FORESTRY IDEAS, 2022, vol. 28, No 2 (64): 287–297

Chemical composition and antioxidant activity of *Fomitopsis pinicola* growing on coniferous and deciduous substrates

Irina S. Kiseleva[1*], Alexander A. Ermoshin[1,2], Boris A. Galishev[1], Duan Shen[3], Chaomei Ma[3], and Jiangfeng He[2]

[1]Ural Federal University, 620000, Ekaterinburg, Russia. *E-mail: irina.kiseleva@urfu.ru
[2]Inner Mongolia Academy of Agriculture and Animal Husbandry Science, 232813, Hohhot, China.
[3]Inner Mongolia University, 010031, Hohhot, China.

Received: 30 December 2021 / Accepted: 04 August 2022 / Available online: 26 September 2022

Abstract

Xylotrophic fungi are widespread in all types of forests. The composition of their primary and secondary metabolites including biologically active compounds depends on species and growth substrate; therefore, fungi can become a valuable raw material for biotechnology, pharmaceuticals, and food industry. *Fomitopsis pinicola* is one of the common species in Russia and Europe, growing predominantly on *Pinus sylvestris* L., but in mixed forests of the Urals it is also found on *Betula pendula* Roth. As metabolism of angiosperms and gymnosperms, and their timber properties are different, growth substrate could affect the chemical composition of fungi fruit bodies; therefore our study aimed at metabolite composition of extracts, obtained from *F. pinicola* collected from birch and pine, and their antioxidant activity. Qualitative analysis revealed alkaloids, phenolics, and anthraquinones. Saponins were found only in the samples obtained from pine. Thin layer chromatography of extracts revealed the same qualitative composition of phenolics, but their amount was higher on birch – 4.2 mg g^{-1} then on pine – 3.1 mg·g^{-1}. In ABTS test extracts showed the same antiradical activity. The metabolomics profile obtained by UHPLC-MS totally revealed 116 compounds, and each fungi sample contained more than 70 of them. Thus, the type of substrate influenced on the profile of metabolites and quantitative composition in *F. pinicola* fruit bodies.

Key words: *Betula pendula*, fungal methanol extracts, *Pinus sylvestris*, TLC, UHPLC-MS.

Introduction

Forests occupy 46.6 % of the Russian Federation territory, which is about 20 % of all forests on the planet. They are not only a source of wood, but also a powerful sequester of carbon, regulating the climate both in local and global scales. Forests provide us with medicinal and food plants, mushrooms, etc. Sverdlovsk Region is one of the ten regions in Russia in terms of area (68 %) covered by forests. The largest city in the region – Yekaterinburg – is located on the eastern slope of

the Ural Mountains, in the southern taiga subzone and is surrounded by mixed forests, represented by *Pinus sylvestris* L. with an admixture of *Betula pendula* Roth. (FAO 2012).

Common forest pests in the Urals are presented by parasitic and saprotrophic wood-destroying fungi. Some of them being parasites control the number of host trees, others being saprotrophic decay dead wood and return CO_2 to the atmosphere. One of the most typical species in the Urals is *Fomitopsis pinicola* (Sw.) P. Karst., which mainly inhabits pine, but also occurs on birch (Gründemanna et al. 2020). Large fruit bodies, wide distribution, and specific form and colour make it easily recognizable. This species damages timber, causing brown wood rot. At the same time it is used in traditional medicine in Siberia, China and Korea (Bishop 2020, Gründemanna et al. 2020).

In recent decades, there has been an increasing interest to new natural raw materials, including fungi that contain biologically active compounds for medical purposes. The search for biologically active substances (BAS) as bioprotectors and plant growth regulators for agriculture is also relevant (Ermoshin et al. 2021b). Large stocks and ease identification make *F. pinicola* promising for study as a raw material for the obtaining BAS for pharmaceuticals and biotechnology. *In vitro* experiments have shown that the chloroform extract of *F. pinicola* fruit bodies negatively affects the growth of some cancer cell lines (Gao et al. 2017). For alkaline extracts, the antidiabetic effect has been demonstrated in rats (Lee et al. 2008), as well as an immunomodulating, anti-inflammatory and antioxidant effects of its polysaccharides. It has been shown that fruit bodies contain ergosterol, sesquiterpenes, lanostane triterpenes and their glycosides (Bishop 2020, Janardhanan et al. 2020). Moreover, fungi fruit bodies are rich in phenolics which are known as the powerful reducing agents, and other biologically active substances – anthroquinones, carbohydrates, amino acids (Ermoshin et al. 2021a). However, this species has been studied to a much lesser extent than Reishi or Chaga used by the official medicine (Gründemanna et al. 2020, Janardhanan et al. 2020).

