Study of the chemical composition of the freon extract of the Damask rose (*Rosa damascena* Mill.)

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Rosa damascena Mill. a rather popular variety of roses has anti-inflammatory, wound-healing, and antiseptic effects.

The aim of this study was to establish the component composition of the freon extract of Damask rose grown *in vitro* and to substantiate the advantages of this method and the prospects for the use of phytosubstances in medical practice.

Materials and methods. The object of the study was the freon extract of Damask rose, grown by the method of clonal micropropagation *in vitro*. The qualitative and quantitative determination of the components of the freon extract was determined using the chromato-mass spectrometric method.

Results. According to the results of the chromato-mass-spectrometric study, 60 compounds were identified, 6 of which were in the isomeric state.

The following components prevailed by percentage: phenylethyl alcohol – 64.070 %, citronellol – 6.090 %, nonadecane – 4.636 %, heneicosane – 2.590 %, geraniol – 1.749 %.

Conclusions. The chemical composition of the freon extract of *Rosa damascena* Mill., grown *in vitro*, was investigated for the first time using the chromato-mass-spectrometric method.

The main components of rose petals: phenylethyl alcohol – 64.070 %, citronellol – 6.090 %, nonadecane – 4.636 %, heneicosane – 2.590 %, geraniol – 1.749 %.

Key words: *Rosa damascena* Mill., chromatography-mass spectrometry, chemical composition.

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Дослідження хімічного складу фреонового екстракту троянди дамаської (*Rosa damascena* Mill.)

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Мета роботи – встановити компонентний склад фреонового екстракту троянди дамаської, вирощеної в умовах *in vitro*, та обґрунтувати переваги цього методу, перспективи використання фітосубстанцій у медичній практиці.

Матеріали та методи. Об'єкт дослідження – фреоновий екстракт троянди дамаської, що вирощена методом клонального мікророзмноження в культурі *in vitro*. Якісне та кількісне визначення компонентів фреонового екстракту здійснили за допомогою хромато-мас-спектрометричного методу.

Результати. У результаті хромато-мас-спектрометричного дослідження ідентифіковано 60 сполук, із них 6 в ізомерному стані.

За відсотковим вмістом переважали такі компоненти: phenylethyl alcohol – 64,070 %, citronellol – 6,090 %, nonadecane – 4,636 %, heneicosane – 2,590 %, geraniol – 1,749 %.

Висновки. Уперше за допомогою хромато-мас-спектрометричного методу дослідили хімічну композицію фреонового екстракту *Rosa damascena* Mill., що вирощена в умовах *in vitro*. Основні компоненти пелюстків троянди: phenylethyl alcohol – 64,070 %, citronellol – 6,090 %, nonadecane – 4,636 %, heneicosane – 2,590 %, geraniol – 1,749 %.

Ключові слова: *Rosa damascena* Mill., хромато-мас-спектрометрія, хімічний склад.

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Key words: *Rosa damascena* Mill., chromatography-mass spectrometry, chemical composition.

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Damask rose (*Rosa damascena* Mill.) is a hybrid of *R. gallica* and *R. phoenicia*, a member of the *Rosaceae* family, which has more than 200 species and 18,000 varieties worldwide. Most of them were formed as a result of selection, multiple repeated crossings, and selection [1].

Rosa damascena Mill. is a perennial branchy shrub up to 1.5 m. Homeland is considered to be the Middle East, in particular the city of Damascus in Syria [2]. Despite the large selection of synthetic drugs on the pharmaceutical market, the Damask rose has not lost its popularity as a source of biologically active substances [3,4]. Thus, Iranian scientists proved the positive effect of Damask rose extract in the treatment of liver diseases [5]. Further studies of the antioxidant effect confirmed the prospects of using this extract in Alzheimer's disease [6]. Available data on the analgesic properties of rose oil in patients with migraine [7]. It is worth noting the presence of antimicrobial and anti-inflammatory effects. For example, in an article by Turkish scientists [8], the effect of an alcoholic extract against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) was investigated.

In turn, Japanese researchers were able to argue the feasibility of using rose water in the treatment of inflammatory skin infections [9].

