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ABSTRACT: This paper presents a review of the realm of medicinal applications of *Inonotus obliquus* raw materials, sterile conks *I. obliquus*, based on the bibliographies of chemical studies of the fungus. The experimental part of the paper is devoted to the presentation of methods of estimating the resource potential of this fungus based on data obtained in the comfort zone of this species. A new form, *I. obliquus* f. *sterilis*, is formally described.

KEY WORDS: medicinal mushrooms, *Inonotus obliquus* medicinal mushroom, Chaga mushroom, antitumor and immune-modulating activity, resource potential, coenological preferences

I. INTRODUCTION

Nearly 130 pharmaceutical activities are revealed in medicinal mushrooms including antitumor, immunomodulating, gene-protective, and antiseptic components.¹ So-called Chaga mushroom, which presents a sterile conk of basidiomycete *Inonotus obliquus* is a well-known source of traditional medicine since Avicenna. However, the pharmacological study of active substances of the fungus was initiated in the middle of the 20th century, when folk medicine claimed medicinal benefits of fungi in cancer research. Currently, the *I. obliquus* is considered as a non-specific drug for gastritis, stomach ulcer, polyposes, and for pre-cancer therapy, which releases “Infusum fungus betulinus” in liquid form, tablet form, and as a complex preparation, “Befunginum,” composed of concentrated fungal extract enriched by 0.175% CoCl₂ and 0.2% CoSO₄. Introducing *I. obliquus*’s important role as an official medicine belongs to Yakimov and Bulatov, who have introduced this fungus in the State Pharmacopoeia of URSS.²

The purpose of the present paper is the generalization of data on the authenticity of the Chaga producer, on the knowledge of its chemical composition to date, on its main clinical effects, and the proposition of approach to the estimation of the resource potential of *I. obliquus* in the comfort geographical zone of the fungus.

II. MATERIALS AND METHODS

The material for experimentation in the paper was birch tree stands growing in the Barysh, Veshkaimsk, and Ulyanovsk forest areas in the Ulyanovsk region of Russia. Within the middle European belt of mixed forests, the area in question is characterized by optimal conditions for birch stand development in forest dynamics.³ The birch species (*Betula* spp.) were elected as a model subject due to the clear preference of this host by *I. obliquus* and the fact that all pharmacological studies of the fungus were based on birch-associated material.

The forests under consideration correspond to *Betuletum parviherbosum* and *Betuletum pteridio- sum* (according to the so-called dominant classification), of IV–V age class and of I–Ia class of bonity scale. The composition of the tree stand was varied
from 6 birch:4 pine to 8 birch:6 pine. The forests studied were in the second stage of anthropogenic digression. The taxation of tree stands was estimated by eye according to Forest Management Instruction. All the sites were found according to standard 56–69–83. An estimation of the stage of forest digression was realized by the transect method.

The revealing of frequency of the fungus was carried out using the method of stretch samples. Taking into consideration a low frequency of the species throughout the forests, we increased the number of trees to check by 10 times and the test’s multiplicity from 6 to 7. As a result, the frequency of I. obliquus in birch forests was expressed through the number of infected trees within 1000 specimens per test.

In the second step, the age of infected trees was estimated using the Pressler drill. All results obtained were statistically elaborated.

III. RESULTS AND DISCUSSION

A. Identity and Affinities of Chaga Producer

A good illustration of the Chaga can be seen in the “Album of the Most Harmful Wood-Parasitizing Fungi” by Myasoedov. The fungus was attributed by this author to Polyporus laevigatus Fr. [the modern name Phellinus laevigatus (P. Karst.) Bourdot et Galzin], although the editor of the album, Prof. Borodin, wrote the following note: “The identification of fungus illustrated as P. laevigatus seems to be rather ambiguous, since it does not correspond to current descriptions. Therefore, an exact identification of the fungus illustrated is highly desirable” (l.c.).

Bondartsev on the basis of hyphal structure considers the Chaga as a sterile form of Fomes igniarius (L.) Fr. [the modern name Phellinus igniarius (L.) Quél.]. Yachevskii gave a similar interpretation of the fungus’ identity.

Vanin formally described this fungus as Fomes igniarius f. sterilis Vanin. In his work he adhered to this position: “…Due to the structure of internal tissue as well as the appearance of produced rot, this form belongs to F. igniarius.” The line of attribution of the Chaga producer to representatives of Phellinus spp. was terminated by Kuznetsova-Zarudnaya, who concluded that the Phellinus affiliation of the fungus was based on cultural studies.