Most of the works do not indicate the type of growth substrate for studied species, though it is known that the composition of the fruit body is influenced by the growth substrate, and partially the fruit bodies are made of fragments of wood on which the fungus grows. Thus, the purpose of our work was to compare the chemical composition of ethanol and methanol extracts and the antioxidant activity of ethanol extracts from tinder fungus *F. pinicola* collected from the most typical growth substrates – pine and birch.

Materials and Methods

Biological material was collected in a mixed forest in the vicinity of the biological station of the Ural Federal University, Sverdlovsk region, Sysertsky district (56°36'4" N 61°3'25" E). Fruit bodies were collected by route method from all tree trunks on the transect. The number and the mass of the collected fruit bodies were assessed in the laboratory (Ermoshin et al. 2021a).

The chemical composition and *in vitro* antioxidant activity of fruit bodies were studied in ethanol (95%) extracts. Ethanol (3 mL) was added to a dry biomass of fruit bodies (150 mg). The mixture was treated with ultrasound for 15 min (570 W) at 50 °C. Then the mixture was centrifuged.

The supernatant was poured into a Falcon tube. Extraction was repeated 3 more times, supernatants were pooled together, and the extract volume was adjusted to 15 mL. The qualitative composition of the main groups of BAS was assessed using standard pharmacopeial tests (Zhu et al. 2017, Shaikh and Patil 2020, Ermoshin et al. 2021a). The amount of the total phenolics was determined spectrophotometrically in the reaction with Folin-Checolteu reagent and expressed in terms of gallic acid (Sulkowska-Ziaja et al. 2012). The antioxidant activity *in vitro* was determined in three tests and expressed as the total reduction potential, as the antiradical activity in ABTS-test, and antioxidant activity in membrane lipid peroxidation model (Ermoshin et al. 2021a, b).

Ethanol extracts were also studied for the qualitative characteristics of the birch and pine bark metabolites by thin layer chromatography (TLC) in a solvent system toluene: ethyl acetate: formic acid (30:18:2), and phenolic compounds were visualised with ferric chloride, flavonoids with aluminum chloride and terpenes with phosphoric-tungstic acid (Bertrams et al. 2013, Hamad et al. 2019, Blondeau et al. 2020, Ermoshin et al. 2021a).

The identification of individual phenolics and terpenoid compounds was carried out in extracts prepared from 50 mg of dry material in 1 mL of methanol. The mixture was treated with ultrasound at 40 °C for 30 min, and then extracts were separated by centrifugation, purified by passing through a reverse-phase column (C-18) and a microfilter with pore size of 0.02 μm. The identification of compounds was done using UHPLC-DAD-MS method. UHPLC-DAD was performed with an Agilent 1290 UHPLC coupled with a 6430 triple quadrupole mass spectrometer (Agilent Technologies, Singapore). Separations were performed on an Agilent ZORBAX RRHD Eclipse Plus C18 column (50×2.1 mm, i.d. 1.8 μm). UHPLC mobile phase was composed of 0.1 % formic acid as A and methanol as B. The A:B ratio was varied to the following program: 0 min, 85:15; 2 min, 73:27; 4 min, 65:35; 7 min, 50:50; 10 min, 40:60, 12 min, 25:75, 17 min, 0:100. The follow rate was 0.3 $mL \cdot min^{-1}$, the injection volume was 2 μL, and the column temperature was 30 ºC. The list of standards was compiled based on the literature data (Kim et al. 2008, Gao et al. 2013, Ermoshin et al. 2022).

Statistical data processing was done using Statistica 8 (StatSoft). The significance of differences in the abundance of fruit bodies on different substrates was determined by Fisher's criterion. The assessment of chemical composition similarity was carried out according to Jaccard coefficient. Differences in quantitative indicators are shown by the nonparametric U-Mann-Whitney test. Analyzes were carried out in 3–4 analytical and 2–3 biological replicates.

