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Chemical Composition and Biological Activity of Triterpenes and Steroids of Chaga Mushroom

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Abstract—Data on the chemical composition of triterpenic and steroid compounds, isolated from the chaga mushroom grown in natural environment or in a synthetic culture have been summarized. Special attention has been paid to the biological activity of chaga mushroom extracts and these particular compounds against various cancer cell lines in vitro and in vivo. This analysis has demonstrated some common features in inhibition of growth of various cell lines by chaga mushroom components. In this context, the most active are triterpene compounds containing OH group at C-22 and a side chain unsaturated bond.

Keywords: chaga mushroom, triterpenes, steroids, biological activity, in vivo, in vitro

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INTRODUCTION

Many publications deal with results of studies on triterpene and steroid compounds of chaga mushroom; they describe isolation of the new compounds directly from this mushroom, elucidation of their structure and determination of their pharmacological activity [1–38]. The authors of some studies propose mechanisms of action of these compounds. However, results on this problem obtained in various laboratories have not been summarized so far.

First triterpene compounds were detected in chaga mushroom by Polish scientists, Ludwiczak and Wrecino [2]. They identified lanosterol—3β-hydroxylanosta-8,24-diene (**A**) and its derivative 3β,22-dihydroxy-lanosta-8,24-diene or inotodiol (**A1**) (figure).

Currently, about 40 triterpene compounds of the lanostane series have been isolated. These include:

—acids—trametenolic (3β-hydroxylanosta-8,24-dien-21-oic, **A2**) and obliquinolic (3β-hydroxylanosta-8-en-21-oic, **A3**), 3β-hydroxy-25,26,27-trinorlanosta-8,22*E*-dien-24-oic acid (**A4**);

—aldehydes—3β-hydroxylanosta-8,24-dien-21-al (**A5**), 3β-hydroxy-25,26,27-trinorlanosta-8,22*E*-diene-24-al (**A6**);

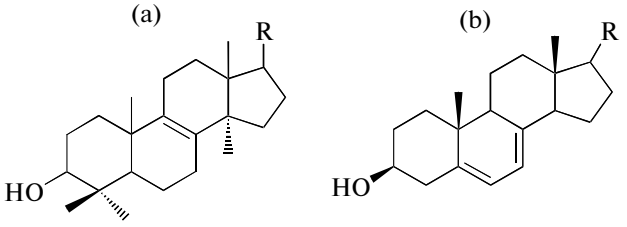
—ketones—3β,22*R*-dihydroxylanosta-8,24-dien-11-one (**A7**), 3,7-dihydroxy-7(8 → 9)-abeo-lanost-24-ene-8-one (**A8**); 3β,22-dihydroxylanosta-8,24-dien-7-one (**A9**), 21,24-cyclopenta-3,11,15,21,25-pentahydroxylanosta-8-ene-7-one (**A10**, **A11**), 21,24-cyclopenta-3,11,21,25-tetra-hydroxylanosta-8-ene-7-one (**A12**), 3β,22-dihydroxylanosta-8,25-dien-24-one (**A13**);

—lactones—3β-hydroxylanosta-8,24-diene-21,23-lactone (**A14**), 24-methyl-3β-hydroxylanosta-8,24-diene-21,23-lactone (**A15**);

—peroxides—3β,22α-dihydroxylanosta-8,23*E*-dien-25-peroxide (**A16**), 3β,22α-dihydroxylanosta-8,24-dien-25-peroxide (**A17**);

—compounds with several double bonds—3β,11β-dihydroxylanosta-8,24-diene (**A18**), 3β,22-dihydroxylanosta-7,9(11),24-triene (**A19**), 3β,22,25-trihydroxylanosta-8-ene (**A20**), 3β,22α,25-trihydroxylanosta-8,23-diene (**A21**), 3β,22,24-trihydroxylanosta-8,25-diene (**A22**), 3β,21-dihydroxylanosta-8,24-diene (**A23**), 3β,22α,25-trihydroxylanosta-8,24-diene (**A24**), 3β,22*R*,25-trihydroxylanosta-8,23*E*-diene (**A25**), 3β,22*R*,25-trihydroxylanosta,7,9(11)23*E*-triene (**A26**);

—compounds with a five-membered ring—21,24-cyclopentalanosta-3β,21,25-triol-8-ene (**A27**), 25-methoxy-21,22-cyclopentalanosta-8-ene-3β,21α-



The general structural formula of lanostane triterpenes (a) and steroid compounds (b).

