

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/358363638>

GC-MS analysis of lipophilic Chaga mushroom constituents

Conference Paper · January 2022

DOI: 10.1063/5.0070158

CITATIONS

0

READS

31

6 authors, including:



Dmitry Shcherbakov

State Research Center of Virology and Biotechnology VECTOR

94 PUBLICATIONS 186 CITATIONS

[SEE PROFILE](#)



Tatyana Kukina

Novosibirsk Institute of Organic Chemistry

52 PUBLICATIONS 199 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Using phage display technology to search for peptides specifically interacting with immune checkpoints [View project](#)

GC-MS analysis of lipophilic Chaga mushroom constituents

Cite as: AIP Conference Proceedings **2390**, 030083 (2022); <https://doi.org/10.1063/5.0070158>
Published Online: 04 February 2022

D. N. Shcherbakov, T. P. Kukina, I. A. Elshin, et al.



[View Online](#)

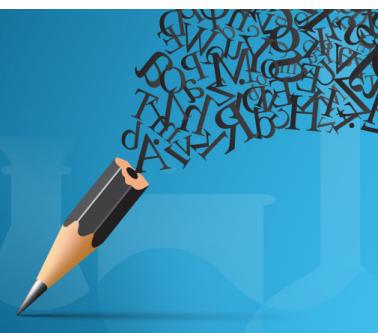


[Export Citation](#)

AIP Publishing Author Services

English Language Editing
High-quality assistance from subject specialists

[LEARN MORE](#)



GC-MS Analysis of Lipophilic Chaga Mushroom Constituents

D. N. Shcherbakov ^{1, 2, a)}, T. P. Kukina ^{3, b)}, I. A. Elshin ^{3, c)}, N. V. Panteleeva ^{2, d)},
T. V. Teplyakova ^{2, e)} and O. I. Salnikova ^{3,f)}

¹Altai State University, Barnaul 656049, Russia

²State Research Center of Virology and Biotechnology Vector, Koltsovo 630559, Novosibirsk oblast, Russia

³Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences,
Novosibirsk 630090, Russia

^{a)}Corresponding author: dnshcherbakov@gmail.com

^{b)}kukina@nioch.nsc.ru

^{c)}kumatoid@nioch.nsc.ru

^{d)}nino4ka_panteleeva@mail.ru

^{e)}Teplyakova@vector.nsc.ru

^{f)}olga@nioch.nsc.ru

Abstract. The composition of Chaga mushroom lipophilic 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 laboratory solvent diethyl ether, two lipophilic extracts with similar compositions were 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 three triterpene and forty aliphatic acids with chain lengths 14 to 30 carbon atoms, including saturated, unsaturated, dibasic, and hydroxy-acids, as well as a number of constituents of the cinnamic series and benzoic acid derivatives. In addition to the components known from the literature, eight triterpene compounds were detected in the unsaponifiable residue for the first time: lanost-8,24-diene-3-one, 9,10-seco-ergost-5,7,10(19), 22-tetraene-3 β -ol, neoergosterol, lanost-7,9(11), 24-triene-3 β -ol, 25-epi-aplisterol, anthraergostatetraenol and 9(11)-dehydroergosterol, stigmastanol. Twenty-four aliphatic components were discovered in this raw material for the first time.

INTRODUCTION

The mushroom *Inonotus obliquus* (Ach. ex Pers.) Pilát, also known as Chaga, is a parasitic fungus belonging to the Hymenochaetaceae Donk family, and infesting birches at cold latitudes of Europe and Asia [1]. This mushroom extract is used as a traditional remedy to cure various diseases such as cancer, cerebrovascular diseases, diabetes, and gastrointestinal diseases [2–3]. Chaga has been used as an effective folk medicine in Russia and Northern Europe to treat several human malicious tumors and other diseases in the absence of any unacceptable toxic side effects since the 16th century. The pharmacological study of active substances of this fungus was initiated in the middle of the 20th century, when folk medicine claimed medicinal benefits of fungi mostly in cancer research.

Polysaccharides of *I. obliquus* are capable of reducing blood levels of glucose, triglycerides, fatty acids, and cholesterol [3]. Mizuno et al. [4] show a certain hypoglycemic action of *I. obliquus* tinctures in combination with an oncostatic effect. Consequent studies on the antidiabetic and antioxidant activities of *I. obliquus* [5–7] demonstrate that the polyphenolic complex was the main substance in scavenging free radicals. Various studies on the biological activities of this mushroom mainly focus on its anticancer activity [8–22].

