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ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF THE METHANOLIC EXTRACT OF THE EDIBLE MUSHROOM *XEROCOMUS SUBTOMENTOSUS*

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Abstract: This study investigates the antimicrobial and antioxidant potential of methanolic extract of mushroom *Xerocomus subtomentosus*. The antimicrobial potential was assessed by microdilution method (MIC values ranged from 5 to 40 mg/mL). Antioxidant activity was evaluated using DPPH, ABTS and reducing power assays, alongside total phenol and flavonoid content measurements. The extract demonstrated strong antioxidant potential (IC_{50}: 80.842 μg/mL for DPPH, 6.587 μg/mL for ABTS, while absorbance for reducing power ranged from 0.380 to 1.168). Total phenolics and flavonoids were 40.439 μg PE/mg and 3.899 μg RE/mg. This study confirms that *X. subtomentosus* is a valuable source of natural antioxidants.

Keywords: mushroom, *Xerocomus subtomentosus*, antimicrobial, antioxidant

Introduction

The use of natural compounds as antimicrobial and antioxidant agents has attracted considerable interest in recent years, especially in medicine, pharmacy, and biotechnology, due to their potential therapeutic benefits. The exploration of the antimicrobial and antioxidant properties of mushrooms has garnered significant attention, reflecting a growing interest in natural bioactive compounds as alternatives to synthetic agents. Oyetayo (2009) highlights the importance of mushrooms in this context, emphasizing their safety and health-promoting properties, which include a range of therapeutic effects such as antioxidant and antimicrobial activities.

Mushrooms, a rich source of bioactive compounds, have been investigated for their potential to serve as natural preservatives. One such mushroom, *Xerocomus subtomentosus* (L.) Quél. 1887 (Boletaceae), commonly known as the

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yellow-cracking bolete, is a wild-growing species traditionally used in various culinary and medicinal applications (Gatto et al., 2011).

Despite the extensive research conducted on edible mushrooms across various countries, only a limited number of studies have investigated the biological properties of this specific species of edible mushroom (Martins et al., 2023). Therefore, the present work aims to evaluate the antimicrobial and antioxidant activity of the methanolic extract of *X. subtomentosus* found in Serbia. The total phenolic and flavonoid contents of the extract were also evaluated, as phenolic compounds are known to contribute to the antimicrobial and antioxidant activities of natural extracts.

Materials and methods

Mushroom samples of *X. subtomentosus* were collected from Gledić mountains, Godačica village (Kraljevo, Serbia), in the summer of 2023. Finely dry ground dried thalli of the examined mushrooms (100 g) were extracted using methanol (500 mL) in a Soxhlet extractor, Quickfit, England. The dry extracts were dissolved in 5% dimethyl sulfoxide (DMSO).

The antimicrobial activity of methanolic extract of *X. subtomentosus* was tested against five bacterial (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*) and ten fungal species (*Aspergillus niger*, *Penicillium italicum*, *Candida albicans*, *Mucor mucedo*, *Trichophyton mentagrophytes*, *Geotrichum candidum*, *Microsporum gypseum*, *Fusarium solani*, *Trichoderma viride*, *Cladosporium cladosporoides*), all obtained from the American Type Culture Collection (ATCC). Bacteria were cultured on Müller-Hinton agar, and suspensions (~10^8 CFU/mL) were prepared in sterile distilled water. Fungi were maintained on potato dextrose and Sabouraud dextrose agar, with spore suspensions (~10^6CFU/mL) standardized spectrophotometrically at 530 nm following NCCLS guidelines.

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method in 96-well plates (Sarker et al., 2007). Resazurin was used to assess bacterial growth, while fungal growth was visually detected. Streptomycin and ketoconazole served as positive controls, while DMSO was the negative control.

The antioxidant activity of the extract was evaluated using DPPH, ABTS, and reducing power assays, along with the determination of total phenolic and flavonoid contents.

DPPH and ABTS radical scavenging assays were performed according to Dorman et al. (2004) and Pontiki et al. (2014), with modifications. Extracts at various concentrations (1000, 500, 250 and 125 μg/mL) were incubated with DPPH or ABTS solutions, and absorbance was measured spectrophotometrically at 517 nm for DPPH and 734 nm for ABTS. Ascorbic acid was used as positive control, while the negative control was DMSO and DPPH or ABTS. The free radical scavenging activity of extracts was evaluated by the following equation (1):

$$\text{DPPH/ABTS scavenging ability (\%)} = \frac{A_0 - A_1}{A_0} \text{ x } 100 \qquad (1)$$

where A_0 is the absorbance of negative control and A_1 is the absorbance of the samples or the standard. IC_{50} (the half-maximal inhibitory concentration) was used to present the radical scavenging activity of extract.

Reducing power assay was assessed following Oyaizu (1986) method by measuring absorbance at 700 nm. A blank sample was prepared with all the reaction agents without extract. Ascorbic acid was u

sed as a positive control.