Campbell and Davidson were the first researchers to associate the Chaga conks formation with Poria obliqua [modern name Inonotus obliquus (Pers.) Pilát], the solution of which was supported by Zabel, partly by Bondartsev, and later—by all the taxonomists.

The generic placement of I. obliquus stays stable within the core-Inonotus, despite the attempt to restore the genus Phaeopus J. Schröt. for the species under consideration.

Corfixen described a new species, I. ulmcola, which differs from I. obliquus by huge setae and the absence of sterile conks formation.

Concerning the anatomical nature of produced conks, this formation combines the structure of the granular core type in the medullar zone and a pseudosclerotial plate type in the cortical zone (Fig. 1). The current position of I. obliquus is the family Hymenochaetaceae (Hymenochaetales, Agaricomycetes).


Basidiomes annual, resupinate, growing under bark, 0.3–1 cm thick, and widely effused up to 0.5 or 1 m in diam., in living state coriaceous and hygrophanous, brittle when dry. Margin thinning out, fertile, concolorous with the hymenophore. Hymenophore tubular; tubes one-layered, up to 7 mm long, positively geotropic with formation of lacerate pores; pore surface at first is honey or goldencinnamon, later dark-reddish brown; the pores lacerate, 4–8 per mm, circular in cross section. Context coriaceous, floccose, yellowish-brown, slightly zonate, up to 5 mm thick.

The hyphal system is pseudodimitic. The generative hyphae are 2.5–4 μm in diam., hyaline, simple-septate, regularly branched; pseudoskeletal hyphae 3–8 μm in diam., moderately thick-walled,
golden-yellow to red-brown, with collapsed branches. Setae are 15–30×4.5–7 μm, of hymenial origin, subulate to ventricose, slightly projecting or enclosed in hymenium. Basidia are 15–20×7–10 μm, short-clavate, without a basal clamp. Basidiospores are 9–10×5.5–6.5 μm, ovoid, hyaline to golden-yellow, even, thin-walled, inamyloid, indextrinoid, cyanophilous.

Pathogen mainly on Fagaceae trees, causes a white rot.

*Inonotus obliquus* f. *sterilis* (Vanin) Balandaykin et Zmitr. comb. nova (MB809726) (ut ‘Nikol.’ sensu auct.)—Bas.: *Fomes igniarius* f. *sterilis* Vanin, Forest Phytopathology (Leningrad): 197, 1934 (Fig. 1).

A bark-erupting conk produced by *I. obliquus* is characterized by an outer structure of pseudosclerotal plate type and internal structure of granular core type. The conks vary from 3 to 35 cm in diam., hemispherical, tripartite or of irregular shapes, with cracked black-brown to black surface. The internal part of the conk is yellow-brown, with granules of white mycelium. Hyphae in pseudosclerotal plate are 3–5 μm in diam., but near the cortical structure they have inflated segments up to 8.5 μm wide, brown, densely packed. The hyphae in the granular core are of the same sizes and shapes as in the subiculum of the basidiome.

**B. Review of Chemical Studies of the Chaga Material**

In a monograph by Shashkina et al.\(^2^6\) the chemical composition of *I. obliquus* conks and all pharmacological preparations and their clinical effects recorded during the last 50 years are considered.

Sysoeva et al.\(^2^7\) have carried out complex investigations on the antioxidant properties of the water solution of *I. obliquus* material in comparison to their alcohol tincture. Also, the fractioning of water *I. obliquus* tincture using ethyl acetate was realized, and the methods of intensification of the antioxidant activity of melanin-containing water tinctures were presented. It was shown that during impact on *I. obliquus* water tincture by solutions of hyper-ramified polymers H30 and H40 the splitting of melanin occurred and the antioxidant activity increased 2–4 times. Further works by Sysoeva et al. revealed that the polyphenoloxicarbonic complex of *I. obliquus* contained two to three types of polymer structures of various molecular movability and water *I. obliquus* extracts slightly varied according to a combination.
of compounds such as phenolic substances, carbohydrates, steroid substances, non-saturated fat acids, vitamin K, coenzyme Q, phospholipids, and glycolipids. Ethyl acetate extract of *I. obliquus* contains phenol-carbonic acids, simple phenols, flavonoids, iridoids, and azulenes.

