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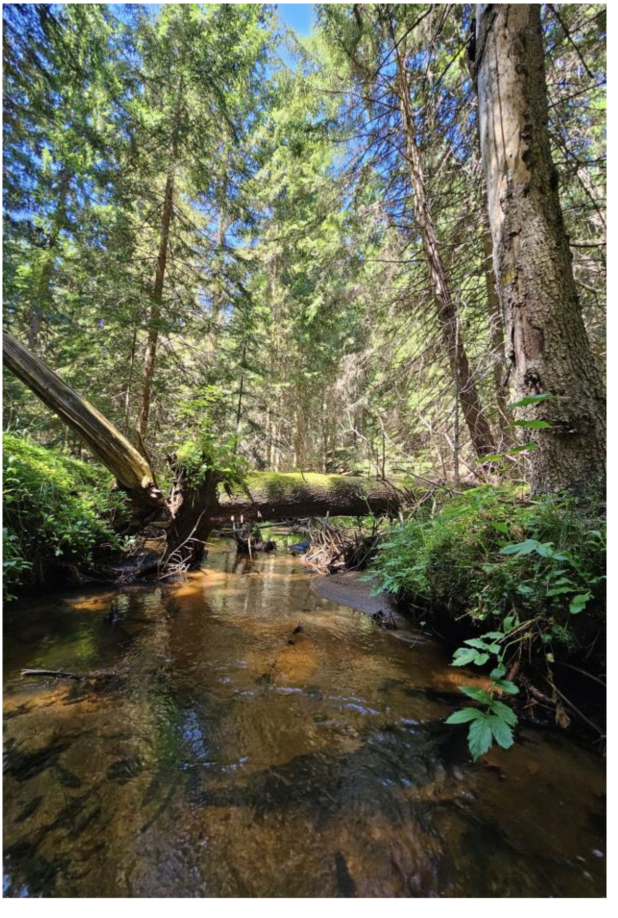

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ANALYSIS OF THE CHEMICAL COMPOSITION OF THREE FUNGAL SPECIES WITH MEDICINAL PROPERTIES TO INVESTIGATE THEIR MEDICAL AND ECOLOGICAL POTENTIAL

Jelena BOŽOVIĆ[1], Zlatan RADULOVIĆ[1], Bojan KONATAR[1], Snežana STAJIĆ[1], Nevena ČULE[1], Radojica PIŽURICA[1], Dragana ŽIVOJINOVIĆ[2]*

Abstract: *This study analyzes the chemical composition of three medicinal fungal species: Fomes fomentarius, Ganoderma lucidum, and Trametes versicolor, with the aim of examining their mineral content, essential elements, trace elements, macroelements, and pH in order to evaluate their medical and ecological potential. The fungi are rich in bioactive compounds, including polysaccharides, triterpenes, and phenolic compounds, which are being studied for their potential therapeutic properties, such as anticancer, anti-inflammatory, and immunomodulatory effects. Additionally, the presence of essential minerals and trace elements, such as calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn), contributes to their nutritional value. The study revealed that the pH values of these fungi vary. Furthermore, the analysis indicated the presence of toxic metals, such as cadmium (Cd), lead (Pb), and mercury (Hg), which may pose health risks if the fungi are consumed or used for medicinal purposes in pharmaceutical preparations. This paper also discusses the potential of these fungifungis within the context of circular bioeconomy and bioremediation, as well as their role in sustainable production and biodiversity conservation. The results suggest that these fungi have a wide range of potential applications, though further research is required to optimize their use in medicine and ecological processes.*

Keywords: medical fungi, chemical composition, medical potentional, ecological potentional.