Total phenolic content was determined using the Folin–Ciocalteu method (Slinkard and Singleton, 1997), with results expressed as μg pyrocatechol equivalents (PE) per mg of dry extract using the formula obtained from the calibration curve of a standard solution of pyrocatechol ($y = 0.0057 x - 0.01646$, $R^2 = 0.9203$).

The total flavonoid content was quantified by the aluminum chloride colorimetric method (Meda et al., 2005) and expressed as μg rutin equivalents (RE) per mg of dry extract, using the formula obtained from the calibration curve of a standard solution of rutin ($y = 0.0296 x + 0.0204$, $R^2 = 0.9992$).

All absorbance measurements were taken on a "Jenway" UK spectrophotometer.

Results and discussion

The results of testing the antimicrobial activity are presented in Table 1. The antimicrobial activity of the methanolic extract of *X. subtomentosus* was evaluated against various bacterial and fungal strains. The minimum inhibitory concentrations (MIC) ranged from 5 to 40 mg/mL, depending on the tested microorganism. Among bacterial species, *B. subtilis* and *K. pneumoniae* exhibited the highest sensitivity to the extract (MIC = 5 mg/mL). In contrast, *E. coli* and *P. mirabilis* showed lower susceptibility. The extract also demonstrated antifungal activity, particularly against *T. mentagrophytes*, *G. candidum*, and *M. gypseum*

(MIC = 10 mg/mL). However, the extract exhibited generally weaker activity against fungal strains compared to bacteria. This could be attributed to the structural complexity of fungal cell walls, which contain chitin and other polysaccharides, making them more resistant to bioactive compounds. The standard antibiotic exhibited significantly lower MIC values, confirming its superior efficacy compared to the mushroom extract.

Table 1. Minimum inhibitory concentration (MIC) of extract of *X. subtomentosus*

Fungal species	*X. subtomentosus* methanol	Antibiotics S - K
Tested organisms	MIC (mg/mL)	
B. subtilis	5	0.016
S. aureus	10	0.031
E. coli	20	0.062
P. mirabilis	20	0.062
K. pneumoniae	5	0.016
A. niger	40	0.078
P. italicum	20	0.156
C. albicans	40	0.039
M. mucedo	40	0.156
T. mentagrophytes	10	0.078
G. candidum	10	0.078
M. gypseum	10	0.039
F. solani	40	0.156
T. viride	40	0.078
C. cladosporioides	20	0.078

Antibiotics: K – ketoconazole, S – streptomycin

Due to the absence of previous reports on the antimicrobial activity of *X. subtomentosus*, this study provides novel insights, highlighting the need for further research on this species.

The DPPH and ABTS radical scavenging activity, reducing power, and the total phenolic and flavonoid content of the examined extract are summarized in Table 2. The methanolic extract of *X. subtomentosus* demonstrated moderate to strong antioxidant activity, with a performance not significantly lower than ascorbic acid, especially in the ABTS assay. In the DPPH assay, it showed a modest IC_{50} value (80.842 μg/mL), indicating superior radical scavenging ability. However, in the ABTS assay, the extract demonstrated notable activity with an IC_{50} value being 6.587 μg/mL. As shown in Table 2, reducing power was concentration-dependent (values of absorbance varied from 0.380 to 1.168). The phenolic and flavonoid content (40.439 μg PE/mg and 3.899 μg RE/mg, respectively) suggests that these compounds contribute to its antioxidant potential.

Table 2. Antioxidative activity of *X. subtomentosus* and total total phenolic and flavonoid contents

Tested extract	DPPH	ABTS	Reducing power			Phenolic content	Flavonoid content
	IC50 (μg/mL)		1000 μg/mL	500 μg/mL	250 μg/mL	(μg PE/mg)	(μg RE/mg)
X. subtomentosus methanol	80.842	6.587	1.168 ± 0.024	0.670 ± 0.015	0.380 ± 0.015	40.439	3.899
Ascorbic acid	6.420	6.373	2.421 ± 0.091	1.936 ± 0.063	1.097 ± 0.037	-	-

Similarly, Martins et al. (2023) reported promising antioxidant activity of *X. subtomentosus*, supporting the findings of this study. Also, Robaszkiewicz et al. (2010) reported strong ABTS activity for *X. subtomentosus*, along with a comparable total phenolic and flavonoid content to that found in our study.

Conclusion

The use of mushrooms presents multiple benefits over synthetic chemical compounds, mainly due to their diverse bioactive properties, which make them valuable in fields such as medicine, nutrition, and environmental sustainability. This study has provided evidence that the methanolic extract of *X. subtomentosus* exhibits moderate antimicrobial and significant antioxidant effects. Due to the widespread availability and abundance of this edible species, it is strongly recommended to include it in one's diet, and in the pharmaceutical industry. Further investigation is necessary to identify the specific active compounds responsible for the bioactive observed in the selected mushroom species.

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