Lee and Lee\(^2^8\) had received sigmoid isotherms in diapason of 20−50°C of water sorption by *I. obliquus* material. It was reliably established that a higher degree of water sorption is characteristic for material characterized by the highest degree of homogenization. These authors, in collaboration with Seog,\(^2^9\) show that frozen exsiccation of fungus material leads to a higher degree of homogenization than hot and vacuum exsiccation.

Kukulyansaya et al.\(^3^0\) showed the difference between *I. obliquus* material obtained in nature and that obtained *in vitro* in chemical structure and biological activity of melanins.

Babitskaya et al.\(^3^1\) had investigated endo- and exomelanins of *I. obliquus* having molecular weight 35−50 kDa. The results showed that endo- and exomelanins are similar in C, N, and H content as well as a quantity of metoxylic, aliphatic, and phenolic groups, but differed by content of carboxylic groups: 0.5% in exomelanins and 1.4% in endomelanins. Also, it was shown that the hydrolysates of *I. obliquus* melanin complexes have amino acids such as lysine, arginine, histidine, aspartate, glutamate, treonine, serine, proline, glycine, alanine, valine, cysteine, methionine, leucine, isoleucine, tyrosine, and phenylalanine. The total of amino acids in the exomelanine complex of *I. obliquus* consisted of 36.40%, whereas in the endomelanine complex it is 32.2%.

The regulatory role of insolation on polyphe- nolic biosynthesis and antioxidant potential of *I. obliquus* was shown by Zheng et al.\(^3^2\) The full spectrum of light had an inhibitory action on biosynthesis and antioxidative potential in contrast to red and blue parts of the spectrum as well as under conditions of darkness.

An interesting result was obtained by Zheng et al.\(^3^3\) who showed that intensification in the production of melanins and triterpenoids by *I. obliquus* was connected to the joint merged cultivation with *Phellinus punctatus*.

Many triterpenoids of *I. obliquus* were identified during the last few decades.\(^3^4\)

Steroid substances produced by *I. obliquus* were studied by Zheng et al.\(^3^5\) It was shown that natural environmental influences (mainly insolation) are capable of suppressing the activity of ergosterol synthesis in comparison with such activity *in vitro*.

The volatile organic compounds of *I. obliquus* were investigated by Ayoub et al.\(^3^6\) These authors established the contents in *I. obliquus* as sesqui- terpenes such as α-santalen, epichlorhydrine−β-santalen, and photosantalol for the first time.

Earlier studies were carried out by Platonova\(^3^7\) concerning *I. obliquus* carbohydrates. Water-soluble polysaccharides of *I. obliquus* were attributed by Platonova to glucosans, because their hydrolysates contain mainly glucose. Further investigations of this author\(^3^8\) showed the minor presence of arabinose and xylose in *I. obliquus* hydrolysates. Mizuno\(^3^9\) describes 21 kinds of polysaccharides in the *I. obliquus* water tincture, including pharmacologically active xylogalactoglucanes. These data correspond with those of Bernier— that arabinosugars and uronic acids are characteristic to polysaccharide hydrolysates of many higher Basidiomycetes.

In conclusion, it is necessary to mention the significance of the results, describing *I. obliquus*’ chemical contents from applying analytical methods. This aspect was discussed by Rhee et al.\(^4^0\) using the example of alkali-soluble β-glucan.

**C. Clinical Effects of the Chaga Material**

The general immunology and cell biology aspects of action of fungal metabolites were reviewed by Moradali et al.\(^4^2\) and Chang and Wasser.\(^1\) These reviews give a framework to the present review, which were divided into the following activities.

**1. Direct Antitumor Activity**

Zhong et al.\(^4^3\) described an effect of growth inhibition of lung carcinoma cells by inotodiol triterpenoid. An anti-cancerogenic effect of *I. obliquus* tinctures was studied also by Burczyk et al.\(^4^4\) Lee
et al., Youn et al., Nomura et al., Chung et al., Nakajima et al., and Park et al. In the cited works, a positive antitumor action of *I. obliquus* extracts was demonstrated in hepatoma, leukemia, colon, and cervical carcinomas. Nakajima et al. show that phenolic compounds of metanolic *I. obliquus* extract demonstrate a target toxicity against several lines of cancer cells and the absence of cytotoxic effects against normal cells. The studies by Nakajima et al. show that within the triterpenoid pool of *I. obliquus* only inotodiol had an inhibitory effect on cancer cell proliferation. Due to demonstrated oncostatic activities of *I. obliquus*, Song et al. proposed the usage of polysaccharide-triterpenoid complexes of *I. obliquus* as a component of heterogeneous inhibitors of cancer cell proliferation. The optimum concentration of these complexes was estimated as 150 mg/mL. The data in question were supported by Won et al. who showed that per-oral doses of polysaccharide complexes of *I. obliquus* suppressed the growth of melanoma.