ANALIZA HEMIJSKOG SASTAVA TRI VRSTE GLJIVA SA LEKOVITIM SVOJSTVIMA U CILJU ISPITIVANJA NJIHOVOG MEDICINSKOG I EKOLOŠKOG POTENCIJALA

Apstrakt: *U ovom radu analiziran je hemijski sastav tri vrste lekovitih gljiva, F. fomentarius, G. lucidum i T. versicolor, s ciljem ispitivanja sastava ovih gljiva u smislu sadržaja minerala, esencijalnih elemenata, elementa u tragovima i makroelemenata kao i pH u svrhu ispitivanja njihovog medicinskog i ekološkog potencijala. Gljive su bogate bioaktivnim komponentama, uključujući polisaharide, triterpene i fenolna jedinjenja, koji se istražuju zbog svojih potencijalnih terapeutskih svojstava, kao što su antikancerogena,*

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antiinflamatorna i imunomodulatorna delovanja. Takođe, prisustvo esencijalnih minerala i elemenata u tragovima poput kalcijuma (Ca), magnezijuma (Mg), gvožđa (Fe) i cinka (Zn) doprinosi njihovim hranljivim svojstvima. Istraživanje je pokazalo da su pH vrednosti ovih gljiva različite. Takođe, analiza je ukazala na prisustvo toksičnih metala, poput kadmijuma (Cd), olova (Pb) i žive (Hg), koji mogu predstavljati rizik za ljudsko zdravlje ako se gljive konzumiraju ili upotrebljavaju u lekovite svrhe za lečenje i pripremu nekih farmaceutskih preparata.. Ovaj rad takođe razmatra potencijal ovih gljiva u kontekstu cirkularne bioekonomije i bioremedijacije, kao i njihovu ulogu u održivoj proizvodnji i zaštiti biodiverziteta. Rezultati pokazuju da ove gljive mogu imati širok spektar primene, ali je potrebno sprovesti dalja istraživanja kako bi se optimizovala njihova primena u medicini i ekološkim procesima.

Ključne reči: lekovite gljive, hemijski sastav, medicinki potencijal, ekoloski potencial.

1. INTRODUCTION

Most lignicolous fungi play a crucial role in the decomposition of organic materials within ecosystems, significantly influencing nutrient cycling. Beyond their ecological role, many fungal species, including *Fomes fomentarius* (L.) Fr., *Ganoderma lucidum* (Fr.) P. Karst., and *Trametes versicolor* (L.) Lloyd, are recognized for their potential therapeutic properties and are frequently used in traditional medicine (Karadžić, D., et al., 2022). These fungi contain bioactive compounds that may contribute to human health, but they also possess a chemical composition with significant ecological implications. Analyzing the chemical composition of these fungi, with particular emphasis on essential minerals and trace elements, provides insight into their potential for human health, as well as their possible impact on ecological systems.

In addition to their health-promoting potential, concerns have been raised regarding the accumulation of heavy metals in fungi, which may pose adverse effects on human health. Given the growing interest in biotherapy and bioremediation, it is crucial to conduct thorough analyses of various fungal species to ensure their safe and effective medical and ecological potential (Nicola, D., 2023).

According to Venturela et al. (2021) many species of fungi contain bioactive substances, such as polysaccharides, triterpenes, and phenolic compounds, which are being studied for their potential antibacterial, antifungal, anti-inflammatory, antioxidative, antiviral, cytotoxic, immunomodulating, antidepressive, antihyperlipidemic, antidiabetic, digestive, hepatoprotective, neuroprotective, nephroprotective, osteoprotective, and hypotensive activities.

Minerals are fundamentally metals and other inorganic substances, present in all body tissues and fluids, and necessary for the maintenance of certain physicochemical processes essential to life (Gupta & Gupta, 2014). Minerals can be classified as either major mi-nerals (that are required in the diet each day in amounts of >100 mg) or trace elements (that are required in the diet each day in amounts of <100 mg). The major minerals include calcium, potassium, sodium, magnesium, and phosphorus, which are present in edible fungi in sufficient quantity. On the other hand, the trace minerals encompass iron, zinc, selenium, copper, manganese, iodine, cobalt, chromium, and molybdenum (Awuchi, 2020).