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diol (**A28**), (20*R*,24*S*-cyclopentalanosta-8-ene-3β, 21*R*,25-triol (**A29**), 20*R*,24*R*-cyclopentalanosta-8-ene-3β, 21*R*,25-triol (**A30**), 20*R*, 24*S*-cyclopentalanosta-7:9(11)-diene-3,21*R*,25-triol (**A31**), stereoisomer 21,24-cyclopenta-1α,3β,21α,25,28-pentahydroxy-5α-lanosta-7,9(11)-diene (**A32**, **A33**);

—epoxides—22*R*,25-epoxylanosta-8-ene-3β, 24*S*-diol (**A34**, **A35**);

—compounds with an oxygen-containing five-membered heterocycle—3β,25-dihydroxylanosta-8-ene-20*R*,24*S*-olide (**A36**, **A37**), 3β,25-dihydroxylanosta-7,9(11)-diene-20*R*,24S-olide (**A38**) [1–27].

Natural chaga mushroom contains trace amounts pentacyclic triterpenes of the lupine series: betulin (**B**), lupeol (**C**) lupenon (**D**); their content is one order of magnitude lower than that of tetracyclic triterpenes, lanosterol derivatives [8, 38].

Natural chaga mushroom also contains steroid compounds. Ergosterol content (**E**) is about 10 times lower than that of triterpenes. Diversity of ergosterol derivatives is also narrower; 3β-hydroxyergosta-7,22-diene (**E1**) and 3β-hydroxyergosta-7-ene (fungisterol **E2**) were found in trace quantities. Such typical plant steroid compounds as sitosterol (**F**), stigmasterol (**G**), sitostanol (**H**), and cholesterol (**I**) characteristic of animals and humans, also exist in trace amounts shown [8, 38].

Thus, natural chaga predominantly accumulate lanostane type triterpenes, small amounts of steroid compounds (mainly **E**). The spectrum of lanosterol derivatives is rather wide; most frequently, compounds with several double bonds in the side chain, and also containing a ketone group or a five-membered ring are detected. These numerous oxygenated lanosterol derivatives are typical for the fungi causing white rot, to which chaga mushroom belongs.

Since chaga mushroom is a rather poorly replenishable source of triterpene and steroid compounds, many researchers have tried to cultivate it on various media (malt, solid mineral and liquid media supplemented with chitosan and cysteine, with birch sawdust, with $AgNO_3$) to increase the yield of these compounds [8, 38–40].

Being cultivated in artificial conditions chaga mushroom also produces more triterpenes than steroids (as in natural conditions). This qualitative composition of tetracyclic triterpenes remains basically unchanged. In naturally grown chaga mushroom **A** and **A1** mainly prevail, while in the artificial culture conditions, the content and composition of the compounds depends on the medium composition. The cultivation conditions have been optimized, so that artificially grown chaga mushroom accumulates the same triterpenes as the naturally grown fungus but in somewhat smaller quantities [8]. In the culture chaga mushroom accumulates a bit more **E** and expands the spectrum of its derivatives: ergosterol peroxide (**E3**), 3β-hydroxyergosta-5,7-diene (**E4**), 3β-hydroxyergosta-5,22-diene (**E5**) appear and higher quantities of **B**, **F**, **G**, **H**, **I** are detected more frequently [8, 39]. Composition of steroid compounds in artificial chaga cultures also depends on cultivation conditions, such as temperature, pH, UV radiation, etc. [8, 38, 40].

BIOLOGICAL ACTIVITY OF TRITERPENES AND STEROIDS OF CHAGA MUSHROOM

In folk medicine chaga mushroom and its aqueous extracts are used from ancient times for treatment of cancer and gastrointestinal tract diseases [41, 42]. The presence of triterpene and steroid compounds was demonstrated in chaga aqueous extracts and meal, remained after their preparation; small amounts of these compounds were also detected in the filtrate obtained after the precipitation and separation of melanin [43–46].

Currently, preparations based on aqueous extracts of chaga mushroom are used in cases where surgery and chemotherapy are not recommended. In the context of oncological diseases available literature is most frequently focused on the effect of water extracts on various animal species and humans.

Long-term (6–9 months) application of preparations based on chaga aqueous extracts significantly improved conditions of stage III–IV cancer patients regardless to the tumor location. In most patients, the use of chaga preparations for 3–4 weeks decreased pain, which subsequently could even disappear. Researchers suggested that chaga lacking any specific effect on the tumor, exhibits a tonic effect on the central nervous system; during longer treatment it can normalize impaired metabolic processes in the body and thus has an inhibitory effect on tumor growth [47–52].

More recent studies have shown that the use of an aqueous extract of the fungus reduced the size of sarcoma tumors MOP and S180, Lewis lung carcinoma and Ehrlich carcinoma, melanoma B16-F10, glioblastoma U-87 MG [33, 34, 53–56], and had the antimetastatic effect in vivo on sarcoma cells, HeLa cervical adenocarcinoma, Ehrlich carcinoma, and in vitro on hepatoma, colon cancer, and sarcoma 180 cells [15, 30, 41, 53].