It is the combination of antimicrobial and anti-inflammatory action that determines the fact that rose essential oil is included in many skin care products.

Rosa damascena Mill. was grown by the method of clonal micropropagation in vitro. The advantage of this method is obtaining healthy planting material identical to the original one with the preservation of all properties of the variety (genetically homogeneous, free from viruses), rapid plant reproduction, and acceleration of the transition of plants from the juvenile to the reproductive phase.

Aim

The aim of this study was to establish the component composition of the freon extract of Damask rose grown *in vitro* and to substantiate the advantages of this method and the prospects for the use of phytosubstances in medical practice.

Materials and methods

The object of the study was the freon extract of Damask rose, grown by the method of clonal micropropagation *in vitro*.

Parts of shoots with rose buds were used for introduction into *in vitro* culture. The whole process was carried out according to the methods generally accepted in biotechnology [10]. Explants were cultured *in vitro* from March to May. They were cultivated on a modified nutrient medium of Murashige and Skoog with growth regulators at an air temperature of 22–24 °C and relative humidity of 65–70 %. The nutrient medium was sterilized in an autoclave under a pressure of 0.11 MPa for 25 minutes. The duration of the passage was 28–30 days.

For introduction into *in vitro* culture, nutrient medium MS with the addition of 2.0 mg/l BAP, 0.2 mg/l IAA and 25.0 mg/l ascorbic acid was used. Explants 0.8–1.2 cm in size with one node were planted.

Removal of apical dominance and induction of the development of axillary buds were used as the main method of propagation at the subcultivation stage. The best morphometric indicators were recorded on the MS medium with the addition of 2.0 mg/l BAP, 0.2 mg/l IAA and 0.5 mg/l adenine. Under such cultivation conditions, the reproduction ratio ranged from 1:7 to 1:12 per passage, while the length of the shoots reached from 11 to 28 mm.

Freon extract was obtained by the Soxhlet method using a low-boiling solvent Freon 12 (difluorodichloromethane).

The analysis of freon extract was carried out by the chromato-mass-spectrometric method. For this, standard methods of determining chemical compounds were applied [11,12]. The resulting dilution was analyzed on a high-performance gas chromatograph Agilent 7890B GC System (Agilent, SantaClara, CA, USA) with a mass spectrometric detector Agilent 5977 BGC/MSD (Agilent, SantaClara, CA, USA). DB-5 ms chromatographic column 30 m long × 250 μm × 0.25 μm. The speed of the carrier gas (helium) was 1.3 ml/min. The injection volume was 0.5 μl. Flow division – 1:5. The temperature of the sample introduction unit was 200 °C → 12 °C/s → 265 °C. Thermostat temperature: programmable, 70 °C (1 min delay) → 10 °C/min → 270 °C (4 min delay). The temperature of the GC/MS interface was 275 °C; ion sources – 230 °C; of a quadrupole mass analyzer – 150 °C. Ionization type: EI at electron energy 70 eV 30–700 m/z. The NIST14 mass spectrum library was used to identify the components.

Biosafety ethics were observed by all scientists during the entire study.

Results

According to the results of the chromato-mass-spectrometric study, 60 compounds were identified, 6 of which were in the isomeric state. The following components prevailed by percentage: phenylethyl alcohol – 64.070 %, citronellol – 6.090 %, nonadecane – 4.636 %, heneicosane – 2.590 %, geraniol – 1.749 %.

Discussion

A review of information in the professional literature showed that phenylethyl alcohol exhibits broncholytic, antiseptic, antimicrobial activity, and is used for the treatment of chronic bronchitis, or other bronchopulmonary diseases [13]. It stimulates the central nervous system, stimulates blood circulation, metabolic processes, and breathing.

Citronellol is widely used in perfumery, cosmetics and has an antifungal effect against *Candida albicans* [14].

Geraniol exhibits bacteriostatic, antiseptic, and fungistatic effects. It is part of many ointments that are prescribed for rheumatism, neuralgia, and colds, inhibits inflammation; affects bronchial secretion, increases or decreases the amount of sputum [15,16]. The works of scientists contain data on a wide range of antimicrobial and antifungal effects of nonadecane and heneicosene [17].