To date, more than 40 triterpene compounds of the different lanostane series have been isolated from chaga: trametenolic acid (3 β -hydroxy-lanosta-8,24-dien-21-oic acid), obliquinic acid (3 β -hydroxy-lanosta-8-en-21-oic

acid), 3β -hydroxy-25,26,27-trinor-lanosta-8,22-dien-24-oic acid; trametenolic aldehyde (3β -hydroxy-lanosta-8,24-dien-21-al), 3β -hydroxy-25,26,27-trinor-lanosta-8,22E-dien-24-al; $3\beta,22R$ -dihydroxy-lanosta-8,24-dien-11-one, 3,7-dihydroxy-7(8-9)-abeo-lanosta-24-en-8-one, $3\beta,22R$ -dihydroxy-lanosta-8,24-dien-7-one, 21,24-cyclopenta-3,11,15,21,25-pentahydroxy-lanosta-8-en-7-one, 21,24-cyclopenta-3,11,21,25-tetrahydroxy-lanosta-8-en-7-one, $3\beta,22R$ -dihydroxy-lanosta-8,25-dien-24-one; 3β -hydroxy-lanosta-8,24-dien-21,23-lactone, 24-methyl- 3β -hydroxy-lanosta-8,24-dien-21,23-lactone; $3\beta,22R$ -dihydroxy-lanosta-8,23-dien-25-peroxide, $3\beta,22R$ -dihydroxy-lanosta-8,24-dien-25-peroxide; $3\beta,11\beta$ -dihydroxy-lanosta-8,24-dien, $3\beta,22$ -dihydroxy-lanosta-7,9(11),24-trien, $3\beta,22,25$ -trihydroxy-lanosta-8-en, $3\beta,22\alpha,25$ -trihydroxy-lanosta-8,23-dien, $3\beta,22,24$ -trihydroxy-lanosta-8,25-dien, $3\beta,2$ -dihydroxy-lanosta-8,24-dien, $3\beta,22\alpha,25$ -trihydroxy-lanosta-8,24-dien, $3\beta,22R,25$ -trihydroxy-lanosta-8,23-dien, $3\beta,22R,25$ -trihydroxy-lanosta-7,9(11),23-trien; 21,24-cyclopentalanosta-8-en- $3\beta,21,25$ -triol, 25-methoxy-21,22-cyclopentalanosta-8-en- $3\beta,21\alpha$ -diol, 20R,24S-cyclopentalanosta-8-en- $3\beta,21R,25$ -triol, 20R,24R-cyclopentalanosta-7,9(11)-dien- $3\beta,21R,25$ -triol; $3\beta,25$ -dihydroxy-lanosta-8-en-20R,24S-olide, $3\beta,25$ -dihydroxy-lanosta-7,9(11)-dien-20R,24S-olide, 22R,25-epoxy-lanosta-8-en-3,24S-diol, etc. [13].

Pentacyclic triterpenes of the lupane series (betulin, lupeol and lupenone), ergosterol and related compounds, phytosterols (β -sitosterol, stigmasterol, and sitostanol) and cholesterol have been isolated as well [13, 17–18].

Some types of activity are due to the presence of lanostane and other triterpenoids [23–32].

Despite the extensively characterization of Chaga composition, its lipophilic fraction has not been well studied, especially, the aliphatic components. Authors identified 14 saturated and unsaturated compounds with the numbers of carbon atoms from 8 to 20 in the aliphatic components of the Chaga [33]. *Inonotus rheades* also contains heneicosanoic and behenic acids [34]. The aim of this work was to study the lipophilic substances of Chaga extracted by methyl *tert*-butyl ether.

EXPERIMENTAL PART

Natural raw sclerotia of Chaga mushroom *Inonotus obliquus* (Ach. ex Pers.) Pilát was collected from Altai Krai, Russia. The raw materials were grounded using an electric mill and extracted by methyl *tert*-butyl ether.