Furthermore, trace elements such as zinc (Zn), iron (Fe), magnesium (Mg), and calcium (Ca) may contribute to their nutritional value. In terms of their ecological role, fungi can be a significant factor in maintaining ecosystem balance due to their impact on soil structure and pH.

This study examines the chemical composition of *F. fomentarius*, *G. lucidum*, and *T. versicolor*, focusing on beneficial minerals and trace elements, pH values that may influence the environment, and the potential of these fungi in the context of circular bioeconomy and biodiversity.

2. MATERIAL AND METHODS

Fruiting bodies samples (carpophores) were collected in the area of National Park Tara. Species identification was performed based on the appearance of the fruiting bodies (carpophores), type of decay, and the appearance of the obtained pure cultures. To confirm the fungi identification, descriptions of these species provided in the publications of the following authors were used: Breitenbach, J., Kränzlin, F. (1986), Hagara, L. (2014), Jahn, H. (1979), and Karadžić, D., Anđelić, M. (2002).

The fruiting bodies sampling was conducted in accordance with standardized procedures to minimize contamination risk and preserve the integrity of the samples. Three species of fungi were used for analysis: *F. fomentarius*, *G. lucidum*, and *T. versicolor*, collected between September and November 2022, from the aforementioned site, which is minimally exposed to anthropogenic influences, allowing for the analysis of pure natural samples.

The fruiting bodies samples were subjected to microwave digestion using the Ethos Easy device (Milestone) for complete mineralization and preparation for analysis. This method allows for efficient and rapid extraction of minerals from biological samples, enabling precise determination of concentrations of various elements and trace elements.

After digestion, the concentrations of minerals in the samples were analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES Varian Vista pro), a standard method for quantifying metals and other trace elements in complex samples. This technique enables highly accurate determination of concentrations of elements such as aluminium (Al), calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn), and other minerals present in the fungi.

Chemical samples were analyzed following standardized procedures, and the pH value of the fungi was measured using a pH meter (Kacjan et al., 2020). All experiments were performed in triplicate, and the data were statistically processed using cluster analysis to compare mineral concentrations between different fungal species.

3. RESULTS AND DISCUSSION
3.1. Chemical Composition of the fungi

These data provide insight into the nutritional potential of three analysed fungi, which are used in both traditional and modern medicine for their numerous health benefits. The following table presents the laboratory analysis results of the mineral composition of the three fungal species.

Table 1. *Mineral Composition of Three fungal Species*

mg/kg	Al	Ba	Ca	Cd	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	P	Pb	S	Se	Sr
FF	33.37	301.6	4408	0.496	0.474	4.76	42.03	0.045	1831	562.3	4.191	19.372	861	2.473	383.3	1.332	3.198
GL	57.17	121.3	2345	10.56	0.282	4.068	154.3	0.001	2054	638	10.43	60.28	1817	0.42	1197	4.514	0.183
TV	62.03	33.83	3952	0.005	0.087	3.155	122.3	0.001	2660	528.6	16.49	33.16	1457	0.001	633.4	1.617	0.218

FF-*F. fomentarius;GL- G. lucidum; TV-T. versicolor*

Toxins in the Fungi

Although medicinal fungi offer numerous health benefits, caution is advised due to the possibility of accumulating toxic elements, such as heavy metals. Although the concentrations of heavy metals like cadmium (Cd), lead (Pb), and mercury (Hg) in all analyzed species are not high, additional attention is needed when using these fungis in diet or medicine.

The presence of cadmium in Ganoderma lucidum (10.56 mg/kg) and F. fomentarius (0.496 mg/kg) can pose a significant health risk, especially when consumed in large amounts over extended periods. Cadmium is known for its nephrotoxic effects (kidney toxicity) and can lead to long-term health issues such as kidney damage, hypertension, and osteoporosis. Careful dosage and controlled use of these fungi for therapeutic purposes are recommended.

