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Quantification of Phenols, Flavonoids, and Sugars in Two Medicinal Fungi: *Fomes fomentarius* (L.) and *Schizophyllum commune* (Fr.)

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Abstract: This study focuses on the chemical analysis of bioactive constituents in the fungi *Fomes fomentarius* (L.) and *Schizophyllum commune* (Fr.), with the aim of identifying and quantifying phenolic compounds, flavonoids, and total sugars as metabolites of significance in medicinal chemistry. Phenols and flavonoids are key factors in cellular protection against oxidative stress, which is crucial for the prevention and therapy of cardiovascular diseases, neurodegenerative disorders, and inflammatory conditions. Furthermore, the presence of sugars (polysaccharides, particularly β -glucans) additionally indicates the immunomodulatory and antitumor effects of these fungi. These chemical components act synergistically in protecting the organism from oxidative and inflammatory damage, thereby confirming their medicinal role in traditional medicine and modern therapies. In this research, fungal extracts were analysed using spectrophotometric methods to determine total phenols and flavonoids (Folin-Ciocalteu for phenols, AlCl_3 and quercetin procedure for flavonoids), as well as the phenol-sulfuric procedure for identifying the presence of sugars (polysaccharides). The analysis confirmed that both fungi contain significant amounts of phenols and flavonoids, demonstrating their potential application in the development of natural drugs and dietary supplements. Future research will focus on the identification of specific molecules of the bioactive components of these fungal species, providing a basis for further research in the field of medicinal chemistry and the pharmaceutical industry.

Keywords: medicinal fungi, phenols, flavonoids, polysaccharides, spectrometry determination

1. Introduction

Medicinal fungi represent a significant group of natural resources with a wide range of bioactive compounds that have potential applications in medicine and pharmacy. *Fomes fomentarius* (F. *fomentarius*) and *Schizophyllum commune* (S. *commune*) are two species of fungi that have been traditionally used in folk medicine due to their anti-

inflammatory, antimicrobial, and antitumor properties. Phenolic compounds and flavonoids are key metabolites contributing to antioxidant activity, while polysaccharides, particularly β -glucans, exhibit immunomodulatory and antitumor effects.

These compounds are recognized as key agents in combating various pathological conditions, including oxidative stress, inflammation, tumors, and cardiovascular diseases. Among numerous species, *F. fomentarius* and *S. commune* draw particular attention due to their wide distribution and historical use.

Phenolic compounds are well-known for their ability to neutralize free radicals, thereby reducing oxidative stress and preventing the onset of chronic diseases. Flavonoids, on the other hand, exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer activities. Polysaccharides, particularly β -glucans, stimulate the immune system and have a direct antitumor effect.

F. fomentarius, commonly known as tinder fungus, is a member of the Polyporaceae family traditionally used for stopping bleeding and as an antiseptic. Modern research confirms that this fungus contains numerous bioactive components, including triterpenoids, polysaccharides, and phenolic compounds, which demonstrate antioxidant, antitumor, and antibacterial properties.

S. commune is a cosmopolitan species of fungus that is highly valued in traditional Asian medicine. Its main bioactive compound is β -glucan, known as schizophyllan, which exhibits strong immunomodulatory and antitumor properties. Additionally, this fungus contains other bioactive components, including phenols and flavonoids, which contribute to its therapeutic potential.

Although the chemical composition of these fungi has been studied globally, data on their chemical profiles depending on geographic origin are often limited. Given that the chemical composition of plants and fungi can vary significantly depending on habitat, climate, and specific environmental conditions, the aim of this study is to analyze the chemical composition of *F. fomentarius* and *S. commune* collected from various locations in Serbia (collected from six different locations in Serbia). Quantification of total phenols, flavonoids, and sugars will provide insight into the potential biological activity of these species and serve as a basis for further pharmacological research.

2. Methodology

2.1 Sample Collection and Preparation

Fungi of *F. fomentarius* and *S. commune* were hand-collected from various geographical locations in Serbia: Crni Vrh, Rtanj Boljevac, Kukavica, Sjenica, Tara, and Golija Javor. After collection, the samples were cleaned, air-dried at room temperature, and ground into a fine powder using coffee grinders. The dry powder was stored in airtight containers until further analysis.

After drying, bioactive substances were extracted using ethanol at room temperature. Approximately 1 g of dried fungi powder was immersed in 10 mL of 96% ethanol for 48 hours at room temperature. Following extraction, the mixture was centrifuged at 4500 rpm for 10 minutes, and the supernatant was collected and filtered through filter paper. The extracts were stored at +4 °C until further analysis.

