

GC-MS analysis of lipophilic components of rhizomes of plant *Rhodiola rosea* L.

Cite as: AIP Conference Proceedings **2419**, 020011 (2021); <https://doi.org/10.1063/5.0069867>
Published Online: 29 October 2021

T. P. Kukina, D. N. Shcherbakov, V. O. Korsakov, et al.



View Online



Export Citation

ARTICLES YOU MAY BE INTERESTED IN

[Application of atomization of liquid smoking preparations in the course of production of whole-muscle meat products](#)

AIP Conference Proceedings **2419**, 020001 (2021); <https://doi.org/10.1063/5.0070937>

[Theoretical and practical aspects use of viburnum opulus berries in confectionery production](#)

AIP Conference Proceedings **2419**, 020004 (2021); <https://doi.org/10.1063/5.0070382>

[Prevention of stress and ensuring the broiler meat quality with lithium-based feed additives](#)

AIP Conference Proceedings **2419**, 020002 (2021); <https://doi.org/10.1063/5.0070940>



Webinar
Quantum Material Characterization
for Streamlined Qubit Development



Register now

GC-MS Analysis of Lipophilic Components of Rhizomes of Plant *Rhodiola Rosea* L.

T P Kukina^{1, a)}, D N Shcherbakov^{2, 3, b)}, V O Korsakov^{2, c)}, I A Elshin^{1, d)} and
Ts Sandag^{4, e)}

¹*Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Russian Academy of Science n.a. N.N. Vorozhtsov, 630090, ave. Academician Lavrentyev, 9, Novosibirsk, Russia*

²*Altai State University, 656049, ave. Lenina, 61, Barnaul, Russia*

³*FBUN SSC VB "Vector", 630559 settlement Koltsovo, Novosibirsk region, Russia*

⁴*School of Biomedicine, Health Sciences University of Mongolia, S. Zorig st. 3, Sukhbaatar district, Ulaanbaatar 14210, Mongolia*

^{a)} *Corresponding author: kukina@nioch.nsc.ru*

^{b)} *dnshcherbakov@gmail.com*

^{c)} *korsakovvlad07@gmail.com*

^{d)} *elshin2014@ngs.ru*

^{e)} *tsogtsaikhan.s@mnums.edu.mn*

Abstract. *The composition of Rhodiola rosea L. lipophylic substances was studied. Acidic and neutral components were identified by gas-chromatography-mass-spectrometry. With methyl-tert-butyl ether as an extractant instead of the volatile solvent diethyl ether, lipophylic extract was obtained. Methyl-tert-butyl ether used as an extraction solvent for raw materials has all the advantages of diethyl ether, being free of its disadvantages. It does not form peroxides or produce elevated partial gas pressure due to its higher boiling point. As a result, comparison with databases identified some triterpene and aliphatic acids with chain lengths 12 to 30 carbon atoms, including saturated, unsaturated, and dibasic acids. In addition to the components known from the literature, more than 50 triterpene and aliphatic compounds were detected in the unsaponifiable residue for the first time.*

INTRODUCTION

Rhodiola rosea L., a golden root is included in folk and officinal medicine due to its powerful adaptogenic effect [1]. It is applied also as substitute of tea and additive in tonics. Generally, plant roots are used as medicinal raw materials. In addition to adaptogenic activity, it exhibits tonic, anti-inflammatory, wound healing, diuretic, antipyretic properties. It is used for vegetative-vascular dystonia, asthenia, neurasthenia, overwork, fevers of various etiologies, diseases of the reproductive system in both men and women, diarrhea, dysentery, tuberculosis, periodontal disease, respiratory infections, diabetes, scurvy, anemia. Antitumor activity was also revealed [2]. To achieve a therapeutic effect, mainly water, water-alcohol and alcohol extracts are used [2]. Therefore, the study of bioactive components was mainly focused on substances that are readily soluble in polar solvents. As a result, flavonoids, alkaloids, glycosides, γ -lactones, carbohydrates, cyanogenic compounds, anthraquinones, phenols, phenolic glycosides, phenolic acids were identified. Low-polarity compounds are limited by some sterols and triterpenes [3-4], as well as essential oil components; in some cases, its yield reaches 0.9% of the raw material load, which was used as the plant rhizome [5-7]. Aliphatic components are identified mainly in essential oil; therefore, the true content of hydrocarbons and alcohols with a chain length above 10, and acids above 16 carbon atoms is greatly underestimated due to their low volatility [2, 6, 8]. Therefore, our task was to study lipophilic components: sterols, triterpenoids, and aliphatic metabolites.