Lead and mercury are also heavy metals that can accumulate in fungi, and their presence in concentrations exceeding permissible limits can lead to neurotoxic effects, nervous system problems, and cardiovascular damage. The use of fungi with high concentrations of these metals can pose a long-term threat, especially to sensitive populations such as children and pregnant women.

Chemical Composition of the Fungi and Its Significance for Their Use

Fomes fomentarius (FF): This fungi is rich in calcium (4408 mg/kg) and potassium (1831 mg/kg), which are beneficial for bone health and electrolyte balance. Additionally, the concentrations of zinc (50.21 mg/kg) and manganese (4.191 mg/kg) confirm its role in the immune system and enzyme activity. However, the presence of cadmium (0.496 mg/kg), although within permissible limits, may pose long-term adverse effects on human health if consumed in excess.

Ganoderma lucidum (GL): While GL is rich in iron (154.3 mg/kg) and phosphorus (1817 mg/kg), which are useful for red blood cell production and energy metabolism, the presence of cadmium (10.56 mg/kg) presents a serious challenge for its safe use. The cadmium content in GL could cause severe toxic effects, particularly for individuals who consume it regularly, so controlling the doses and methods of application for therapeutic purposes is critical.

Trametes versicolor (TV): TV appears to be the most favorable fungi sample in terms of toxic metal concentrations, while being rich in calcium (3952 mg/kg) and potassium (2660 mg/kg), essential for electrolyte balance and bone health. Due to low cadmium and lead concentrations, TV can be considered safer for consumption and potential use in bioremediation.

According to research by El Sheikh, A.F. (2022), G. lucidum contains the highest amounts of potassium (432 mg/100 g), phosphorus (225 mg/100 g), and sulfur (129 mg/100 g), while iron (2.22 mg/100 g) and zinc (0.7 mg/100 g) are present in trace amounts.

According to Hobbs (2005), Schizophyllum commune contains phosphorus (408 mg/100 g), magnesium (277 mg/100 g), calcium (188 mg/100 g), iron (12.3 mg/100 g), magnesium (8.8 mg/100 g), zinc (5.7 mg/100 g), copper (0.9 mg/100 g), and chromium (133 μg/100 g).

Ahlawat, O.P., et al. (2016) state that Volvariella bombycina contains vitamin D (107 IU/g), calcium (25.61 mg/kg), phosphorus (4.12 mg/kg), iron (72.5 mg/kg), copper (50.2 mg/kg), zinc (119.95 mg/kg), and magnesium (0.12 mg/kg).

High concentrations of minerals such as calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), and zinc (Zn) in fungi like F. fomentarius and G. lucidum indicate their potential as sources of nutrients that could benefit human health. For example, calcium is critical for bone and dental health, while iron is essential for oxygen transport in the blood, and magnesium plays a role in muscle function and metabolism. Additionally, the presence of minerals like manganese (Mn) and copper (Cu) can contribute to the body's defence against oxidative stress, which is often associated with the prevention of chronic diseases (Agarwal et al., 2021).

3.2. pH Values of the Fungi

In this study, the pH values of the fungi were analyzed, and the results were as follows:

- ***Fomes fomentarius* (FF):** pH 4.63
- ***Ganoderma lucidum* (GL):** pH 4.1
- ***Trametes versicolor* (TV):** pH 5.32

Significant differences between the species are evident: F. fomentarius and G. lucidum exhibit an acidic character, while T. versicolor shows a weaker acidic pH value.

3.3. Cluster Analysis as a Method of Grouping Parameters

Cluster analysis is a statistical method that enables the grouping of samples based on similarities in various chemical parameters, such as the content of polysaccharides, phenolic compounds, and micronutrients in medicinal fungi. This method is useful for identifying patterns in the chemical composition of fungi, which can help in understanding their therapeutic active properties and determining pH values that influence their biological activity. Cluster analysis allows for a detailed examination of the variations in composition between different species and their potential medicinal benefits (Zhou et al., 2020).