2.2 Determination of Total Phenols

For quantification, the Folin-Ciocalteu method was used with gallic acid as the standard substance. The total phenolic content in the fungi extracts was determined according to a standard protocol [1]. Folin-Ciocalteu reagent and sodium carbonate solution were added to the extracts. After incubation, absorbance was measured using a spectrophotometer at 765 nm. Quantification was performed based on the calibration curve of gallic acid (GAE), and the results were expressed as mg gallic acid equivalent per gram of dry fungi (mg GAE/g).

2.3 Determination of Total Flavonoids

The total flavonoid content was determined using a colourimetric method with aluminium chloride (AlCl₃), following the procedure described by Chang et al. (2002) [2]. Samples were mixed with aluminium nitrate and sodium acetate solutions. Absorbance was measured at 415 nm. Quantification was performed based on the calibration curve of quercetin (QE), and the results were expressed as µg quercetin equivalent per gram of dry fungi (µg QE/g).

2.4 Determination of Total Sugars

Total sugars were determined by the phenol-sulfuric acid method, according to Dubois et al. (1956) [3]. The samples were mixed with 5% phenol and concentrated sulfuric acid. Absorbance was measured at 490 nm. The results were expressed as a percentage (%) of sugars (percentage by weight of sugar in the dry mass of the sample).

3. Results and Discussion

The results of the determination of total phenols, total flavonoids, and total sugars in the *F. fomentarius* and *S. commune* samples are presented in Table 1.

Table 1. Content of total phenols, flavonoid and sugar in fungi from different locations

Location	<i>Fomes fomentarius</i>			<i>Schizophyllum commune</i>		
	Total phenol (mg GAE/g)	Total flavonoid (µg QE/g)	Total sugar (%)	Total phenol (mg GAE/g)	Total flavonoid (µg QE/g)	Total sugar (%)
Crni vrh	1.85	581.7	1.174	1.09	101.7	2.446
Rtanj Boljevac	0.81	110.2	0.315	0.85	45.2	2.891
Kukavica	0.97	102.6	1.031	0.80	70.6	2.636
Sjenica	2.38	1006.2	1.619	0.33	63.1	2.382
Tara	1.11	330.4	0.999	0.70	21.7	1.985
Golija Javor	0.96	318.2	1.126	0.64	17.9	1.969

This study revealed significant differences in the content of bioactive compounds between the two fungal species, as well as among different locations. The variability in the chemical composition of the fungi reflects the impact of geographical climate, ecological conditions, and anthropogenic factors.

Total phenols in *F. fomentarius* ranged from 0.81 mg GAE/g (Rtanj Boljevac) to 2.38 mg GAE/g (Sjenica), while *S. commune* showed lower values, from 0.33 mg GAE/g (Sjenica) to 1.09 mg GAE/g (Crni Vrh) (Table 1). The higher phenolic content in *F. fomentarius* could be attributed to greater exposure to UV radiation and stress conditions, as phenolic compounds act as natural antioxidants.

Total flavonoids were dominant in *F. fomentarius*, with the highest value recorded at the Sjenica location (1006.2 µg QE/g), while the highest value for *S. commune* was recorded at Crni Vrh (101.7 µg QE/g). Flavonoids in fungi can be considered secondary

metabolites supporting antioxidant functions, so higher concentrations at certain locations suggest their biological adaptation to specific ecological conditions.

Variations in the concentration of phenols and flavonoids in fungi can be due to genetic factors, as well as the influence of habitat, including substrate type, climate, and altitude.

The results clearly show that *S. commune* contains significantly higher concentrations of total sugars compared to *F. fomentarius*. Values for *S. commune* ranged from 1.969% to 2.891%, while for *F. fomentarius* they ranged from 0.315% to 1.619%. This aligns with the well-known fact that *S. commune* contains significant amounts of β -glucans, polysaccharides with recognized immunomodulatory and antitumor effects. The high sugar concentration in *S. commune* confirms its role as a source of therapeutic polysaccharides and opens the way for further research focused on isolating and characterizing specific β -glucans from samples of different locations.

Differences in the biochemical composition of fungi at different locations may reflect environmental conditions, including soil, altitude, climate factors, and biological interactions. Additionally, *F. fomentarius* is generally richer in phenols and flavonoids, while *S. commune* has a higher polysaccharide content, which confirms their complementary roles in pharmacological applications.

4. Conclusions

The analysis of bioactive compounds in *F. fomentarius* and *S. commune* fungus collected from six locations in Serbia confirmed that both species are rich sources of bioactive molecules of pharmacological significance. The study revealed that *F. fomentarius* is generally richer in phenols and flavonoids, with the highest concentration recorded in the sample from the Sjenica location. On the other hand, *S. commune* contains significantly higher concentrations of total sugars (Rtanj Boljevac), confirming its potential as a source of therapeutic polysaccharides.

Variations in the chemical composition between samples from different locations indicate the influence of ecological factors on the biosynthesis of secondary metabolites. This highlights the importance of geographical origin in assessing the quality and biological potential of these biomaterials.

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