The biological activity of various organic chaga extracts (ethanol, methanol, petroleum ether, ethyl acetate, chloroform), was investigated in vitro.

The ethanol extract of chaga mushroom had antiproliferative action on B16F1 melanoma cells; it caused 60%-inhibition of growth of lung cancer cell NCI-H460, gastric cancer HT-29 cells [56, 57]. At a concentration of 20–40 μg/mL the chloroform extract of this fungus reduced proliferation of P388 leukemia cells; its activity was much higher than the activity of an aqueous extract of the chaga mushroom against hepatoma and cervical cancer [32]. This indirectly indicates that chaga steroid compounds in the

chloroform extract are more active than melanin of the aqueous extract.

Petroleum ether and ethyl acetate extracts obtained during subsequent separation of the ethanol extract by means of petroleum ether and ethyl acetate, respectively, reduced in vitro development of prostate carcinoma cells PC3 and breast carcinoma MDA-MB-231. Active concentrations of these extracts against PC3 prostate carcinoma cells were 29.57 ± 12.18 and 19.22 ± 0.46 μg/mL, respectively, in the case of breast carcinoma MDA-MB-231 these were 57.39 ± 14.46 and 46.49 ± 13.21 μg/mL [36]. Petroleum ether extract activity against both cancer cell lines and activity of the ethyl acetate extract against cells MDA-MB-231 were comparable to that of a known cytostatic, doxorubicin, and in the case of PC3 cells they were even three times lower than for doxorubicin [36]. The highest activity of the petroleum ether extract is probably related to the preferential enrichment with triterpene and steroid compounds.

The high anti-tumor activity of chaga triterpenes was originally demonstrated in vitro experiments using Ehrlich ascites carcinoma and Crocker sarcoma [3, 4]. Compound **A1** demonstrated a marked effect on tumor cells, while compound **A** had a very weak effect (initial change in cells).

Table summarizes results of studies of individual triterpene and steroid compounds against different cancer cell lines in vitro.

A, **A1**, **A2**, **A5**, **A7**, and **E3** were the most active compounds acting on a wide range of cancer cells (table). At the same time, some compounds demonstrated certain specificity. **E**, **E3**, **A7**, and **A5** were the most active against carcinomas. The inhibitory concentration of **E** against prostate carcinoma cells was five times lower than for other compounds, thus indicating its high activity. Compounds **A** and **A1** were the most active against adenocarcinomas, both substances were active against breast adenocarcinoma MCF-7 at their minimal concentrations of 1 μg/mL. Compounds **A1**, **A5** and **A7** were more active against leukemia and **A1** was the most potent of them against the P388 leukemia cell: **A1** acted in the concentration of 6 μg/mL, which is one order of magnitude lower than the concentration of other compounds.

Thus, steroid compounds were more effective than triterpenes in the case of carcinomas. In the case of carcinoma and leukemia cells triterpene **A1** was more effective possibly due to the presence of OH-group at C-22, which is obviously crucial for manifestation of the anti-proliferative effect [32]. The high activity of compound **A7**, may be also attributed to the presence of OH-groups at this carbon atom. It should be noted that all compounds demonstrating anti-tumor activity, contain an unsaturated bond in the side chain, which can also contribute to the anti-tumor activity.

Results obtained in vitro studies were confirmed by studies employing these compounds in vivo. For example, compounds **A1** and **A5** induced death of papilloma cells applied superficially to mice [62], and also reduced the growth of sarcoma S180 by 18 and 34%, respectively. Compound **A** reduced the size of S180 tumor by 23% [60].

Intraperitoneal administration of **A1** to CDF1 mice with grafted P388 leukemia significantly increased the lifespan of mice without evident side effects (e.g., weight loss or diarrhea) by 20.8% in the case of mice treated with 10 mg/kg of this compound [32].

In addition to anti-tumor activity some compounds also exhibit other types of biological activities. For example, **A**, **A2**, **A7**, and **A15** demonstrated a marked hepatoprotective effect in vitro, representing 74.8, 81.2, 75.0, and 71.9%, respectively, versus bicyclol, a hepatoprotector agent used as control [26]. **A1** and **A5** exhibited hypoglycemic properties in vitro [55, 59]; **A2**, **A5**, and **A24** demonstrated antifungal properties in vitro; **A1**, **A2**, **A5**, **B**, and **B3** exhibited anti-inflammatory properties in vitro [35], while **A1**, **A5**, and **A23** behaved as antioxidants [36]. **A1** and **A5** demonstrated an antimutagenic effect, reduced levels of mutagens MNNG, 4NQO, in *Salmonella typhimurium* TA98 and TA100 [63]. Almost all lanosterol derivatives may regulate cholesterol biosynthesis [63], and betulin derivatives isolated from birch bark, and other natural sources, exhibit anti-tumor properties in vitro against melanoma cells, lung carcinoma, neuroblastoma, medulloblastoma, glioblastoma and Ewing sarcoma, prostate adenocarcinoma PC3, leukemia K562 and HeLa cervical adenocarcinoma cells [65, 66].