2. Immune Modulation Activity

Immune-modulation properties of *I. obliquus* within many higher Basidiomycetes were presented by Wasser. These data were confirmed by special clinical research. For example, an immune modulation activity of water tincture of *I. obliquus* was revealed in experiments on hanger cells. Also, Kim et al. have a series of further experiments on immune modulation properties of *I. obliquus* polysaccharides. It was shown that exopolysaccharides have more prominent action in comparison to endopolysaccharides.

3. Anti-mutagenic Activity

The anti-mutagenic effect of chaga tincture was demonstrated on an example of induced mutagenesis of *Salmonella typhimurium*. These data present important results in oncology and therapy, too, because they consider the components of *I. obliquus* as having certain gene-protective properties.

4. Anti-hyperglycemic and Antioxidant Activity

This type of fungal activity was revealed in experiments on mice with alloxane diabetes. It was shown that polysaccharides of *I. obliquus* are capable of reducing glucose, triglycerides, fat acids, and cholesterol levels in blood. Also, experts in histology have suggested a regeneration of pancreatic tissue by polysaccharides found in *I. obliquus*. Mizuno et al. show a certain hypoglycemic action of *I. obliquus* tinctures in combination with an oncostatic one.

The consequent studies on antidiabetic and antioxidant activities of *I. obliquus* demonstrate that (1) polyphenolic complex is the main substance in catching of free radicals and (2) the productivity of polyphenolic biosynthesis is higher in the *in vivo* state.

5. Antimicrobial Activity

Sharikov and co-workers have revealed that undisclosed metabolites of *I. obliquus* demonstrate a clear bactericidal effect on a range of *Mycobacterium smegmatis* and *Francisella tularensis* strains. The bactericidal effects of *I. obliquus* extracts support its successful use in stomatology. Particularly, Galchenko et al. have attained a positive effect in the treatment of the erosion of hard tissues of the tooth by the desiccated *I. obliquus* extract. Zyubr et al. have proposed the mode of production of Chaga-based stomatological phytofilms for the treatment of inflammatory bacterial diseases of the mucous membrane of the mouth cavity. The high levels of regenerative rate can be observed during treatment.

D. Coenological Optimum and Resource Potential of *Inonotus obliquus*

The substrate range of *I. obliquus* includes *Acer platanoides, Alnus glutinosa, A. incana*, and various *Fagus, Quercus, Populus*, and *Betula* species as the main hosts (the *Ulmus*-associated sister taxon was segregated as an independent species,
Inonotus ulmicola, Corfixen"). The fungus mainly infests living trees and causes a chronic heart white-rot, producing sterile conks during a series of vegetation periods and subcortical fruit bodies in final developmental stages. A general geographical pattern of the fungus appears to be Holarctic.

As it was revealed by Konev, the birch-mixed taiga forests of West and East Siberia are characterized by maximum productivity of *I. obliquus*, where the mass of sterile conks reaches 0.5–1 kg/10,000 m².

On the material of Sayanes (Central Siberia) taiga forests, Sinadsky had revealed a positive correlation between Chaga frequency and forest altitude. According to the author, the open one-layered stands represent an optimal forest environment for Chaga distribution.

The birch represents the main host tree of *I. obliquus*. Due to their wide-range adaptation to edaphic and climatic conditions, *Betula* species are widely distributed from subarctic open forests up to the mountain regions of Transcaucasia, Altai, Pamir, throughout both the Eurasian and North American continents. In the forest zone of Eastern Europe, the birch stands are distributed over nearly 25% forest areas, whereas in forest-steppe zones their distribution consists of only 6.6%. The clear and moderately moist conditions are optimal for birch development; however, in southern regions the birch demonstrates a zero-tolerance tendency. Also, the birch successfully survives hard winters and exhibits a certain tolerance for industrial emissions and saline soil conditions.

The Ulyanovsk region of Russia represents a rather good area for quantitative studies of potential sources of Chaga-producing *I. obliquus* because it is located in a central part of Eastern Europe, where nemoral and boreal forests are intermixed and optimal conditions for growth of productive birch stands exist. Two species of *Betula*—*B. pendula* and *B. pubescens*—as well as their hybrids are distributed over this area. Both species can become infested with *Inonotus obliquus* without any preferences. The results of investigations of *I. obliquus* frequency in various structural types of birch stands are given in Table 1.