Results and Discussion

The studied species was found mainly on pine. However, the species is also known to inhabit deciduous species, including birch, but with a significantly lower abundance (Bishop 2020, Gründemanna et al. 2020). The biomass and the number of collected fruit bodies are presented in Table 1. Most of the basidiocarps were collected from pine trunks – 4 times more often than on birch. By mass 90 % of all fruit bodies were collected from pine. Basidiocarps on birch were twice smaller in mass than on pine.

Table 1. Frequency and total weight of basidocarps on different substrates.

Species	Number of trees and percent of trees	Total dry mass of the collected fruit bodies, g/%	Average dry mass of fruit bodies per tree, g
Pine	19 (79.2 %)	665/89.9	35
Birch	5 (20.8 %)*	75/10.1*	15
Total	24 (100 %)	740/100	

Note: * – the difference is significant at $p < 0.05$ (Fisher's test).

The bark of trees is the outer layer of the trunks, and its chemical composition directly depends on the composition of the wood. Despite the difference in composition, at least a quarter of the wood components are found in the bark (Ghavidel et al. 2021), therefore, we have studied the chemical composition of the bark.

The extracts from pine and birch bark contained the main groups of BAS (Table 2) as phenolics, flavonoids, triterpenes in both species; tannins and alkaloids were not detected, which corresponds to literature data (Bertrams et al. 2013, Hamad et al. 2019). Anthraquinones were found in the pine bark and saponins were presented only in the birch bark.

A quantitative analysis was performed for phenolics and flavonoids. They are known as important BAS with multiple health effects and activities (Selamoglu 2017). There was no significant difference in the content of phenolic compounds between species; however, the content of flavonoids in the pine bark was more than 5.5 times higher than in the birch bark.

Table 2. The main groups of BAS in the bark of birch and pine.

Sample (extract)	Phenolics, $mg \cdot g^{-1}$	Flavonoids, $mg \cdot g^{-1}$	Tannins	Anthraquinones	Alkaloids	Terpenes	Saponins
Pine bark	32.3 ±1.0	13.2 ±0.8	-	+	-	+	-
Birch bark	30.6 ±0.6	2.3 ±0.3**	-	-	-	+	+
Basidiocarps from pine	3.1 ±0.1	n.d.	-	+	+	+	+
Basidiocarps from birch	4.2 ±0.2*	n.d.	-	+	+	+	-

Note: (+) – component detected; (-) – qualitative reactions are negative; n.d. – not determined, below the limit of detection, in a quantitative method; * – differences between pine and birch are significant at $p < 0.05$ (U-criterion); ** – differences between pine and birch are significant at $p < 0.01$ (U-criterion).

The analysis of individual compounds was carried out by the method of TLC (Table 3). In birch bark 8 spots were found (presumably 5 terpenes, 2 phenols and 1 flavonoid), and 10 spots – in pine (presumably 2 terpenes, 2 phenols, 2 flavonoids and 4 four spots were not identified to class of chemicals). Tree spots were common to both substrates. Two of them were terpenes, and one corresponded to the quercetin standard. Salicylic acid was found in the birch bark, according to the standard. The index of similarity for the chemical composition of substrates was 0.2 according to Jaccard criterion. Thus, pine as a substrate had a greater variety of metabolites than birch. Not only were a greater number of individual compounds observed in the pine bark, but also a higher content of phenolics and flavonoids.

The results obtained do not contradict the literature data (Hamad et al. 2019, Ramadhani et al. 2021). We supposed that *F. pinicola* basidiocarps harvested from pine would also have a wider variety of metabolites.

Table 3. TLC analyses of pine and birch bark.