The triterpene compounds lanosterol and inotodiol were found in chaga melanin [67]. This obviously accounts for the manifestation of chaga anti-tumor effects: moderate on the primary tumor foci and strongly pronounced on metastasis [68].

Currently, the anti-tumor effects of lanostane compounds are attributed to changes in biochemical mechanisms: inhibition of cancer cell proliferation, induction of cell cycle arrest at various stages, increased apoptosis and regulation of signaling pathways associated with impaired expression of key enzymes (caspases) and proteins (p53, bax, Bcl-2) [27, 29, 32].

The action of the triterpene compounds of chaga mushroom is mainly associated with a decrease in cancer cell proliferation recognized in vitro studies. The aqueous chaga extract in vitro preferentially caused a cell cycle arrest and increased apoptosis, while in vivo it activated cells of the immune system and reduced the number of metastasis [15, 57].

Chaga polysaccharides also demonstrated a pronounced activity against cancer cells, but their mechanisms of action significantly differed. Polysaccharides influence the immune system through stimulation of lymphocytes and natural killer cells [24, 64].

The anti-tumor activity of triterpene and steroid compounds of chaga mushroom

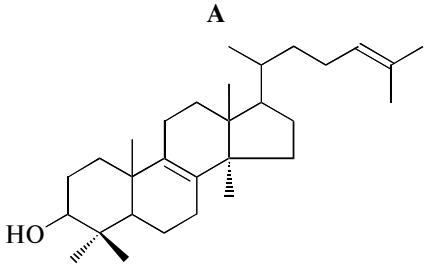
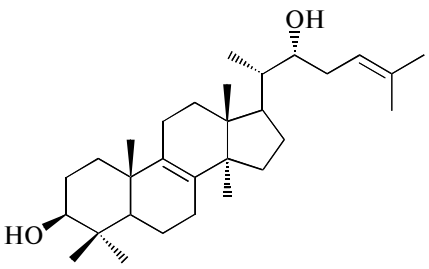
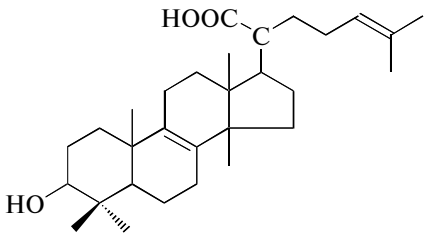
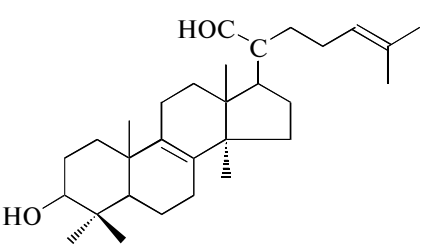
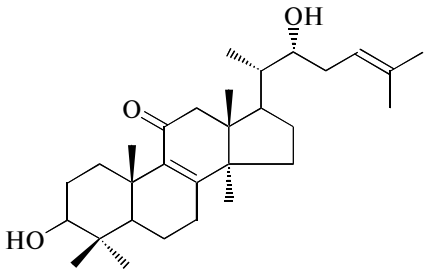
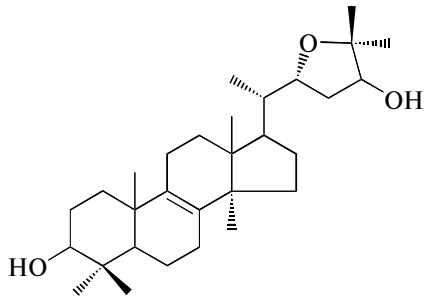
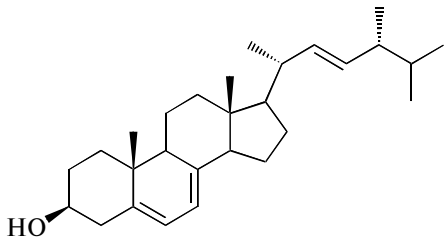
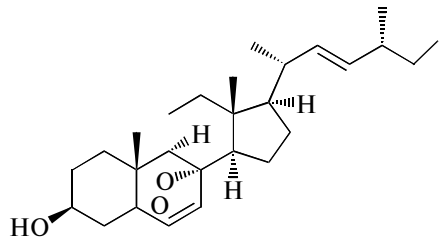
Substance	Cell lines	Inhibitory concentration
A 	Breast adenocarcinoma MCF-7	1 μg/mL; 250 μg/mL [7, 9, 24]
	P388 leukemia	More than 100 μg/mL [23]
	Cervical adenocarcinoma HeLa	250 μg/mL [31]
	Lung carcinoma A-549	250 μg/mL [23]
	Gastric adenocarcinoma AGS	250 μg/mL [31]
	Breast adenocarcinoma MDA-MB-231	More than 40 μg/mL [35]
	Prostate carcinoma PC3	More than 40 μg/mL [35]
	L1210 leukemia	More than 80 μg/mL [23]
	Gastric adenocarcinoma COLO205	More than 40 μg/mL [23, 25]
A1 	Breast adenocarcinoma MCF-7	1 μg/mL; 250 μg/mL [7, 9, 24]
	Walker carcinosarcoma 256	10 μg/mL [9]
	P388 leukemia	6 μg/mL; more than 40 μg/mL [20, 24]
	L1210 leukemia	49 μg/mL [23]
	Gastric adenocarcinoma COLO205	75 μg/mL [23]
	Breast adenocarcinoma MDA-MB-231	More than 40 μg/mL [35]
	Prostate carcinoma PC3	More than 40 μg/mL [35]
	Cervical adenocarcinoma HeLa	250 μg/mL [31]
	Lung carcinoma A-549	250 μg/mL [23, 24]
	Gastric adenocarcinoma AGS	250 μg/mL [31]
A2 	Breast adenocarcinoma MCF-7	5 and 10 μg/mL [9, 7]
	P388 leukemia	12 mg/mL [20]
	L1210 leukemia	16 μg/mL [23]
	Breast adenocarcinoma MDA-MB-231	25 μg/mL [35]
	Prostate carcinoma PC3	29 μg/mL [35]
	Gastric adenocarcinoma COLO205	More than 90 μg/mL [23, 24]
	Lung carcinoma A-549	More than 90 μg/mL [23, 24]
A5 	Breast adenocarcinoma MCF-7	250 μg/mL [7, 9]
	Cervical adenocarcinoma HeLa	250 μg/mL [31]
	P388 leukemia	9 mg/mL [20]
	Breast adenocarcinoma MDA-MB-231	16 μg/mL [35]
	L1210 leukemia	28 μg/mL [23]
	Prostate carcinoma PC3	33 μg/mL [35]
	Gastric adenocarcinoma COLO205	More than 80 μg/mL [23]
	Gastric adenocarcinoma AGS	250 μg/mL [31]
	Lung carcinoma A-549	250 μg/mL [23]

