Scientific Bulletin. Series F. Biotechnologies, Vol. XXII, 2018
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

CHARACTERIZATION OF SOME BIOACTIVE COMPOUNDS RELEASED BY *Inonotus obliquus* IN SUBMERGED CULTURE

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Abstract

Inonotus obliquus (chaga) is a birch phytoparasitic mushroom with an irregular shape that represents dark-colored mycelial agglomeration. It has a high pharmaceutical value because of its rich content in bioactive compounds, polysaccharides, phenols, or triterpene (betulin, for example). The purpose of this study was to determine the composition of the fermented medium in different active compounds, while also identifying the antioxidant potential expressed by the fermented medium of I. obliquus mycelium. In a general analysis of the obtained results, the potential of the I. obliquus mycelium to release in the fermented medium compounds with antioxidant potential is primarily due to the medium composition. Particular attention was determined by the presence of other compounds, such as ascorbic acid, which was identified in a reduced number of samples. The results led to the conclusion that fermentation medium with I. obliquus mycelium had the potential to be cultivated via a technological process to obtain valuable compounds.

Key words*: carbon source, chaga mushroom, phenolic compounds, DPPH.*

INTRODUCTION

Inonotus obliquus (chaga mushroom) grows predominantly on birch trees, as the bark is up to 22% rich in betulin (Todd, 2016). Betulin is deficiently absorbed into the human body, even when administered intravenously (Müllauer, 2011). The fermentative process is not frequently used given the lack of optimization (Singh, 2016). The mushroom composition includes the following: complex polysaccharides (which boost immunity in the cardiovascular system and serves as a liver detoxifier) (Robinson, 2018), polyphenols, vitamins (Sanchez, 2017), minerals, and other antioxidant compounds (Vasile, 2017). Betulin is absorbed from bark and biotransformed into betulinic acid (Liu, 2011). This is a functional compound that has antitumor action. It is assumable that such antitumor effects represent a mixture between the action of betulin and the bioactive compounds that exert antioxidant effects, which can reduce the oxidative stress at the cellular level (Krol, 2015).

The purpose of the study was to characterize the bioactive compounds and antioxidant potential expressed by a medium fermented following inoculation with *I. obliquus* mycelium.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were purchased from Sigma Aldrich GmbH (Sternheim, Germany). All other unlabeled chemicals and reagents were of analytical grade.

Mycelium cultivation and fermentation conditions

I. obliquus mycelium was obtained and authenticated by Mihaela Ene, Bucharest, Romania. The corresponding number for the specimen was 17011723IF. The mycelia were revitalized on a medium of malt extract agar. The inoculum was prepared by cultivating the mushroom on a laboratory rotary shaker at 150 rpm, for a minimum of 5 days, at 23ºC, in 250 mL Erlenmeyer flasks with 100 mL of the culture medium containing the following (g%) (Vamanu, 2014): Sample 1 (glucose 1, fructose 4, malt 1, yeast extract 4, starch 1, and lactose 4); Sample 2 (glucose 1, fructose 4, malt 1, yeast extract 4, starch 4, and lactose 1); Sample

3 (glucose 1, fructose 4, malt 4, yeast extract 1, starch 1, and lactose 4); Sample 4 (glucose 1, fructose 4, malt 4, yeast extract 1, starch 4, and lactose 1); Sample 5 (glucose 4, fructose 1, malt 1, yeast extract 4, starch 1, and lactose 4); Sample 6 (glucose 4, fructose 1, malt 1, yeast extract 4, starch 4, and lactose 1); Sample 7 (glucose 4, fructose 1, malt 4, yeast extract 1, starch 1, and lactose 4); and Sample 8 (glucose 4, fructose 1, malt 4, yeast extract 1, starch 4, and lactose 1).

Bioactive compound quantification

Ascorbic acid was determined using Quantofix® test strips. Exopolysaccharides were determined by precipitation with cold absolute ethanol (Sun, 2015). The total soluble phenolic and flavonoid contents were determined using spectrophotometry, according to previously described methods (Vamanu, 2018).

Antioxidant activity quantification

The antioxidant activity of the fermentation broth was determined using spectrophotometry to assess DPPH scavenging activity (Vamanu, 2013) and lipid peroxidation inhibition (Vamanu, 2014).

Statistical analysis

All parameters for antioxidant activity were assessed in triplicate, and the results were expressed as the mean ± standard deviation (SD) of three observations. The mean values and SD were calculated with the Excel program from Microsoft Office 2016 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSIONS

The overall contents of polyphenols showed some significant variations between the eight medium formulas being tested. The maximum polyphenol levels were reached in Sample 1, whereas the minimum amount was reached in Sample 3. The difference between Samples 7 and 8 was 38.77% higher, on average.

The accumulation of flavonoids was inversely proportional to the accumulation of phenolic compounds, which is correlated with the distinct antioxidant profile (Figure 1). Thus, in the samples that had high phenol content, the flavonoids did not exceeded a maximum value of 10 µg/mL quercitin equivalent. The overall share of flavonoids was 15.5%, on average. In Sample 5, for instance, the overall flavonoid content was 75.04% higher.