No	Spot colour				Rf	Distribution coefficient relative to			Sample		Group of compounds
	Without chemical processing	With $AlCl_3$	With phosphoric-tungstic acid	With $FeCl_3$		Quercetin	Gallic acid	Salicylic acid	Birch bark	Pine bark	
1	-	-	grey	-	0.06	0.14	0.22			+	n.d.
2	brown	brown	-	-	0.07	0.19	0.33	0.12		+	n.d.
3	-	yellow	-	grey	0.12	0.28	0.46	0.18		+	flavonoid
4	rose	brown	rose	-	0.14	0.34	0.57	0.22	+		terpene
5	-	-	-	grey	0.19	0.42	0.68	0.29	+		phenolic
6	grey	grey	-	grey	0.26	0.64	1.09	0.43		+	phenolic
7	violet	grey	-	violet	0.35	0.83	1.42	0.55		+	phenolic
8	yellow	green	yellow	grey	0.45	1.00	1.69	0.67	+	+	quercetin
9	-	-	violet	-	0.54	1.20	1.83		+	+	terpene
10	grey	grey	-	blue	0.63	1.52	2.60	1.00	+		salicylic acid
11	-	-	violet	-	0.71	1.57	2.39		+		terpene
12	-	-	violet	-	0.77	1.71	2.61		+	+	terpene
13	-	-	violet	-	0.81	1.80	2.74		+		terpene
13	-	-	grey	-	0.88	1.97	3.00			+	n.d.
15	-	-	grey	-	0.95	2.11	3.22			+	n.d.

Note: Rf – retention factor, n.d. – not determined, below the limit of detection in a quantitative method.

Comparison of basidiocarp extracts showed that their chemical composition differed from the bark composition. For example, alkaloids were found only in the fruit bodies. Anthraquinones were detected in basidiocarps growing on both substrates, while in the bark they were found only in pine. The presence of saponins depended on the growth substrate. The amount of phenolics in the fruit bodies was significantly less than in the growth substrate, and flavonoids were found in trace amounts. Even though pine bark was richer in phenolics than birch bark, fruit bodies collected from pine contained 25 % less phenolics than basidiocarps from birch.

Our results on the antioxidants such as phenolic compounds and anthraquinones, found in *F. pinicola* basidiocarps, correspond to literature data. (Yen et al. 2000, Sulkowska-Ziaja et al. 2012). We compared *in vitro* antioxidant activity of extracts from basidiocarps growing at the same territory and having the same age but differing in the growth substrate (Table 4).

Table 4. *In vitro* antioxidant activity of extracts.

Substrate	ABTS-test, %	Total reduction potential, %	Inhibition of lipid peroxidation, %
Pine	92.9 ±0,1	553 ±12	44.9 ±11,7
Birch	92.8 ±0,2	795 ±24*	32.3 ±19.0*

Note: * – differences between pine and birch are significant at $p < 0.05$ (U-criterion).

Basidiocarps growing on birch had a higher level of phenolics and reduction potential than those growing on pine. In the model of membrane lipid peroxidation the opposite pattern was observed – the extract from fruit bodies growing on birch inhibited the formation of malondialdehyde by a quarter weaker than the extract of fruit bodies from pine. Probably, the mechanism of LPO inhibition was not directly related to the antioxidant effect of phenolics since they are hydrophilic molecules and do not dissolve in the lipid phase of membranes. Regardless of the growing substrate, the fungi extracts had the same high antiradical activity in the ABTS test. Thus, *F. pinicola* extracts from both substrates had a high antioxidant activity, which corresponds to literature data (Sulkowska-Ziaja et al. 2012, Nowacka et al. 2015, Onar et al. 2016, Sevindik et.al. 2017) and makes it interesting for biotechnology, pharmaceutical and nutraceutical usage as the possible sources of antioxidants.

TLC revealed only three metabolites in basidiocarp extracts, which could not be reliably determined to class. The method turned out to be uninformative for distinguishing the composition of the fruit bodies extracts for one species of fungi growing on different substrates, therefore, to compare the composition of the fruit bodies, HPLC was done in combination with mass spectrometry. We focused on the search for the most common phenolic compounds with pronounced antioxidant activity and biological effects, such as anti-cancer, antiviral, antibacterial, hypoglycemic, and others (Sulkowska-Ziaja et al. 2012, 2018). Except phenolic compounds, we have paid attention to terpenoids known for their anticancer activity (Gao et al. 2013, Cör et al. 2018, Dasgupta and Acharya 2019). The results are presented in Table 5.

Table 5. Content of individual phenols and terpenes in fruit bodies.