Table. (Contd.)

Substance	Cell lines	Inhibitory concentration
A7 	Nasopharyngeal cancer KB[1]	4.5 μg/mL [25]
	HL-60 leukemia	6.2 mg/mL [25]
	P388 leukemia	6.4 mg/mL [25]
	L1210 leukemia	9 mg/mL [25]
	Lung carcinoma A-549	More than 4 μg/mL [35]
	Hepatoma Bel-7402	More than 4 μg/mL [35]
A34 	P388 leukemia	13 mg/mL [20]
E 	Prostate carcinoma PC3	3.8 μg/mL [35]
	Breast adenocarcinoma MDA-MB-231	More than 40 μg/mL [35]
E3 	Walker carcinosarcoma 256	5 μg/mL [38]
	Breast adenocarcinoma MDA-MB-231	13 μg/mL [35]
	Prostate carcinoma PC3	16.3 μg/mL [35]
	Gastric adenocarcinoma COLO205	More than 80 μg/mL [23]
	Lung carcinoma A-549	More than 80 μg/mL [23]
	L1210 leukemia	More than 80 μg/mL [23]
	Breast adenocarcinoma MCF-7	More than 80 μg/mL; 10 μg/mL [38, 23]

Thus, among 40 triterpene and steroid compounds currently detected in chaga mushroom six compounds exhibit the highest anti-tumor activity; these include: lanosterol, inotodiol, trametenolic acid, 3β-hydroxylanosta-8,24-dien-21-al, 3β,22*R*-dihydroxylanosta-8, 24-dien-11-one, 22*S*,25-epoxylanost-8-ene-3β, 24*S*-diol.

It has been shown that individual triterpene and steroid compounds are more active than extracts with respect to cancer cell lines. The concentration at which the individual compounds begin to have an effect on tumor cells is about 1 μg/mL. The compounds have insignificant effect (if any) on somatic cells. The inhibitory effect on kidney cells is not more than 20%, while known natural cytotoxic agents exert a potent toxic effect on these cells (e.g., vinblastine, vincristine, etoposide) [69].