Extraction was carried out in a Soxhlet extractor for 27 h (9×3). The extract was divided into acidic and neutral components to facilitate the analysis. Free acids were isolated from the total extract by alkaline extraction with 2% aqueous sodium hydroxide. Also, an additional step of fractioning and saponification was required: fractions of total acids and unsaponifiable substances (USs) were obtained by alkaline hydrolysis. Following extraction, concentrate of the extract was obtained using a rotatory evaporator (Büchi, Switzerland). Thin-layer chromatography was performed on Sorbfil and Armsorb plates in the hexane – methyl *tert*-butyl ether (MTBE) system (6:1 and 1:1 for the analysis of the fraction of low polar compounds and the remaining fractions, respectively). The chromatogram was developed by spraying the plates with a mixture of vanillin–sulfuric acid–ethanol in the ratio of 1:10:90 with subsequent heating of the plate. Target compounds were purified by column chromatography on silica gel from Sigma-Aldrich (Merck Grade 7734, 70–230 mesh). Gradient of 1 to 50% diethyl ether in hexane was used as the mobile phase. Mass spectra were recorded on a Hewlett Packard G 1800 A device consisting of an HP 5890 series II gas chromatograph and an HP 5971 mass selective detector, equipped with a column 30 m×0.25 mm×0.25 μm with HP-5 MS sorbent (5% diphenyl, 95% dimethylsiloxane). Helium was used as a carrier gas (1 mL/min). Column temperature mode: 2 min at 50 °C, then increase the temperature to 300 °C at the rate of 4%/min, and 30 min at 300 °C. For analysis, the acidic fractions were converted to their methyl esters. The unsaponifiable residue was submitted to analysis without derivatization and as acetyl-derivates.

RESULTS AND DISCUSSION

In this work methyl *tert*-butyl ether (MTBE) was selected as the extraction solvent over diethyl ether (DE) due to its low-toxicity, high yield of lipophilic components of plant raw materials. Additionally, MTBE does not form peroxides or create increased gas content and produces extract showing similar chemical composition as those obtained using DE. The extract yield was 4%. The extract consisted of acidic and neutral components. The neutral part was thus the sum of hydrocarbons and aliphatic and triterpene alcohols in free form and in the form of esters with aliphatic acids.

TABLE 1. Distribution of components in the fraction of USs from Chaga mushroom (ChM), mg/100g of the sample weight.

Component	Content in ChM(mg/100g)	Component	Content in ChM(mg/100g)
Squalene	0.1	24-Methylene-lanost-8-en-3-ol	0.8
Tricosanal	0.7	β -Sitosterol	0.5
9(11)-Dehydroergosterol	4.0	Lupenone	0.2
9,10-Secoergosta5,7,10(19),22-tetraen-3-ol	3.6	Ergosta-4,6,8(14),22-tetraen-3-one	1.4
Cholesterol	0.8	25-Epiaplysterol	1.8
Ergosta-5,7,9(11),22-tetraen-3-ol	2.1	Antraergostatetraenol	0.2
Ergosterol	13.4	Neoergosterol	1.2
Ergosta-7,22-dien-3-ol	6.5	Ergosta-5,8-dien-3-ol	1.4
7-Ergostenol	6.5	Inotodiol	167.8
Lanosta-8,24-dien-3-one	0.5	Trametenolic aldehyde	65.7
Lanosterol	78.6	Betulin	16.8
D:C-Friedooleana-7,9(11)-dien-3-ol	0.5	(22E)-Ergosta-8(14),15,22-trien-3-ol	0.3
Lanosta-7,9(11),24-trien-3-ol	1.2	Ergosta-5,8,22-trien-3-ol	0.5
Lupeol	4.1	Stigmastan-3-ol	0.1

Table 1 shows the qualitative and quantitative compositions of triterpenoic constituents of Chaga mushroom USs obtained from GC-MS analyses normalized to the masses of fractions from the chromatographic separation. The main constituents are inotodiol, lanosterol and trametenolic aldehyde. The neutral aliphatic components are squalene and tricosanal. In addition to the previously identified components, we identified 8 triterpene compounds for the first time in the unsaponifiable residue: lanosta-8,24-diene-3-one, 9,10-seco-ergosta-5,7,10(19),22-tetraen-3 β -ol, neoergosterol, lanosta-7,9(11),24-trien-3 β -ol, 25-epi-aplysterol, anthraergostatetraenol, 9(11)-dehydroergosterol, and stigmastanol. Analysis of acetylated sample also showed the presence of minor isomer of inotodiol-diacetate with the same mass-spectrum overlapping to database (ratio is 1:20).

TABLE 2. Distribution of components in the fraction of total aliphatic and triterpenoic acids, mg/100g of the ChM sample weight.