MATERIALS AND METHODS

The raw material of the rhizome of the plant *Rhodiola rosea* L. was harvested in the phase of dying off of flower shoots in August 2020 in the Altai Territory and dried at room temperature in a room without access to direct sunlight. Air-dry raw materials are ground on a screw grinder and sieved through a sieve with holes of 2 mm. A portion of the raw material was loaded into a Soxhlet nozzle and extracted with methyl tert-butyl ether (MTBE) for 20 hours (3×6-7 hours). The extract yield is 1.24% by weight of the raw material. The processing of the extract and the chromatographic purification of the components of the unsaponifiable residue (UR) was performed in the same way [9]. Sample preparation for GC-MS analysis included the release of free acids with an alkaline extractant (2% aqueous sodium hydroxide solution) and hydrolysis of the extract freed from free acids with a 15% aqueous alcoholic solution of potassium hydroxide. Three fractions were obtained: free acids, bound acids, and unsaponifiable residue (HO). The acidic components were methylated with diazomethane, neutral UR substances were subjected to chromatographic separation on a silica gel column using hexane as an eluent with diethyl ether increasing from 0 to 50%. Fractions were collected in 7-8 ml penicillin vials. The fractions were pooled pursuant to the results of thin layer chromatography on Sorbfil and Silufol plates. For the development of the chromatogram, a mixture of hexane with MTBE with a content of the latter from 10 to 50% was used. A mixture of vanillin with sulfuric acid and ethanol in a ratio of 1:10:90 was used as a developing reagent, followed by heating. In this case, fractions were obtained, the analysis of which was performed using GC-MS at the Chemical Research Center for Collective Use of the Siberian Branch of the Russian Academy of Sciences. Low-polarity compounds (hydrocarbons, ketones) were analyzed without derivatization. For the most representative fractions enriched in triterpene components, acetylation with acetic anhydride in pyridine was performed. Chromatomass spectra were recorded on a Hewlett Packard G 1800 A instrument consisting of an HP 5890 Series II gas chromatograph and an HP 5971 mass selective detector. Column 30 m × 0.25 mm × 0.25 µm with HP-5MS sorbent (5% diphenyl, 95% dimethylsiloxane). The carrier gas is helium (1 ml/min). Column temperature: 2 min. at 50°C, then the temperature rises at a rate of 10° per minute up to 280°C, 30 min. at 280°C. Temperature of evaporator 280°C, source of ions 170°C. FAME identification was performed using the NIST 08 mass spectra library similarly to [10]. 6 main fractions were obtained: hydrocarbons, ketones, 3 fractions of mixtures of aliphatic and triterpene alcohols, sterols. Based on the results of GC-MS analysis, the content of each component in the analyzed mixtures was calculated in mg/100 g of raw material (mg/%).

TABLE 1. Content of groups of substances in the HO of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Group of substances	Contents	Group of substances	Contents
Aliphatic hydrocarbons	40.1	Triterpene ketones	21.1
Aliphatic ketones	2.4	Triterpene alcohols, including sterols	215.8
Aliphatic alcohol	112.7	Free acids	218.7
Triterpene hydrocarbons	2.8	Bound acids	325.7

RESULTS AND DISCUSSION

Analysis of acidic and neutral components of extractive substances from the raw material of the rhizome of the plant *Rodiola rosea* L., obtained by saponification of the extracts, showed the following results. The content of extractive lipophilic substances is 1.24%, unsaponifiables are 40% of the weight of the extract. For saponification, additional fractionation and purification of groups of compounds were performed. As a result, concentrates of hydrocarbons, epoxides, ketones, aliphatic and terpene alcohols, including sterols and diols, were obtained. The contents of the identified components are summarized in tables.