CONCLUSIONS

Chaga mushroom triterpenes and steroids represent about 50 compounds among which lanosterol

derivatives with several double bonds in the side chain or containing a ketone group and a five-membered ring predominate.

Studies in vitro and in vivo have shown that in contrast to chaga extracts individual triterpene and steroid compounds are more effective in cancer therapy. Triterpene compounds containing OH group at C-22 and an unsaturated bond in the side chain exhibit the highest anti-tumor activity. For example, inotodiol is effective against carcinosarcoma, adenocarcinoma, and leukemia cells, while 3β-hydroxylanosta-8,24-dien-21-al is active against carcinomas. Among the steroid compounds isolated from an artificial culture of chaga mushroom ergosterol and ergosterol peroxide exhibit pronounced and moderate anti-tumor activity against prostate carcinoma, respectively.

Triterpene and steroid compounds of chaga mushroom also demonstrate hepatoprotective, hypoglycemic, anti-fungal, anti-oxidant and anti-inflammatory properties; in addition they can regulate cholesterol biosynthesis.

REFERENCES

1. Kier, L.B., *J. Pharmac. Sci.*, 1961, vol. 50, pp. 471–474.
2. Ludwiczak, R.-S. and Wrecino, U., *Rocz. Chem.*, 1962, vol. 36, pp. 497–502.
3. Lovyagina, E.V. and Shivrina, A.N., *Biokhimiya* 1962, vol. 27, no. 5, pp. 794–800.
4. Lovyagina, E.V. and Shivrina, A.N., in *Kormovyie belki i fiziologicheski aktivnye veshchestva dlya zhivotnovodstva*, (Feed Proteins and Physiologically Active Substances for Animals), Moscow-Leningrad, 1965, pp. 59–64.
5. Kahlos, K., Hiltunen, R., Schantz, M.V., *Planta Medica*, 1984, vol. 50, pp. 197–198.
6. Kahlos, K. and Hiltunen, R., *Planta Medica*, 1986, vol. 52, pp. 495–496.
7. Kahlos, K., Kangas, L., and Hiltunen, R., *Planta Medica*, 1986, vol. 52, p. 554.
8. Kahlos, K., *Biotechnology in Agriculture and Forestry,* 1994, vol. 26, pp. 179–198.
9. Shin, Y., Tamai, Y., and Terazawa, M., *Euras. J. Forest Res.*, 2000, vol. 1, pp. 43–50.
10. Shin, Y., Tamai, Y., and Terazawa, M., *Int. J. Med. Mushrooms*, 2000, vol. 2, pp. 201–207.
11. Shin, Y., Tamai, Y., and Terazawa, M., *J. Wood Sci.,* 2001, vol. 47, no. 4, pp. 313–316.
12. Shin, Y., Tamai, Y., and Terazawa, M., *Int. J. Med. Mushrooms*, 2001, vol. 3, p. 250.
13. He, J., Feng, X.Z., Lu, Y., and Zhao, B., *Chinese Chemical Letters*, 2000, vol. 11, no. 1, pp. 45–58.
14. He, J., Feng, X.Z., Lu, Y., and Zhao, B., *J. Asian Nat. Prod. Res.*, 2001, vol. 3, pp. 55–61.
15. Kim, E.J., Lee, Y.J., Shin, H.K., Park, J.H., *J. Korean Soc. Food Sci. Nutr.*, 2006, vol. 35, no. 5, pp. 516–523.
16. Nakata, T., Taji, S., and Yamada, T., *Bioorg. Med. Chem.*, 2007, vol. 15, no. 1, pp. 257–264.