Acid component	Content in ChM(mg/100g)	Acid component	Content in ChM(mg/100g)
Suberic	0.3	2-Hydroxy-docosanoic	19.4
Lauric	0.2	15-Tetracosenoic	1.8
Myristic	0.2	2-Hydroxy-tricosanoic	3.2
Pentadecanoic	0.9	Pentacosanoic	5.9
Pentadecenoic	0.2	16-Pentacosenoic	0.1
Palmitic	29.1	2-Hydroxy-tetracosanoic	52.2
Palmitoleic	1.9	17-Hexacosanoic	2.8
Margaric	1.2	Cerotic	3.8
Heptadecenoic	0.2	Heptacosanoic	1.9
Stearic	15.1	Montanic	0.9
Oleic	31.5	Nonacosanoic	0.4
Vaccenic	1.2	Melissic	0.8
Linoleic	54.2	2-Hydroxy-pentacosanoic	7.4
9,11,13-Octadecatrienoic	0.4	2-Hydroxy-hexacosanoic	1.2
2-Hydroxyhexadecanoic	1.3	Trametenolic	148.4
Nonadecanoic	0.1	Hexadecanedioic	3.3
Arachidic	4.6	Betulinic	2.0
Heneicosanoic	3.5	Betulonic	2.8
Behenic	13.2	Octadecanedioic	2.2
Tricosanoic	8.6	Eicosanedioic	2.0
Tricosenoic	1.0	Docosanedioic	1.2
Lignoceric	13.0		

Table 2 presents the qualitative and quantitative compositions of triterpenoic and aliphatic acid constituents of Chaga mushroom. As shown, we identified 3 triterpene and 14 aliphatic acids with chain lengths from 8 to 30 carbon atoms, including saturated, unsaturated, dibasic, and hydroxy-acids, as well as a number of constituents of

the cinnamic series and benzoic acid derivatives, this finding was comparable to the identified components of this mushroom in database. Noteworthy to state that we identified 24 aliphatic components in chaga extract for the first based on our extraction procedure. We found trametenolic acid as the most prominent aliphatic component of chaga, and the 2-hydroxy acids content was also prominent.

CONCLUSION

The chemical composition of Chaga mushroom (*Inonotus obliquus* (Ach. ex Pers.) Pilát) lipophilic constituents was identified by gas chromatography–mass spectrometry. Eight triterpene compounds were detected in the unsaponifiable residue and twenty four aliphatic acid components were discovered in this raw material at first time. The high percentages of triterpenoic compounds, as well as sterols, triterpenols, and triterpenoic acids, and increased concentration of the lipophilic constituents of Chaga mushroom (*I. obliquus*) were observed.