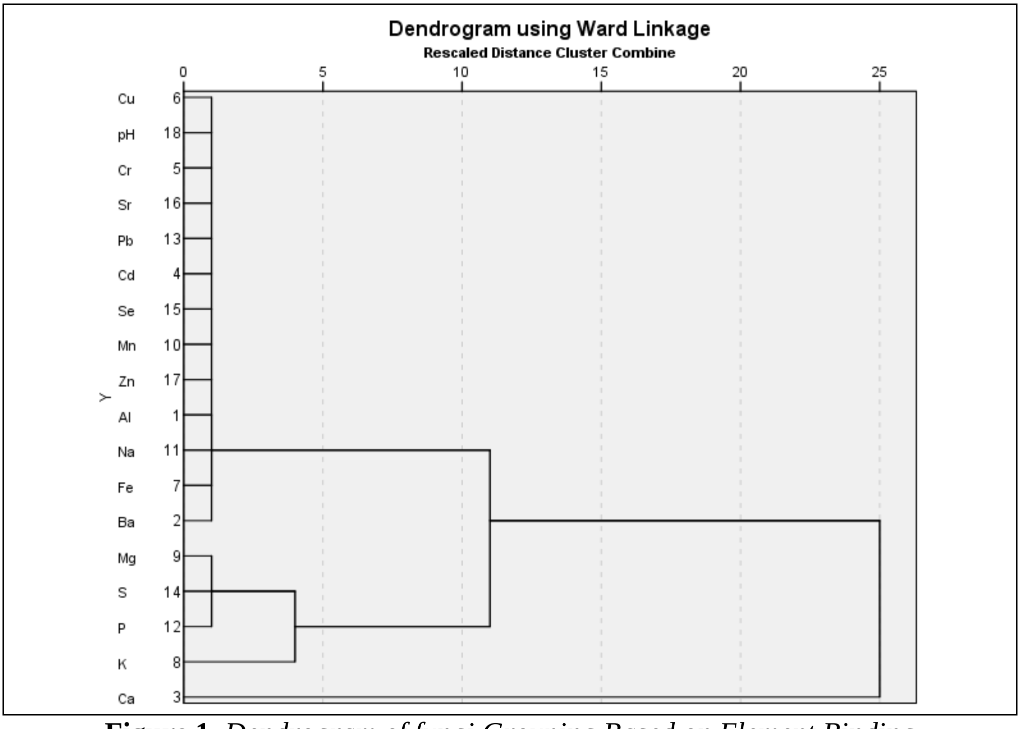

Figure 1. *Dendrogram of fungi Grouping Based on Element Binding*

As shown in Figure 1, different species of fungi are primarily grouped based on the binding of the investigated parameters. In the first cluster, pH value is grouped with most microelements, rather than with macroelements (Ca, Mg, K, P, S), due to the more significant impact of pH on the solubility and presence of microelements, as opposed to macroelements.