17. Tajia, S., Yamada, T., In, Y., Wada, S., Usami, Y., Sakuma, K., and Tanaka, R., *Helvetica Chimica Acta*, 2007, vol. 90, pp. 2047–2057.
18. Tajia, S., Yamada, T., and Tanaka, R., *Helvetica Chimica Acta*, 2008, vol. 91, pp. 1513–1524.
19. Taji, S., Yamada, T., Wada, S., Tokuda, H., and Sakuma, K., *Eur. J. Med. Chem.*, 2008, vol. 43, pp. 2373–2379.
20. Nakata, T., Taji, S., and Yamada, T., *Bulletin of Osaka University of Pharmaceutical Sciences*, 2009, vol. 3, pp. 53–64.
21. Zhong, X., Ren, K., Lu, S., Yang, S., and Sun, D., *Chin. J. Integr. Med.,* 2009, vol. 15, pp. 156–160.
22. Handa, N., Yamada, T., and Tanaka, R., *Phytochemistry*, 2010, vol. 71, pp. 1774–1779.
23. Kim, Y.J., Park, J., Min, B.S., and Shin, S.H., *J. Korean Soc. Appl. Biol. Chem.*, 2011, vol. 54, no. 2, pp. 287–294.
24. Zheng, W., Miao, K., Liu, Y., Zhao, Y., Zhang, M., Pan, S., and Dai, S., *Appl. Microbiol. Biotechnol.*, 2010, vol. 87, pp. 1237–1254.
25. Handa, N., Yamada, T., and Tanaka, R., *Phytochem. Lett.*, 2012, vol. 5, pp. 480–485.
26. Liu, C., Zhao, C., Pan, H.-H., Kang, J., and Yu, X., *J. Nat. Prod.*, 2014, vol. 77, pp. 35–41.
27. Song, F.Q., Liu, Y., Kong, X.S., Chang, W., and Song, G., *Asian Pac. J. Cancer Prev.*, 2013, vol. 14, pp. 1571–1578.
28. Zhao, F., Mai, Q., Ma, J., Xu, M., Wang, X., Cui, T., Qui, F., and Han, G., *Fitoterapia*, 2015, vol. 101, pp. 34–40.
29. Ríos, J.L., Andujar, I., Recio, M.K., and Giner, R.M., *J. Nat. Prod.*, 2012, vol. 75, pp. 2016–2044.
30. Chen, C., Zheng, W., Gao, X., and Xiang, X., *Am. J. Pharmacol. Toxicol.*, 2007, vol. 2, pp. 10–17.
31. Illana-Esteban, C., *Bol. Soc. Micol*, 2011, vol. 35, pp. 175–185.
32. Nomura, M., Takahashi, T., Uesugi, A., Tanaka, R., and Kobayashi, S., *Anticancer Res.*, 2008, vol. 28, pp. 2691–2696.
33. Youn, M., Kim, J., Park, S., Kim, Y., and Park, C., *J. Ethnopharmacol.*, 2009, vol. 121, pp. 221–228.
34. Mazurkiewicz, W., Rydel, K., Pogocki, D., Lemieszek, M.K., Langner, E., and Rzeski, W., *Acta Poloniae Pharmaceutica—Drug Research*, 2010, vol. 67, no. 4, pp. 397–406.
35. Mazurkiewicz, W., *Acta Poloniae Pharmaceutica*, 2006, vol. 63, pp. 497–501.
36. Ma, L., Chen, H., Dong, P., and Lu, X., *Food Chemistry*, 2013, vol. 139, pp. 503–508.
37. Zheng, W., Zhang, M., Zhao, Y., Miao, K., Pan, S., Caoa, F., and Daic, Y., *Phytochem. Anal.*, 2011, vol. 22, pp. 95–102.
38. Gao, Y., Xu, H., Lu, Z., and Xu, Z., *Chin. J. Chromatogr.*, 2009, vol. 29, pp. 745–749.
39. Shin, Y., Tamai, Y., and Terazawa, M., *Euras. J. Forest Res.*, 2001, vol. 2, pp. 27–30.
40. Nizkovskaya, O.P., Milova, N.M., Shivrina, A.N., Lovyagina, E.V., and Platonova, E.G., *Trudy Instituta*