Taking into account all the above, *Rosa damascena* Mill., grown *in vitro*, can be recommended for further research as a promising plant with a wide range of therapeutic effects.

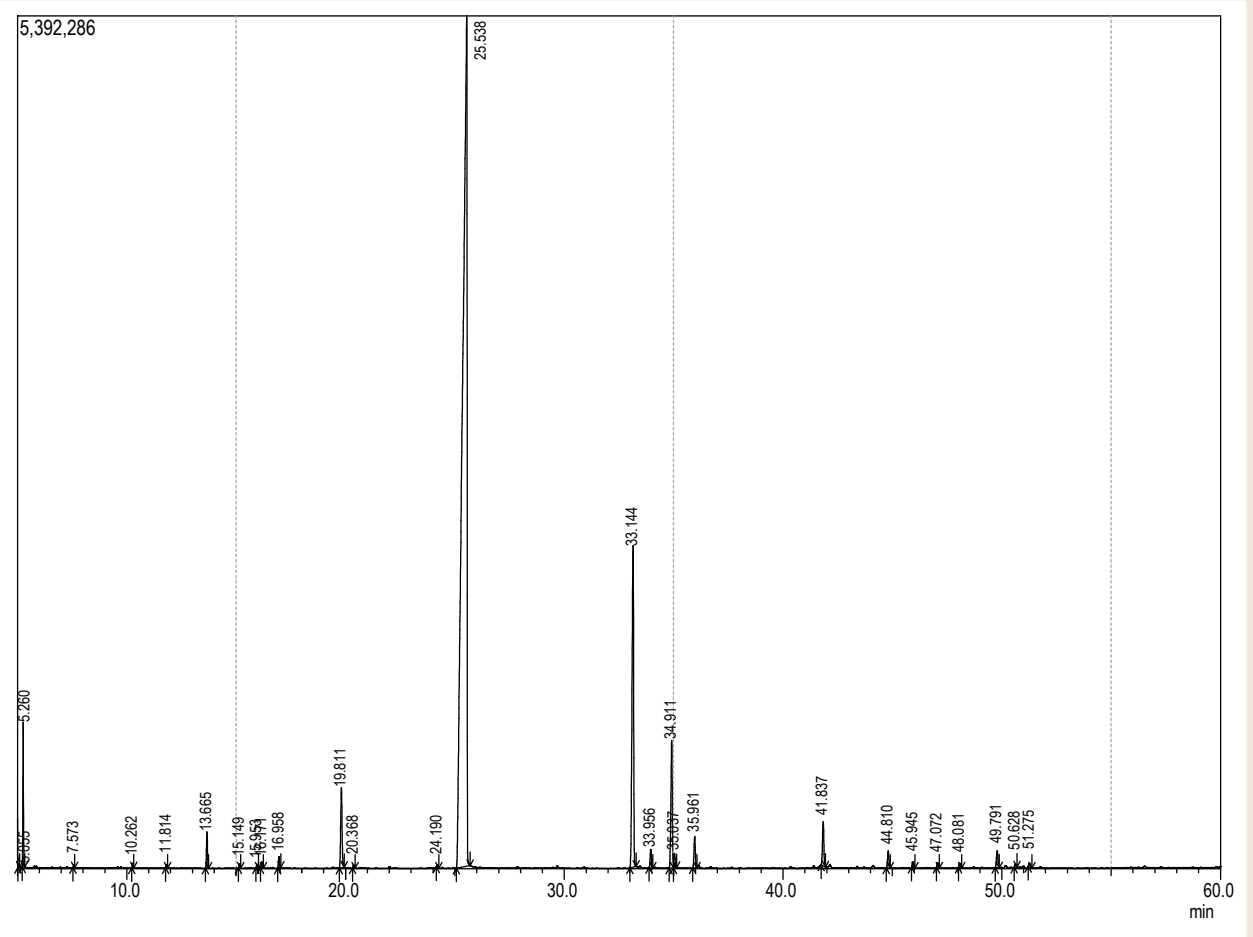

Fig. 1. GC–MS chromatogram of the freon extract of *R. damascena* Mill.