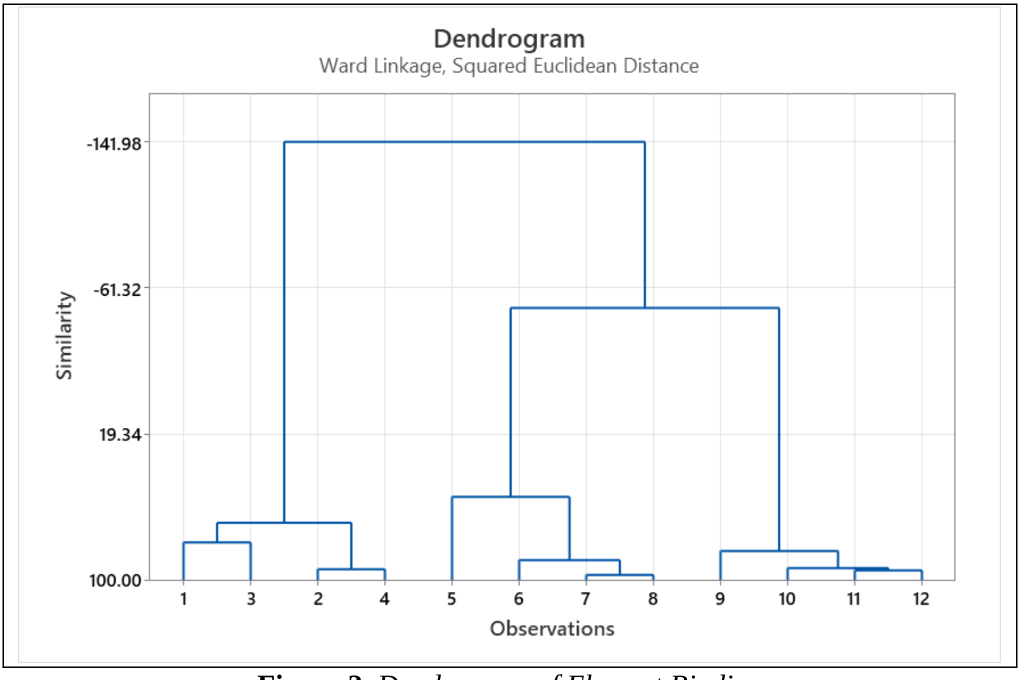

Figure 2. *Dendrogram of Element Binding*

Figure 2 illustrates how the fungi are grouped based on their mineral composition. Primarily, three clusters emerged, each representing a specific species of fungi, i.e. *F. fomentarius*, *G. lucidum*, and *T. versicolor*. Each cluster contains samples of only one species, indicating clear distinctions in their mineral content.

3.4. Circular Bioeconomy and the Role of Fungi in Sustainable Production

Circular bioeconomy promotes the use of biological resources in a way that minimizes waste and supports renewable energy and material sources. Fungi such as *F. fomentarius*, *G. lucidum*, and *T. versicolor* play a key role in this context, as they are utilized in bioremediation, biotechnology, and the pharmaceutical industry (Kavanagh, K., 2018). Due to their ability to break down organic matter, these fungi can aid in waste recycling and soil regeneration, reducing the need for artificial fertilizers and pesticides. Their application in biotechnology, such as the production of bioactive compounds, also contributes to reducing the ecological footprint of industrial production (Zhang et al., 2022).

Based on previous research, all three fungi possess medicinal properties and have potential applications in the pharmaceutical industry. Due to their solid fruiting bodies, they can be used after drying as powdered form, in tincture form (extraction of the fruiting bodies in alcohol), and in the form of an infusion (after pouring boiling water, allowing it to steep for 25 minutes).

According to Karadžić et al. (2022), *F. fomentarius* also exhibits medicinal properties, such as: antibacterial activity; inhibition of the growth of gastric cancer cells SGC-7901 and MKN-45; inhibition and apoptosis of lung cancer cells (A549); antiviral effects (against the H1N1 influenza virus and herpes simplex virus type

HSV-2, strain BH); immunomodulatory effects; antioxidant activity; and it is beneficial for the correction of hyperglycemia and prevention of additional complications caused by diabetes.

In Europe, *G. lucidum* are sold as dietary supplements. This fungi contains vitamins (primarily C, D, E, B1, B2, B6, and B12) and minerals (notably calcium, phosphorus, iron, silver, zinc, manganese, germanium, and barium). The content of natural, organic germanium is ten times higher than that of ginseng (El Sheikha, 2022; Huie et al., 2000). According to Chen (1999), *G. lucidum* is used to treat insomnia, anorexia, dizziness, chronic hepatitis, high cholesterol, coronary diseases, high blood pressure, cancer, and bronchial asthma. It is also used as an antidote for poisoning from certain toxic fungi and for prevention of altitude sickness in mountaineering.