Mikrobiologii Akad. Nauk SSSR, 1959, vol. 6, pp. 277–285.

41. Shashkina, M.Ya., Shashkin, P.N., Sergeev, A.V., and Goryainova, L.K., *Chaga, chagovit, chagoluks v lechebnoi i profilakticheskoi praktike* (Chaga, Chagovit, Chagoluks in Therapeutic and Prophylactic Practice), Moscow: EDAS Holding, 2008.
42. Sysoeva, M.A., *Vysokodispersnye kolloidnye sistemy i melaniny chagi* (Highly Dispersed and Colloidal Systems and Melanins of Chaga Mushroom), Kazan, 2013.
43. Sysoeva, M.A., Khabibrakhmanova, V.R., Gamayurova, V.S., and Tazeeva, A.Kh., *Khimiya Rastitel'nogo Syr'ya,* 2008, no. 3, pp. 119–122.
44. Sysoeva, M.A., Nikitina, S.A., and Khabibrakhmanova, V.R., *Vestnik Kazanskogo Universiteta*, 2012, vol. 15, no. 18, pp. 217–219.
45. Yumaeva,L.R., The composition and properties of the extracts of the fungus meal, *Cand. Sci. (Chem.) Dissertation*, Kazan, KGTU, 2009.
46. Sysoeva, M.A., Khabibrakhmanova, V.R., and Gamayurova, V.S., *Khimiya Rastitel'nogo Syr'ya,* 2009, no. 3, pp. 151–156.
47. Berezina, M.P., in *Chaga i ee lechebnoe primenenie pri rake IV stadii* (Chaga Mushroom and Its Therapeutic Use in Stage IV Cancer), Leningrad: Medgiz, 1959, pp. 143–159.
48. Bulatov, P.K., in *Chaga i ee lechebnoe primenenie pri rake IV stadii* (Chaga Mushroom and Its Therapeutic Use in Stage IV Cancer), Leningrad: Medgiz, 1959, pp. 261–270.
49. Martynova, E.Ya., in *Chaga i ee lechebnoe primenenie pri rake IV stadii* (Chaga Mushroom and Its Therapeutic Use in Stage IV Cancer), Leningrad: Medgiz, 1959, pp. 271–278.
50. Bulatov, P.K., and Martynova, E.Ya., in *Kompleksnoe izuchenie fiziologicheski aktivnykh veshchestv* (Complex Study of Physiologically Active Substances of Lower Plants), Moscow–Leningrad: Nauka, 1961, pp. 247–253.
51. Pyaskovskii, S. and Richter, S., in *Kompleksnoe izuchenie fiziologicheski aktivnykh veshchestv* (Complex Study of Physiologically Active Substances of Lower Plants), Moscow–Leningrad: Nauka, 1961, pp. 258–263.
52. Martynova, E.Ya, in *Chaga i ee lechebnoe primenenie pri rake IV stadii* (Chaga Mushroom and Its Therapeutic Use in Stage IV Cancer), Leningrad: Medgiz, 1959, pp. 279–293.
53. Shashkina, M.Ya., Shashkin, P.N., and Sergeev, A.V., *Rossiiskii bioterapevticheskii zhurnal,* 2005, vol. 4, no. 4, pp. 59–72.
54. Shin, S.H., Kim, Y.J., and Park, J., *J. Korean Soc. Food Sci. Nutr.*, 2013, vol. 42, pp. 1022–1028.
55. Youn, M.J., Kim, J.K., Park, S.Y., and Kim, Y., *World J. Gastroenterol.,* 2008, vol. 14, no. 4, pp. 511–517.
56. Lu, X., Chen, H., Dong, P., Fu, L., and Zhang, X., *J. Sci. Food. Agric.*, 2010, vol. 90, pp. 276–280.
57. Park, E., Jeon, K., and Byun, B.H., *Cancer Prevention Research*, 2005, vol. 10, pp. 54–59.
58. Lee, H.S., Kim, E.J., and Kim, S.H., *Nutr. Res. Pract.*, 2015, vol. 9, no. 2, pp. 111–116.
59. Song, K.C., Choi, B.L., and Shin, J.W., *Korean J. Orient. Med.*, 2007, vol. 28, no. 4, pp. 27–41.
60. Zhang, Y., Zhao, Y., Cui, H., Cao, C., Guo, J., and Liu, S., *Biol. Trace Elem. Res.*, 2011, vol. 144, pp. 1351–1357.
61. Chung, M.J., Chung, C.K., Jeong, Y., and Ham, S.S., *Nutr. Res. Pract.*, 2010, vol. 4, pp. 177–182.
62. Koyama, T., Gu, Y., and Taka, A., *Asian Biomedicine*, 2008, vol. 2, pp. 459–469.
63. Sun, Y., Yin, T., Chen, X.H., and Zhang, G., *Int. J. Med. Mushrooms*, 2011, vol. 13, pp. 121–130.
64. Ham, S.S., Kim, S.H., Moon, S.Y., and Chung, M.J., *Mutation Research*, 2009, vol. 672, pp. 55–59.
65. Tolstikov, G.A., Flekhter, O.B., and Schultz, E.E., *Khimiya v interesakh ustoichvogo razvitiya*, 2005, vol. 13, pp. 1–30.
66. Tolstikova, T.G., Sorokina, I.V., Tolstikov, G.A., Tolstikov, A.G., and Flekhter, O.B., *Bioorgan. Khim.*, 2006, vol. 32, pp. 42–55.
67. Burmasova, M.A., Phenolic and related compounds of an aqueous extract of the chaga mushroom, *Cand. Sci. (Chem) Dissertation*, Kazan, KNITU, 2013.
68. Ryzhova, G.L., Kravtsova, S.S., Matasova, S.A., Gribel, N.V., Pashinskii, V.G., and Dychko, K.A., *Khim.-Farm. Zhurn.*, 1997, no. 10, pp. 44–47.
69. Mashkovskii, M.D. *Lekarstvennye sredstva* (Drug Preparations), Moscow: Novaya Volna, 2012.

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