Compound	Precursor ion / Product ion, $m \cdot z^{-1}$	Substrate	
		Pine, $\mu g \cdot g^{-1}$	Birch, $\mu g \cdot g^{-1}$
	Phenolics		
Gallic acid	169/125	8.04±0.40	1.32±0.05*
Chlorogenic acid	353/191.1	0.02±0.00	0.03±0.00
Caffeic acid	179/135	0.81±0.04	1.21±0.05
P-coumaric acid	163/119	0.33±0.00	0.33±0.02
Cinnamic acid	147/103.6	n.d.	3.61±0.20*
Protocatechuic acid	153/109	42.67±2.10	56.87±2.80
Catechin	289/109	n.d.	2.65±0.10*
Epicatechin	289/245	0.05±0.00	n.d.
Rutin	610/300	0.21±0.01	0.44±0.02*
Luteolin	285/133	0.21±0.01	0.36±0.02

Compound	Precursor ion / Product ion, m·z^{-1}	Substrate	
		Pine, μg·g^{-1}	Birch, μg·g^{-1}
Kaempferol	285/133	0.56±0.03	0.64±0.03
Terpenes			
20-OH lucidenic acid A	455/149	n.d.	0.23±0.01
20-OH lucidenic acid N	457/none	0.53 ±0.03	0.10±0.00*
Ganodermanotriol	473/437.3	n.d.	36.76±1.50*
Ganoderiol A	475/457.3	8.60±0.4	15.16±0.70*
Ganoderiol D	489/471.2	33.97±1.40	6.25±0.37*
Ganoderiol F	477/none	1.15±0.05	0.89±0.04
Lucidumol A	473/259.1	0.47±0.02	1.01±0.05
Ergosterol peroxide	429/none	263.49±13.00	167.68±8.30*

Note: * – differences between pine and birch are significant at $p < 0.05$ (U-criterion).

Protocatechuic acid was the predominant phenolics, which is known as a powerful antioxidant. Hydroxybenzoic acid which is often presented in fungi (Kim et al. 2008) was not found in the studied species. The sum of identified phenolic compounds was only 1.5–2 % of the amount of phenolics determined spectrophotometrically. Flavonoids were not detected by qualitative reactions, and spectrophotometrically their content was determined in trace amounts, thus, flavonoids did not play a significant role in the antioxidant activity of *F. pinicola* fruit bodies extracts. However, they were determined by the method of UPHLC-MS. A lot of the compounds in studied extracts were not identified, but most likely they belonged to polyphenols. In terms of the concentration and diversity of identified compounds, birch samples were richer in BAS. This makes the study of basidiocarps collected from birch rather interesting compared to pine.

UHPLC revealed small concentration of terpenes, characteristic to *Ganoderma* (Reishi). They were also found in trace amounts in other fungal species, that are not related to *Ganoderma* genus (Gao et al. 2013). These compounds are known to perform an anticancer activity, and even in small concentration they can inhibit tumor cells (Gao et al. 2013). Ergosterol peroxide which is known as pronounced anticancer compound (Dasgupta and Acharya 2019) dominated among individual terpenes and phenols. Its concentration (0.16–0.26 mg·g^{-1}) was higher in extracts from fruit bodies grown on pines then on birches.

A detailed study of chromatograms (Fig. 1) showed at least 74 compounds (molecular weight individual peaks) in fruit bodies from birch, and 72 in basidiocarps from pine. Thirty of them were common for both fungi extracts (they have the same retention time and ion mass). Jaccard similarity coefficient was 0.26, which was close to the value of the coefficient for chemical composition of bark from TLC analysis.

The search for new antioxidants is relevant and promising, since oxidative stress is a common response to the pathogenesis of many diseases. Reactive oxygen species disrupt the structure of proteins; increase the frequency of mutations, which lead to the tumor risks, the heart diseases, and so on. Therefore, the