T. versicolor has medicinal properties and is used for: reducing the toxicity of oncology treatments in the therapy of metastatic colorectal cancer, against liver, lung, gastrointestinal, and breast cancers, improving the immune system, and against hepatitis C (Hobs, C., 2004).

3.5. Biodiversity and Ecological Impact

Fungi play an irreplaceable role in maintaining biodiversity. By decomposing organic matter, fungi provide essential nutrients for plant and animal species, helping to maintain ecosystem stability. Lignin, second only to cellulose, is one of the most abundant polymers in nature. The dry mass of wood contains between 25% and 30% lignin. Fungi are rare organisms capable of decomposing lignin. The primary role in lignin degradation is played by fungi that cause white rot.

These fungi, as noted by Mount (1978), can completely decompose lignin, sometimes much faster than polysaccharides. In studying lignin degradation by white rot fungi, Kirk and Shimada (2012) report that the key enzymes involved are oxygenases, phenol oxidases (laccase and peroxidase), cellobiose:quinone oxidoreductases, and β-esterases. The biodegradation of lignin is a strictly anaerobic process that occurs in several stages. In the first stage, methoxy groups are removed, followed by oxidation of the polymer side chains and the breakdown of aromatic rings, with the final stage releasing aliphatic molecules (Schmidth, O.,1994). In addition to white rot fungi, some actinomycetes from the genera *Nocardia* and *Streptomyces*, and ascomycetes from the genus *Xylaria*, are also capable of lignin degradation (Priyanga, U., Kannahi, M., 2018).

Fomes fomentarius, *G. lucidum*, and *T. versicolor* cause white rot in hardwoods, and less frequently in conifer species. *Fomes fomentarius* develops on dead, living trees and continues its destructive process on fallen wood, but only in conditions of high humidity. According to research by Schwarze, F.W.M.R., et al. (2000), the hyphae of *F. fomentarius* primarily spread through the vascular tissue and along the medullary rays. The fungal action begins in the early wood inside the tracheids, where the S3 layer is first decomposed, and degradation proceeds toward the outer wall. The middle lamella remains intact in the initial stages of decay.

5. CONCLUSION

The chemical analysis of three fungal species, *F. fomentarius*, *G. lucidum*, and *T. versicolor*, demonstrates their potential as sources of essential minerals and bioactive compounds beneficial for human use, while also highlighting possible ecological implications, especially in terms of pH values and the presence of heavy metals. Given their bioactive components, these fungi have potential applications in biotechnology processes and the pharmaceutical industry. However, further research is needed to fully understand their ecological impacts and their potential use in sustainable production. All three species cause white rot and decompose lignin, thereby playing a significant role in the cycling of matter in nature. They can be utilized in the prevention and treatment of a wide range of diseases. In forestry, however, only *F. fomentarius* can cause substantial damage.

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ANALYSIS OF THE CHEMICAL COMPOSITION OF THREE FUNGAL SPECIES WITH MEDICINAL PROPERTIES TO INVESTIGATE THEIR MEDICAL AND ECOLOGICAL POTENTIAL

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Summary

The results of the analysis of three fungal species, F. fomentarius, G. lucidum, and T. versicolor, indicate that these fungi contain various bioactive components, such as polysaccharides, triterpenes, and phenolic compounds, which are being researched for their potential anticancer, anti-inflammatory, and immunomodulatory properties. In addition, the presence of minerals such as calcium, magnesium, iron, and zinc suggests that these fungi also possess nutritional properties that contribute to bone health, metabolism, and immune function.

However, the analysis also revealed the presence of toxic metals, including cadmium (Cd), lead (Pb), and mercury (Hg), which may pose a risk to human health, particularly if these fungi are consumed in excessive quantities. For example, cadmium concentrations in G. lucidum and F. fomentarius could lead to severe toxic effects on the kidneys, while the presence of lead and mercury may negatively affect the nervous system and heart. Although the concentrations of toxic metals were not high in the analyzed species, caution is advised in their use.