REFERENCES

1. D. L. Hawksworth, P. M. Kirk, B. C. Sutton, and D. N. Pegler, *Ainsworth and Bisby's dictionary of the fungi* (8th ed.) (Cambridge: CAB International, University Press, 1995). p. 616.
2. Y. S. Choi, S. J. Hur, C. S. An, Y. H. Jeon, Y. J. Jeoung, J. P. Bak, and B. O. Lim, *Journal of Biomedicine and Biotechnology*, 1–6 (2010).
3. J. E. Sun, Z. H. Ao, and Z. M. Lu, *Journal of Ethnopharmacology* **118**, 7–13 (2008).
4. T. Mizuno, A. K. Zhuang, H. Okamoto, T. Kiho, Sh. Ukai, S. Leclerc, and L. Meijer, *Int. J. Med. Mushrooms* **1**, 301–316 (1999).
5. W. Zheng, Y. Zhao, M. Zhang Z. Wei, K. Miao, and W. Sun, *Med. Mycol.* **47** (8), 814–823 (2009).
6. Y. K. Park, J.S. Kim, E.J. Jeon and M. H. Kang, *Korean J. Nutr.* **42** (1), 5–13 (2009).
7. H. S. Song, Y. J. Lee, S. K. Kim, K. Y. Moon, W. K. Moon, D. W. Kim, and Y. S. Kim, *Korean J. Pharmacognosy* **35** (1), 92–97 (2004).
8. Q. Van, B. N. Nayak, M. Reimer, P. J. Jones, R.G. Fulcher, and C.B. Rempel, *J. Ethnopharmacol* **125** (3), 487–493 (2009). doi:10.1016/j.jep.2009.06.026
9. H.-G. Kim, D.-H. Yoon, C.-H. Kim, B. Shrestha, W.C. Chang, S.Y. Lim, W. H. Lee, S. G. Han, J. O. Lee, M. H. Lim, G. Y. Kim, S. Choi, W.O. Song, J.M. Sung, K. C. Hwang, and T. W. Kim. *Journal of Medicinal Food* **10** (1), 80–89 (2007).
10. Y.-M. Park, J.-H. Won, Y.-H. Kim, J.-W. Choi, H.-J. Park and K.-T. Lee, *Journal of Ethnopharmacology* **101** (1–3), 120–128 (2005).
11. M. J. Chung, C. K. Chung, Y. Jeong, and S. S. Ham, *Nutrition Research Practice* **4** (3) 177–182 (2010).
12. L. Ma, H. Chen, P. Dong, and X. Lu, *Food Chem.* **139** (1–4), 503–508 (2013).
13. S. A. Nikitina, V. R. Habibrakhmanova, and M. A. Sysoeva, *Biochemistry(Moscow) Supplement Series B: Biomedical Chemistry* **10** (1), 63–69 (2016).
14. M. J. Youn, J. K. Kim, S. Y. Park, Y. Kim, C. Park, E. S. Kim, K. I. Park, H. S. So, and R. Park, *J. Ethnopharmacol.* **121** (2), 221–228 (2009).
15. W. Zheng, K. Miao, Y. Liu, Y. Zhao, M. Zhang, S. Pan, and Y. Dai. *Appl. Microbiol. Biotechnol.* **87**, 1237–1254 (2010).
16. M. J. Chung, *Nutrition research and practice* **4** (3), 177–182 (2010).
17. T. V. Teplyakova and T. A. Kosogova, *Higher mushrooms of Western Siberia-promising objects for drug biotechnology*. (SibNSHB, Novosibirsk, 2014). 297 p. ISBN 978-5-906143-61-7. (In Russian).
18. M. Balandaykin and I. Zdmitrovich, *Int. J. Med. Mushrooms* **17** (2), 95–104 (2015).
19. J. L. Rios and I. Andujar, *Lanostanoids from fungi as potential medicinal agents*, In J. H. Merillon and K.G. Ramawat (eds.), *Fungal metabolites*, (Springer International Publishing Switzerland, 2015). pp. 931–964. Doi 10.1007/078-3-319-19456-1_19-1.
20. Y. O. Kim, H. W. Park, J. H. Kim, J. Y. Lee, S. H. Moon, and C. S. Shin, *Life Sciences* **79** (1), 72–80 (2006).
21. S. P. Wasser, *Applied Microbiology and Biotechnology* **60**, 258–274 (2002).
22. Y. O. Kim, S. B. Han, H. W. Lee, et al., *Life Sciences* **77** (19), 2438–2456 (2005).
23. S. Nakamura, J. Iwami, H. Matsuda, S. Mizuno, and M. Yoshikawa, *Tetrahedron* **65**, 2443–2450 (2009).
24. F. Zhao, G. Xia, L. Chen, J. Zhao, Z. Xie, F. Qiu, and G. Han, *Journal of Natural Medicines* **70**, 721–730 (2016).

25. F. Zhao, Q. Mai, J. Ma, M. Xu, X. Wang, T. Cui, F. Qiu, and G. Han, *Fitoterapia* **101**, 34–40 (2015).
26. N. Handa, T. Yamada, and R. Tanaka, *Phytochemistry Letters* **5**, 480–485(2012).
27. R. Tanaka, M. Toyoshima, and T. Yamada, *Phytochemistry Letters* **4**, 328–332 (2011).
28. K. R. Lee, J. S. Lee, Y. R. Kim, I. G. Song, and E. K. Hong, *Oncology Reports* **31**, 2447–2453(2014).
29. Y. Shin, Y. Tamai, and M. Terazawa, *Journal of Wood Science* **47**, 313–316(2001).
30. K. Kahlos, R. Hiltunen, and M. V. Schantz, *Planta Medica* **50**, 197–198 (1984).
31. K. Kahlos and R. Hiltunen, *Acta Pharmaceutica Fennica* **95**, 71–76 (1986).
32. Y. Shin, Y. Tamai, and M. Terazawa, *Eurasian Journal of Forest Research* **2**, 27–30 (2001).
33. Sh. Yusoo, T. Yutaka, and T. Mioru, *Int. J. of Medicinal Mushrooms* **3**, 250 (2001).
34. T. Nakata, S. Taji, T. Yamada and R. Tanaka, *Bulletin of Osaka University of Pharmaceutical Sciences* **3**, 53–56 (2009).
35. J. L.Ríos, I. Andújar, M. C. Recio, and R. M. Giner, *J. Nat. Prod.* **75**, 2016–2044 (2012).
36. M. A. Sysoeva, V. R. Khabibrahmanova, B. C. Gamayurova, and E. F. Zainetdinova, *Khimiya rastitel'nogo syr'ya (Chemistry of plant raw materials)* **1**, 111–114 (2008). (In Russian)
37. T. G. Gornostay, M. S. Polyakova, G. B. Borovsky, and D. N. Olennikov, *Khimiya rastitel'nogo syr'ya (Chemistry of plant raw materials)* **1**, 105–111 (2018). (In Russian)