ANTIOXIDANT ACTIVITY OF BIOMASS EXTRACTS OF XYLARIA LONGIPES NITSCHKE STRAINS UNDER SUBMERGED CONDITIONS

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Introductions. Fungi have been reported to exhibit significant antioxidant activities due to the content of such compounds as phenolic acids, phenylpropanoids, flavonoids, lignin, melanin, tannins etc. [1]. For that reason, there is an increasing interest in exploring biological activities, including antioxidants of fungal extracts at different developmental stages. Extensive research has been performed on Basidiomycetes [2,3].

However, there is limited information available on the production of antioxidant compounds by xylariaceous fungi cultivated under different conditions, although considerable evidence that these fungi are producers of a variety of bioactive secondary metabolites exists [4,5]. Therefore, the aim of this study was to assess and compare the antioxidant potential of the mycelia of two strains of *Xylaria longipes* Nitschke in submerged culture. In this study, the antioxidant production of the methanolic, ethanol, and ethyl acetate extracts was determined.

Materials and methods. Strains isolation and cultivation. Fungal strains were isolated from the entostromatal tissue of *X. longipes* fruiting bodies, collected in different regions of Ukraine, and preserved in the *IBK* Mushroom Culture Collection [6,7]. The details of the origin and cultural-morphological characteristics of the obtained mycelial cultures have been described by us previously [8].

Mycelial biomass production for the experiment was carried out under submerged cultivation conditions using glucose-yeast-peptone (GYP) nutrient medium as follows (g/L): glucose 25; peptone 3; yeast extract 3; $MgSO_4$ 0,25; KH_2PO_4 1; K_2HPO_4 1; pH 7.

The inoculum was initially prepared by growing out mycelium of *X. longipes* IBK 2718 and 2726 for 7 days at 25 ± 1 °C on a glucose-yeast-peptone agar medium (GYPA) containing 20 g/l agar-agar. The obtained mycelium was homogenized and inoculated in 250 ml Erlenmeyer flasks (in 6 duplicates), containing 50 ml of GYP medium (10% v/v) and cultivated on a laboratory shaker under the following conditions: temperature 23 ± 1 °C, agitation speed 120 rpm, for 9 days. The mycelial biomass was harvested by filtration and subjected to solvent extraction as described below.

Extraction. Mycelial biomass of *X. longipes* was extracted by ethyl acetate, ethanol (96%), and methanol. A known weight of the powdered mycelium was extracted using solvents in a ratio of 1:5 (w/v) for 24 hours at room temperature (20±1ºC) thrice. The resulting extracts were centrifuged to remove insoluble impurities and evaporated using a vacuum rotary evaporator (40°C). The residues were dissolved in solvent in ratio 1:1 (w/v), and then stored at 4°C prior to further analysis.

DPPH antioxidant assay Free-radical scavenging activity of obtained extracts were measured by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay [9]. An aliquot of 100 μl of extract was mixed with 2900 μl of DPPH solution (120 μM) in methanol and incubated in darkness at 37 ºC for 30 min. The absorbance was recorded at 517 nm.

Inhibition of free radical by DPPH in percentage (I%) was calculated according to the equation:

$I(\%) = ((A_0 - A_1)/A_0) \times 100$, where A_0 is the absorbance of the control reaction (containing all reagents except the test compound), and A_1 is the absorbance of the test compound. All tests were carried out in triplicate.

Results and discussion. The DPPH method is based on the reduction of 1,1-Diphenyl-2-picryl-hydrazyl in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form. It was established that all extracts from *X. longipes* IBK 2718 and 2726 notably reduced the concentration of DPPH free radical (Table 1). The antioxidant activity was influenced by the solvent used to extract the biomass and the active compounds extracted from the mycelium.

Table 1. DPPH scavenging activities of *X. longipes* biomass extracts, obtained by different solvents.

Strain	DPPH scavenging activities of extracts (%)		
	Ethyl acetate extract	Ethanol extract	Methanol extract
IBK 2718	41,28	52,63	89,5
IBK 2726	51,92	86,82	88,99

Methanol extracts showed almost double the activity of ethyl acetate extracts. The performance of methanol extracts was the highest for both *X. longipes* strains and amounted to 89,5% and 88,99% for IBK 2718 and 2726, respectively. The higher efficacy of the methanol extract could be attributed to the extraction of more components that are capable of donating hydrogen.

In comparison, extracts of *Xylaria* sp. YX-28, an endophyte living on *Ginkgo biloba*, showed noticeably weaker antioxidant activity [9]. The authors indicated 66,29% and 29,66% of radical-scavenging activities of cultivated fruiting bodies extracted with methanol and ethyl acetate, respectively.

Literature data on another endophyte, *X. feejeensis* HMJAU22039, isolated from *Tectona grandis*, indicate that maximum antioxidant activity of DPPH was obtained by methanolic extraction (73.86%), followed by ethyl acetate and chloroform extract with the values of 69,24% and 60,23% [10]. It is worth noting that in both of the mentioned studies, the authors used other cultivation conditions than those of the present study, which also may have influenced the free-radical scavenging activity.

In our study, a considerable difference in reducing power was noted between ethanol extracts of studied strains, which may be a result of strain specificity. Overall, mycelial biomass of *X. longipes* 2726 showed higher antioxidant activity after extraction with ethanol and ethyl acetate, than strain IBK 2718. However, for the last one, the highest value of free radical reduction was recorded among all the extracts we studied.

Conclusions. From the data obtained, it was established that both strains of *X. longipes* possessed reducing capacity, showing promising activity for further application. It was observed that the reducing ability of fungal extracts varied depending on the solvent, which is assumed to result from the different antioxidant compounds (and their concentrations) isolated by types of extraction. Methanol

extracts demonstrated the strongest reducing power, amounting to 89,5% and 88,99% for strains IBK 2718 and 2726, respectively. Compared to the literature data for other representatives of the genus *Xylaria* cultivated under different conditions, these values appear to be significant. Overall, *X. longipes* strains IBK 2718 and 2726 proved to be promising producers of natural antioxidants, while submerged cultivation was a suitable method for their production.

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