora*	*Xanthomonas campestris*	*Candida albicans*	*Saccharomyces cerevisiae*
	MBC and MFC, µg/mL					
NEO	150	150	150	150	300	300

Table 4

The antimicrobial activity of *R. damascena* Mill. absolute.

Sample	*Bacillus subtilis*	*Pseudomonas Fluorescens*	*Erwinia carotovora*	*Xanthomonas campestris*	*Candida albicans*	*Saccharomyces cerevisiae*
	MBC and MFC, µg/mL					
Lani	300	600	300	600	300	300
Raduga	600	600	300	600	600	300
Ukraina	600	300	300	300	300	600
Krimskaya	300	600	600	600	300	300

The *in vitro* antimicrobial activity of rose absolute samples was performed against the same bacterial strains and fungal species. Compared to *Narcissus* oil, the antibacterial activity of *Rose* absolutes was more moderate.

The most active in this respect were the absolutes obtained from the Ukraina cultivar (300 µg/mL) against three strains, followed by the Lani sample (300 µg/mL) against two strains. The Raduga and Krimskaya samples showed the same antibacterial activity only against one strain of bacteria.

From the perspective of antifungal activity, the Lani and Krimskaya absolutes were the most active (300 µg/mL), while Raduga and Lani absolutes demonstrated activity against only one fungal species at the same concentration (Table 4).

All the samples exhibited a stronger and more specific antimicrobial activity than ethanol and did not interfere with ethanol's antimicrobial effect.

Conclusions

The content of twenty-eight major components of *N. poeticus* L. essential oil and thirty-seven of four *R. damascena* absolute samples of Moldovan origin was determined by GC-MS analysis.

In addition to the previously described constituents of Narcissus oil, lilac alcohols B and D, lilac aldehydes A and C, β-caryophyllene, β-myrcene and several other compounds are reported for the first time. Based on the high content of phenylethyl alcohol, it can be concluded that the absolute samples obtained from the Lani, Raduga, Ukraina and Krimskaya rose cultivars are of high quality and meet the European Union requirements.

The *in vitro* assessment of the Narcissus essential oil on six strains of microorganisms confirmed its high antibacterial and antifungal activity at concentrations ranging from 150 to 300 µg/mL. The antimicrobial evaluation of Rose absolute samples, performed against the same strains and species of bacteria, showed average antibacterial and antifungal activity, with effective concentration between 300 and 600 µg/mL. The observed activity varied depending on both the microorganism tested and the specific sample evaluated.

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