The pH value analysis showed significant differences among the species. F. fomentarius and G. lucidum have acidic pH (around 4.1-4.6), while T. versicolor exhibits a slightly acidic pH value (5.32).

Cluster analysis was employed to group the fungi based on their chemical composition, revealing patterns in mineral concentrations and pH values among the species. The results indicate significant variation in composition, which could be valuable for further research on therapeutic applications.

From an ecological perspective, these fungi have the potential for use in bioremediation, reducing ecological footprints, and promoting sustainable production in the biotechnology industry. Through the breakdown of organic materials, fungi can contribute to soil regeneration and reduce the need for synthetic fertilizers, aligning with the principles of circular bioeconomy.

Based on these findings, this study confirms the considerable medicinal potential of these fungi, but also underscores the need for further research to ensure the safety of their use and optimize their application in medicine, pharmaceuticals, and bioremediation. Future studies should focus on minimizing the risks posed by toxic elements and gaining a better understanding of their ecological implications.

ANALIZA HEMIJSKOG SASTAVA TRI VRSTE GLJIVA SA LEKOVITIM SVOJSTVIMA U CILJU ISPITIVANJA NJIHOVOG MEDICINSKOG I EKOLOŠKOG POTENCIJALA

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Rezime

Rezultati analize tri vrste gljiva, *F. fomentarius*, *G. lucidum* i *T. versicolor*, ukazuju na to da ove gljive sadrže brojne bioaktivne komponente, poput polisaharida, triterpena i fenolnih jedinjenja, koji se istražuju zbog svojih potencijalnih antikancerogenih, antiinflamatornih i imunomodulatornih svojstava. S obzirom na prisustvo minerala kao što su kalcijum, magnezijum, gvožđe i cink, ove gljive takođe imaju hranljiva svojstva, koja doprinose zdravlju kostiju, metabolizmu i imunološkoj funkciji.

Međutim, analiza je pokazala i prisustvo toksičnih metala, kao što su kadmijum (Cd), olovo (Pb) i živa (Hg), koji mogu predstavljati rizik za ljudsko zdravlje, naročito ako se ove gljive koriste u prekomernim količinama. Na primer, koncentracija kadmijuma u gljivama *G. lucidum* i *F. fomentarius* može izazvati ozbiljne toksične efekte na bubrege, dok prisustvo olova i žive može negativno uticati na nervni sistem i srce. Iako koncentracije toksičnih metala nisu bile visoke u analiziranim vrstama, preporučuje se oprez u njihovoj upotrebi.

Analiza pH vrednosti pokazala je značajne razlike među vrstama. *F. fomentarius* i *G. lucidum* imaju kiseli pH (oko 4.1-4.6), dok *T. versicolor* ima slabije kiselu pH vrednost (5.32).

Klasterska analiza je korišćena za grupisanje gljiva na osnovu njihovog hemijskog sastava, što je omogućilo uočavanje obrazaca u koncentracijama minerala i pH vrednostima među vrstama. Rezultati ukazuju na značajnu varijaciju u sastavu, što može biti korisno u daljim istraživanjima vezanim za terapeutske primene.

U kontekstu ekoloških aspekata, gljive imaju potencijal za upotrebu u bioremedijaciji, smanjenju ekološkog otiska i održivoj proizvodnji u biotehnološkoj industriji. Kroz procese razgradnje organskih materija, gljive mogu doprineti obnovi tla i smanjenju potrebe za veštačkim đubrivima, što je u skladu sa principima cirkularne bioekonomije.

Na osnovu rezultata, ovaj rad potvrđuje veliki medicinski potencijal ovih gljiva, ali i potrebu za daljim istraživanjima kako bi se osigurala sigurnost njihove upotrebe i optimizovala njihova primena u medicini, farmaciji i bioremedijaciji. Dalje studije treba da se fokusiraju na smanjenje rizika od toksičnih elemenata i na bolje razumevanje njihovih ekoloških implikacija.

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