Table 1. Chemical composition of the freon extract of *R. damascena* Mill.

No.	RT	Compounds	Percentage, %
1	5.055	Pentanal	0.034
2	5.260	2-Butanone, 3-hydroxy-	1.321
3	7.573	Hexanal	0.028
4	10.262	Formic acid, hexyl ester	0.059
5	11.814	Heptanal	0.040
6	13.665	2-Pinene	0.545
7	15.149	Benzaldehyde	0.056
8	15.953	Sabinene	0.057
9	16.171	Pinene	0.098
10	16.958	Myrcene	0.183
11	19.811	Benzyl Alcohol	1.664
12	20.368	Benzeneacetaldehyde	0.053
13	24.190	Linalool	0.065
14	25.538	Phenylethyl alcohol	64.070
15	33.144	Citronellol	6.090
16	33.956	Neral	0.565
17	34.911	Geraniol	1.749

Cont. of table 1.

No.	RT	Compounds	Percentage, %
18	35.037	Acetic acid, 2-phenylethyl ester	0.286
19	35.961	Geranial	0.625
20	41.837	Eugenol	0.491
21	44.810	Methyleugenol	0.352
22	45.945	Caryophyllene	0.123
23	47.072	Guaiene	0.092
24	48.081	Humulene	0.095
25	49.791	Germacrene D	0.367
26	50.628	*Pentadecane	0.056
27	51.275	Bulnesene	0.089
28	62.149	*Pentadecane	0.696
29	63.487	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	0.502
30	67.484	Heptadecane	0.283
31	71.303	Z-5-Nonadecene	1.309
32	72.609	*Nonadecane	4.636
33	73.580	Geranyllinalool	0.049
34	77.419	*Nonadecane	0.628
35	80.836	*Docosene	0.038
36	81.587	Trifluoroaceticacid,n-tridecylester	0.140
37	82.076	Heneicosane 85	2.590
38	89.183	Ethyl(9Z,12Z)-9,12-octadecadienoate	0.067
39	86.491	Octadecane	0.070
40	87.617	Hexadecanal	0.095
41	89.017	*17-Pentatriacontene	0.203
42	89.275	2-Phenylethyllaurate	0.219
43	89.017	*17-Pentatriacontene	0.591
44	90.295	13-Tetradecen-1-olacetate	0.183
45	90.384	1-Heneicosanol	0.500
46	90.753	Tricosane	1.290
47	91.310	3,7-Dimethyloct-6-enylisobutylcarbonate	0.096
48	92.765	Neryl (S)-2-methylbutanoate	0.051
49	96.045	*Phytolacetate	0.396
50	97.751	Cyclohexanecarboxylicacid, 2-phenylethylester	0.156
51	98.484	*Docosene	0.687
52	98.758	Tetratetracontane	0.429
53	101.598	Nonanoicacid, pentadecylester	0.033
54	103.829	*Phytolacetate	0.287
55	105.314	*Eicosane	0.482
56	105.590	Oxalicacid, 2-phenylethyltridecylester	0.997
57	106.027 1	n-Tetracosanol-1	0.449
58	106.235	*Eicosane	0.444
59	106.857	3,7-Dimethyloct-6-enylisobutylcarbonate	0.596
60	108.335	Nerylbutanoate	0.345

*: These compounds are in the form of isomers.

Conclusions

1. The chemical composition of the freon extract of *Rosa damascena* Mill., grown *in vitro*, was investigated for the first time using the chromato-mass-spectrometric method.

2. Based on the results of research, *Rosa damascena* Mill. is a valuable source of compounds such as phenylethyl alcohol, citronellol, nonadecane, heneicosane, geraniol, etc.

3. The advantages of the method of clonal micropropagation of *R. damascena* Mill. grown *in vitro* and the prospects of using phytosubstances to create new potential bacteriostatic, antiseptic, antimicrobial, anti-inflammatory medicinal, and cosmetic products were substantiated.

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