TABLE 2. The content of aliphatic hydrocarbons in the non-saponified MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Octadecane	0.4	Heptacosane	4.9
Nonadecane	0.3	Octacosane	0.3
Eicosane	0.5	Nonacosane	5.3

TABLE 2. Continued

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Geneicosane	0.8	Triacontane	0.1
Docosane	0.9	Hentriacontane	2.2
Tricosane	8.0	Dotriacontane	0.1
Tetracosane	1.3	Tritriacontane	5.4
Pentacosane	6.5	Pentatriacontane	0.9
Hexacosane	0.9	Squalene	0.6

The table shows that the fraction is dominated by odd components, the main of which, tricosan, pentacosan, heptacosan, nonacosan, and tritriacontane make up 75% of the total weight of the fraction.

TABLE 3. The content of aliphatic ketones in the UR of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
2-Tricosanone	0.4	2-Heptacosanone	0.6
2-tetracosanone	0.3	2-Octacosanone	0.1
2-Pentacosanone	0.3	2-Nonacosanone	0.2
2-Hexacosanone	0.2	10-Nonacosanone	0.3

Comparison of the mass spectra of aliphatic ketones with the database indicates the predominance of compounds with a keto group in the 2-position in the fraction.

TABLE 4. The content of aliphatic alcohols in the UR of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (mg%).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Hexadecanol	0.8	Tetracosanol	42.7
Octadecanol	0.9	Pentacosanol	0.7
Nonadecanol	1.0	Hexacosanol	39.7
Eicosanol	1.7	Heptacosanol	0.3
Heneicosanol	1.0	Octacosanol	3.3
Docosanol	17.8	Triacontanol	0.1
Tricosanol	0.9	Phytol	0.1

The main components of the group - docosanol, tetracosanol and hexacosanol make up 90% of the total weight of alkanols.

TABLE 5. The content of triterpene hydrocarbons in the UR of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Stigmasta-3,5-diene	2.0	28-Nor-17 α -hopan	0.3
28-Nor-17 β -hopan	0.3	17 α -21 β -Hopan	0.2

Triterpene hydrocarbons are few in number and their contribution to the UR composition is small.

TABLE 6. The content of triterpene ketones in the US of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Tarax-14-en-3-one	2.9	α -Amirenone	5.1
Isomultiflorenone	7.0	Lupenone	2.5
β -Amirenone	1.1	Stigmasta-3,5-diene-7-one	2.5

TABLE 7. The content of triterpene alcohols and sterols in the UR of MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Content in raw materials (% mg)	Constituent	Content in raw materials (% mg)
Cholesterol	0.8	Isomultiflorenol	1.4
Campesterol	23.8	Isosvertenol	1.2
β -Sitosterol	168.7	Stigmast-7-en-3-ol	2.0
β -Stigmastanol	3.1	Ergostanol	0.9
β -Amirin	0.7	Ergost-7-en-3-ol	0.4
α -Amirin	1.8	Stigmast-7,16,25-trien-3-ol	0.6
Brassicasterol	2.6	24-Methylene-cycloartanol	0.1
Stigmasterol	1.5	Methylursolate	0.2

The main component of the group, β -sitosterol, was discovered earlier [3], data on the detection of β -amyrin [3] are also known, the rest of the components were detected in the raw material for the first time. In addition to the alcohols listed in the table, small amounts of geraniol, geranylgeraniol, myrtenol and cinnamic alcohol were found; these compounds were found in the raw material earlier [6]. Manool, manoiloxide and epimanoyloxide were found, which were also identified earlier in the composition of essential oil [5-7].

TABLE 8. Content of free and bound acids in MTBE extract of the rhizome of the plant *Rhodiola rosea* L. in terms of the weight of the raw material (% mg).