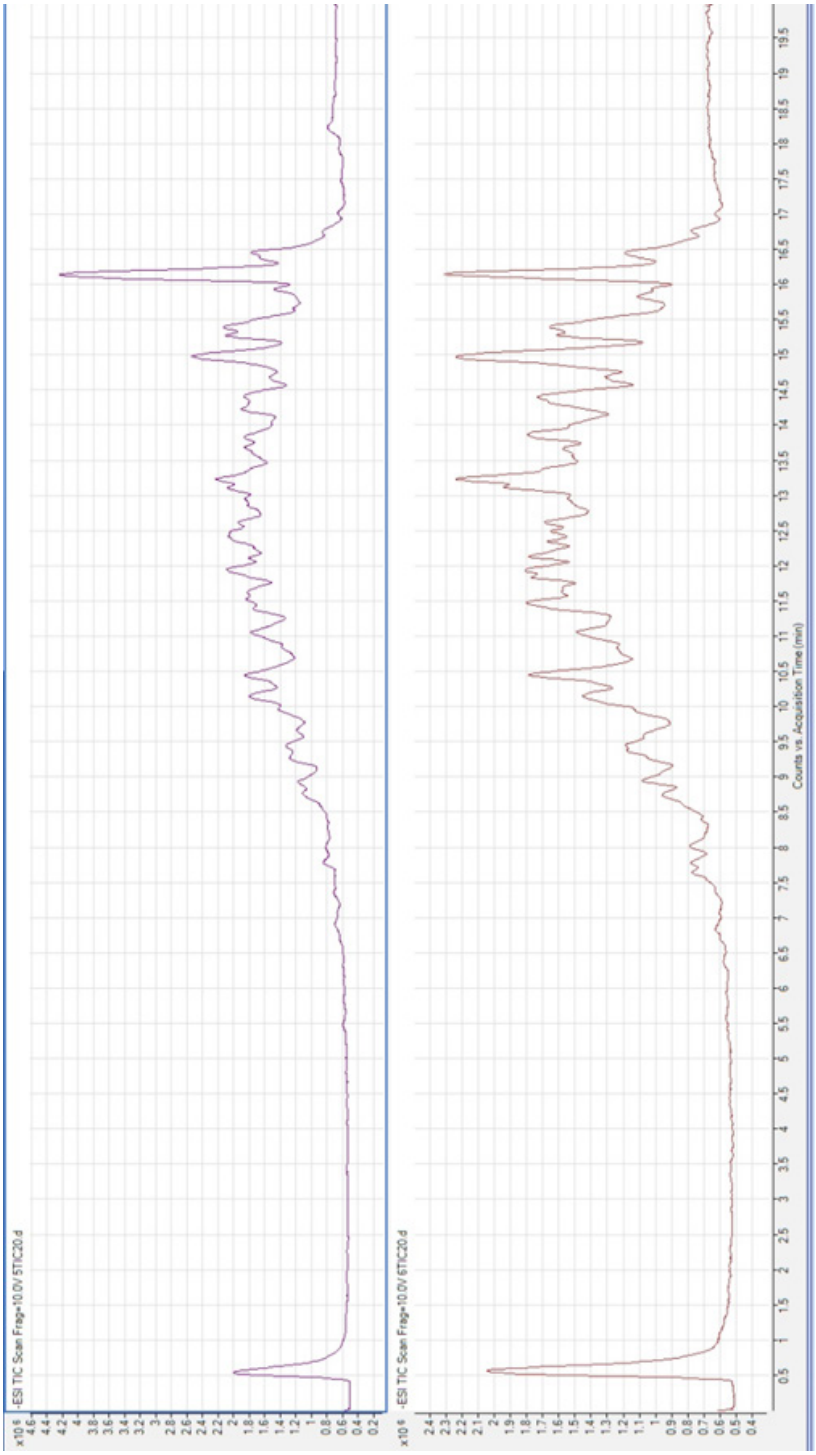

Fig. 1. HPLC-MS analyses: total ionic current, positive ionization (+ TIC).
Note: above – extract from basidiocarps growing on birch, below – on a pine tree.

search for new natural antioxidants is an important task. At the same time, it matters to know from which substrate the fruit bodies of fungi should be collected. In our study raw materials collected from birches had a higher content of phenolic compounds and greater antioxidant activity.

Conclusion

The chemical composition of extracts from *F. pinicola* fruit bodies collected on the same territory at the same time, growing on pine or birch revealed the same groups of BAS, except for saponins, which were found only in basidiocarps from pine. The analyses of the metabolic profiles by finger-printing method revealed the influence of the substrate on the chemical composition of fungi extracts – more than 70 compounds were found in each sample, and only 30 were common. The total amount of phenolics and antioxidant activity were higher in the fruit bodies, collected from birch. Protocatechuic acid, a powerful antioxidant, prevailed among phenolics in both fungi extracts. Both samples contained terpenes, characteristic for *Ganoderma* (Reishi), but in much smaller quantities. Ganoderiol D predominated among others. High concentration of anti-cancer compound ergosterol peroxide found in both fungi extracts.