As can be seen from the data presented, the frequency of *I. obliquus* in various types of birch-containing forests is determined by taxation factors, whereas it would be possible to estimate the power of each factor. For example, an increase in birch yield in a forest is positively correlated (r≈1) to *I. obliquus* frequency, but the increase in stand density is negatively correlated (r=−0.79) to species frequency.

*I. obliquus* is more widely distributed in birch forests of sprout coppice origin than in birch forests of seedling coppice origin (0.43±0.30 findings/1000 trees vs 0.14±0.14 findings/1000 trees, respectively). Similarly, the distribution of *I. obliquus* in one-layered stands is wider than in two-layered stands (0.14±0.14 findings/1000 trees vs 0.29±0.18 findings/1000 trees, respectively).

There is a clear dependence between *I. obliquus* frequency and the age of stand trees: the stands of higher classes are characterized by higher *I. obliquus* infestation. The reason is a sufficiently weaker immune response of such trees on the fungus attack.

The productivity of stand and ground conditions are negatively correlated to Chaga frequency: in rather oligotrophic associations, like *Betula vaccinioso-hylocomiosa*, the frequency of *I. obliquus* sufficiently increases in comparison to herb-rich forest types as well as in order of reaching of the lower (V) growth class.

The degree of the anthropogenic pressing directly influences the distribution of *I. obliquus* within a forest: the frequency of pathogens in the forests on the first digression stage is sufficiently lower than on the fourth digression stage (0.14±0.14 findings/1000 trees vs 0.57±0.20 findings/1000 trees, respectively).

Table 2 shows the quantitative estimation of the impact power of all aforementioned factors on *I. obliquus* frequency, which was made using the ANOVA (analysis of variance) algorithm.

The results obtained show that a high degree of impact on *I. obliquus* distribution is significantly affected by tree age (k=11.25%), followed by the degree of forest recreation pressure (11.11%) and stand composition (10.64%).
The age ranking of infested trees lies between 32 and 89 years, respectively (the median consists of 62.08±4.45 years; \( s=15.84, n=12 \)). The localization of sterile conks on birch stems lies between 0.8–8.1 m (the median consists 4.28±0.52 m; \( s=3.14 \text{ m}; n=37 \)).

Within 37 *I. obliquus* conks, 8 were oriented on the Southeast (21.62%), 6 (16.22%) on the North,
5 (13.51%) on the South, 5 (13.51%) on the Northeast, 4 (10.81%) on the Southwest, 4 (10.81%) on the West, 3 (8.11%) on the East, and 2 (5.41%) on the Northwest.

Obviously these results reflect the distribution of the fungus by air spore flows.

Since the southwestern streams predominant the area investigated,5,69,70 the predominant conks’ orientation do not coincide with the fungal dispersal factor. The more sufficient factor is mechanical bark perforations in birch stands.

IV. CONCLUSIONS

The I. obliquus should be considered as an important issue in medicinal mushroom science. The prevalence of polyphenolic composites in its granular core and pseudosclerotal plate (combined into sterile conk, so-called Chaga) indicates its clear antioxidant and gene-protective—i.e., anti-cancer, anti-microbial, and anti-hyperglycemic—activities. The glucan and triterpenoid profile of the mushroom allows the use of I. obliquus in some cases as a direct antitumor agent. Further investigations of the fertile stage (basidiomes) of this fungus are necessary for precise knowledge on its polysaccharide profile and action.67

For estimations on I. obliquus as a potential resource, a sufficient circumstance is rather specific distributional profile of the fungus: (1) The sprout coppice stands are richer in fungus in comparison to seedling coppice stands; (2) The older birch stands are richer in fungus in comparison to younger ones; (3) The stands on oligotrophic soil conditions are richer in fungus in comparison to eutrophic ones; (4) The anthropogenic digression of birch stand is positively correlated to fungus frequency. All these factors are connected to birch immune characteristics, including the character of bark perforations. The main productive area potential sources of the fungus are south boreal forests containing Betula pendula and B. pubescens on moderately moist soils which can be reduced to Melico nutantis-Piceetum root type. This forest type is widely distributed in Finland, Belarus, the central part of European Russia, Western and South Siberia, the Russian Far East, and middle Canada.

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