Constituent	Free acids	Bound acids
Anisic	3.9	
Suberic (octanedioic)	4.2	
Azelaic (nonanedioic)	12.1	
Veratric	0.9	
Lauric	0.6	1.5
Myristic	1.0	4.9
Pentadecenic	0.1	2.3
Pentadecanoic	0.1	0.7
Palmitic	52.6	65.0
Palmitoleic	0.6	0.5
Caffeic	2.5 (2 isomers 2:3)	
Margarine	0.6	1.3
Oleic	9.9	17.8
Linolenic	9.4	41.2
Linoleic	38.8	102.0
Stearic	6.1	6.7
Conjugated linolenic (9,11,13-octadecatriene, 3 isomers)	0.1	3.4
Isolinoleic	0.1	0.3
Nonadecanoic		2.8
Eicose-11,14-dienoic		1.5
Arachinic	4.9	8.6
Heneicosanoic	0.9	1.2
Behenic	8.8	12.2
Tricosanoic	3.4	5.7
Lignoceric	25.4	24.8
Pentacosanoic	1.8	1.4
Phellogenic (docosandioic)		0.3
Cerotinic	21.4	17.4
Montanic	2.5	1.7
Melissic	1.0	0.5
Betulonic	2.2	

Capric, lauric, myristic, pentadecanoic, palmitic acids appear in the literature [3, 7]. They were identified in the composition of the essential oil; therefore, components with a chain length of more than 16 carbon atoms were not included in the studied fraction due to their low volatility. Caffeic acid has also been identified earlier [3, 4], other acidic components have been found in this raw material for the first time.

CONCLUSIONS

1. The composition of ether-extractive substances of the rhizome of *Rhodiola Rosea* L. was studied by GC-MS method. More than 100 compounds have been identified, most of which have been found in raw materials for the first time.
2. Aliphatic and terpene compounds were found in the composition of neutral components: hydrocarbons, ketones, alcohols, including sterols, including those rarely found in medicinal plants.
3. An increase in the temperature of the analysis of acidic components made it possible to reveal aliphatic components with a chain length of more than 16 carbon atoms, as well as triterpene betulonic acid. Bound and free components differ in both qualitative and quantitative composition.

This work was performed within the framework of the RFBR grant No. 20-54-44016/20.

The authors are grateful to the Chemical Research Center for Collective Use of the Siberian Branch of the Russian Academy of Sciences for the GC-MS analysis.

REFERENCES

1. Pooja, A. S. Bawa and F. Khanu, *Phytother Res.* **23**(8), pp. 1099–1102 (2009).
2. A. L. Budantsev and E. E. Lesiovskaya, *Wild Useful Plants of Russia[in Russian]* (St-Petersburg, Izdatelstvo SPCPA, 2001).
3. L.A. Budantsev, *Plant Resources of Russia: Wild Flowering Plants, Their Constituent Composition and Biological Activity [in Russian]*2(Tovarishchestvo Nauchnykh Izd. KMK, Moscow, 2009).
4. V.A. Bykov, G.G. Zapesochaynaya and V.A. Kurkin, *Pharm. Chem. J.* **33**, pp. 29–40 (1999).
5. S. Shatar, R.P. Adams and W. Koenig, *Journal of Essential Oil Research***19**(3), pp. 215–217 (2007).
6. L. Evstatieva, M. Todorova, D. Antonova and J. Staneva, *Pharmacogn. Mag.* **6** (24), pp. 256–258 (2010).
7. J. Rohloff, *Phytochemistry* **59**(6), pp. 655–661(2002).
8. F. Alperth, I. Turek, S. Weiss, D. Vogt, and F. Bucar, *Sci. Pharm.***87**(8)(2019).
9. T.P. Kukina, T. S. Frolova and O. I. Salnikova, *Chemistry of Natural Compounds* **50**(2), pp. 233–236 (2014).
10. T.P. Kukina, D.N. Shcherbakov, K.V. Gensh, E.A. Tulyshcheva, O.I. Salnikova, A.E. Grazhdannikov and E.A. Kolosova, *Russian Journal of Bioorganic Chemistry***43**(7), pp. 57–61(2017).