Thus, the growing substrate affects the qualitative and quantitative composition of *F. pinicola* fruit bodies. The type of fungal growth substrate can change the profile of the metabolites in them. The data obtained gives the prospects for further study and use of *F. pinicola* in pharmaceuticals. This species is typical for pines, but could be also find on birch trunks in mixed forests in much smaller quantities. More detailed study of *F. pinicola* fruit bodies, growing on a birch is necessary.

Acknowledgements

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, Project No. FEUZ-2021–0014.

References

Bertrams J., Müller M.B., Kunz N. 2013. Phenolic compounds as marker compounds for botanical origin determination of German propolis samples based on TLC and TLC-MS. Journal of Applied Botany and Food Quality 86: 143–153. doi:10.5073/JABFQ.2013.086.020

Bishop K.S. 2020. Characterisation of Extracts and Anti-Cancer Activities of *Fomitopsis pinicola*. Nutrients 12(60), 609. doi:10.3390/nu12030609

Blondeau D., St-Pierre A., Bourdeau N., Bley J., Lajeunesse A., Desgagné-Penix I. 2020. Antimicrobial activity and chemical composition of white birch (*Betula papyrifera* Marshall) bark extracts. MicrobiologyOpen 9, e944. doi:10.1002/mbo3.944

Cör D., Knez Ž., Hrnčič M.K. 2018. Antitumour, Antimicrobial, Antioxidant and Antiacetylcholinesterase Effect of *Ganoderma lucidum* Terpenoids and Polysaccharides: A Review. Molecules 23(3), 649. doi:10.3390/molecules23030649

Dasgupta A., Acharya K. 2019. Mushrooms: an emerging resource for therapeutic terpenoids. 3 Biotech 9(10), 369. doi:10.1007/s13205-019-1906-2

Ermoshin A., Kiseleva I., Nikkonen I., Duan S., Ma C. 2022. Chemical composition and antioxidant activity of two fungi species from the genus *Ganoderma*. AIP Conference Proceedings 2390, 030017. doi:10.1063/5.0069052

Ermoshin A., Sinenko O., Nikkonen I., Novikov

V., Kiseleva I. 2021b. Extracts of xylotrophic fungi reduce toxic effects of cadmium ions in barley. AIP Conference Proceedings 2388, 030006. doi:10.1063/5.0068513

Ermoshin A.A., Kiseleva I.S., Nikkonen I.V., Nsengiumva D.S., Duan S., Ma C., Kurchenko V.P. 2021a. Antioxidant activity and Chemical Composition of Extracts from Fruiting Bodies of Xylotrophic Fungi Growing on Birch. Journal of Siberian Federal University 14(3): 339–353. doi:10.17516/1997-1389-0354

FAO 2012. The Russian Federation Forest sector outlook study to 2030. Food and Agriculture Organization of the United Nations. Rome. 84 p.

Gao J., Sato N., Hattori M., Ma C.-M. 2013. The simultaneous quantification of Ganoderma acids and alcohols using ultra high-performance liquid chromatography–mass spectrometry in dynamic selected reaction monitoring mode. Journal of Pharmaceutical and Biomedical Analysis 74: 246–249. doi:10.1016/j.jpba.2012.11.003

Gao Y., Wang P., Wang Y., Wu L., Wang X., Zhang K., Liu Q. 2017. *In Vitro* and *In Vivo* Activity of *Fomitopsis pinicola* (Sw. Ex Fr.) Karst Chloroform (Fpkc) Extract against S180 Tumor Cells. Cell Physiology and Biochemistry 44: 2042–2056. doi:10.1159/000485944

Ghavidel A., Bak M., Hofmann T., Hosseinpourpia R., Vasilache V., Sandu I. 2021. Comparison of chemical compositions in wood and bark of Persian silk tree (*Albizia julibrissin* Durazz.). Wood Material Science & Engineering 3: 1–12. doi:10.1080/17480272.2021.1953141

Gründemanna C., Reinhardt J.K., Lindequist U. 2020. European medicinal mushrooms: Do they have potential for modern medicine? – An update. Phytomedicine 66, 153131. doi:10.1016/j.phymed.2019.153131

Hamad A., Ates S., Olgun Ç. 2019. Chemical Composition and Antioxidant Properties of Some Industrial Tree Bark Extracts. BioResources 14(3): 5657–5671.

Janardhanan K.K., Ravikumar K.S., Karuppayil S.M. 2020. Medicinal mushroom bioactives: potential sources for anti-cancer drug development. International Journal of Applied Pharmaceutics 12(4): 40–45. http://dx.doi.org/10.22159/ijap.2020.v12s4.40103

Kim M.-Y., Seguin P., Ahn J.-K., Kim J.-J., Chun S.-C., Kim E.-H., Seo S.-H., Kang E.-Y., Kim S.-L., Park Y.-J., Ro H.-M., Chung I.-M. 2008. Phenolic Compound Concentration and Antioxidant Activities of Edible and Medicinal Mushrooms from Korea. Journal Agricultural Food Chemistry 56(16): 7265–7270. https://doi.org/10.1021/jf8008553

Lee S.-I., Kim J.-S., Oh S.-H., Park K.-Y., Lee H.-G., Kim S.-D. 2008. Antihyperglycemic effect of *Fomitopsis pinicola* extracts in streptozotocin-induced diabetic rats. Journal of Medical Foods 11(3): 518–524. doi:10.1089/jmf.2007.0155

Nowacka N., Nowak R., Drozd M., Olech M., Los R., Malm A. 2015. Antibacterial, Antiradical Potential and Phenolic Compounds of Thirty-One Polish Mushrooms. PLoS One 10(10), e0140355. doi:10.1371/journal.pone.0140355

Onar O., Akata I., Celep G.S., Yildirim O. 2016. Antioxidant Activity of Extracts from the Red-Belt Conk Medicinal Mushroom, *Fomitopsis pinicola* (Agaricomycetes), and Its Modulatory Effects on Antioxidant Enzymes. International Journal of Medicinal Mushrooms 18(6): 501–508. doi:10.1615/IntJMedMushrooms.v18.i6.40

Ramadhani F., Girsang E., Florenly F. 2021. The bioactive of *Pinus merkusii* needle and bark extract as antioxidant and antiaging. Jurnal Kimia dan Pendidikan Kimia 6(1): 78–88. doi:10.20961/jkpk.v6i1.45371

Selamoglu Z. 2017. Polyphenolic Compounds in Human Health with Pharmacological Properties. Journal of Traditional Medicine & Clinical Naturopathy 6, e137. doi:10.4172/2573-4555.1000e138

Sevindik M, Akgul H., Akata I., Alli H., Selamoglu Z. 2017. *Fomitopsis pinicola* in healthful dietary approach and their therapeutic potentials. Acta Alimentaria, 46(4): 464–469. doi:10.1556/066.2017.46.4.9

Shaikh J.R, Patil M.K. 2020. Qualitative tests for preliminary phytochemical screening: An

overview. International Journal of Chemical Studies 8(2): 603–608. doi:10.22271/chemi.2020.v8.i2i.8834

Sulkowska-Ziaja K., Muszynska B., Motyl P., Pasko P., Ekiert H. 2012. Phenolic compounds and antioxidant activity in some species of polyporoid mushrooms from Poland. International Journal of Medicinal Mushrooms 14(4): 385–393. doi:10.1615/intjmedmushr.v14.i4.60

Sulkowska-Ziaja K., Szewczyk A., Galanty A., Gdula-Argasińska J., Muszyńska B. 2018. Chemical composition and biological activity of extracts from fruiting bodies and mycelial cultures of *Fomitopsis betulina*. Molecular Biology Reports 45: 2535–2544. doi:10.1007/s11033-018-4420-4

Yen G.-C., Duh P.-D., Chuang D.-Y. 2000. Antioxidant activity of anthraquinones and anthrone. Food Chemistry 70(4): 437–441. doi:10.1016/S0308-8146(00)00108-4

Zhu F., Lu W., Feng W., Song Z., Wang C., Chen X. 2017. Preliminary Investigation on the Chemical Constituents and Antioxidant Activities of Two *Phellinus* Mushrooms Collected in Foshan. International Journal of Organic Chemistry 7(1): 25–33. doi:10.4236/ijoc.2017.71003