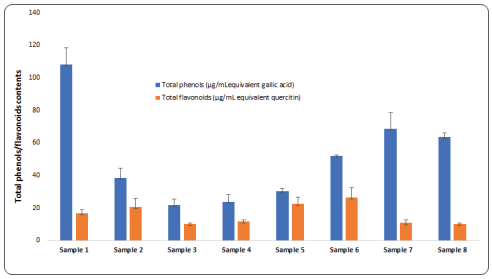

Figure 1. Overall contents of phenols and flavonoids in the fermented medium

This study represents the first assessment of the presence of these bioactive compounds in medium fermented by mycelium *I. obliquus*.

The value of the antioxidant potential (Figure 2) of the medium was determined in relation to the contents of the main bioactive compounds (Figure 1).

The differences noted between the antioxidant activity (specifically, the anti-radical activity) was duly interpreted based upon several distinct phenolic profiles, as determined by the fermented medium formula.

The high accumulation of flavonoids induced antioxidant activity (which was duly expressed under the form of lipid peroxidation inhibition); this effect was inversely proportional to the anti-radical activity (for instance, Sample 4).

However, the lipid peroxidation inhibition did not drop below 50%, which illustrates how valorization of the fermented medium holds great potential for reducing oxidative stress.

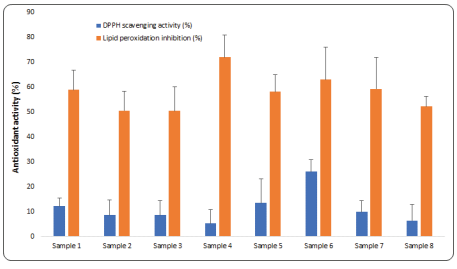

Figure 2. Inhibiting the DPPH radical and lipids peroxidation in the fermented medium

Thus, the value of index R^2 between the overall phenolic contents and anti-radical activity was

-0.499 (Figure 3). Conversely, the flavonoid content showed a very good correlation (R^2 = 0.6169; Figure 4) with the DPPH radical-inhibiting activity. This behavior was duly expressed by an inversely proportional correlation between the overall accumulation of phenols and that of flavonoids, as this was the main method of inducing antioxidant effects.
The correlation between the two methods was high, which showed direct dependency between the composition of the medium and the antioxidant response.
The correspondence between the two activities has proven that the biological value is not directly dependent on the presence of one single biological component.
This is why the polysaccharide ratio is significantly influential; it maintains part of the phenolic ratio, while the response of the samples shall consider this component too. Upon conducting a preliminary analysis, one has noticed that a reduction in the overall phenol content matches this particular fermentative behavior, which was translated by a high level of antioxidant protection (Figure 2).

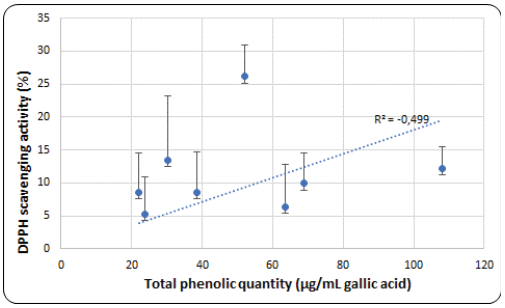

Figure 3. Correlation between scavenging activity and total phenolic quantity

Upon a general review of the results obtained in this study, the fermentative capacity of mycelium *I. obliquus*, in terms of its ability to release antioxidant compounds into the fermented medium, is primarily due to the formula of the medium.
The presence of other compounds, such as ascorbic acid, was also identified in a low number of samples.
This profile was interpreted as an accumulation within the mycelium, which triggered the lowering of the biological response (Moldoveanu, 2015) in the fermented medium.

CONCLUSIONS

The results have led to the conclusion that the medium fermented by the mycelium of *I. obliquus* holds potential as a technical procedure that can facilitate the fast and reproducible production of high-value biological compounds.
Future studies shall examine the division of the phenolic ratio, for instance, in view of identifying the phenolic profile and assessing the metabolic capacity of selecting an optimal substrate for the tested species.

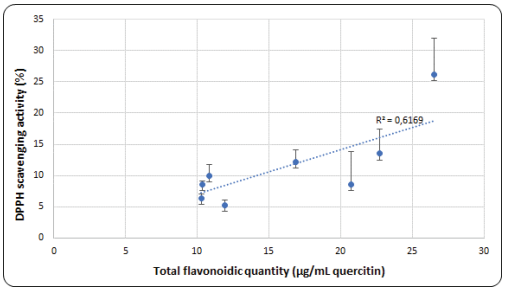

Figure 4. Correlation between scavenging activity and total flavonoid quantity

ACKNOWLEDGEMENTS

This work was supported by the the Executive Agency for Higher Education, Research, Development, and Innovation Funding - Programme 1, Theme 171PED/2017. (http://www.robiomush.ro/biochaga/) English-language editing of this manuscript was provided by Journal Prep.

REFERENCES

Krol S.K., Kiełbus M., Rivero-Müller A., Stepulak A., 2015. Comprehensive review on betulin as a potent anticancer agent. Biomed Research International, 2015, Article ID 584189.

Liu J., Fu M.L., Chen Q.H., 2011. Biotransformation optimization of betulin into betulinic acid production catalysed by cultured armillaria luteo-virens sacc zjuqh100-6 cells. Journal of Applied Microbiology, 110, 1:90-97.

Moldoveanu C., Vasilache V., Risca I.M., 2015. Biological effects of some new imidazole derivatives on spruce (*Picea abies*) germination. Revista de Chimie, 66, 1: 104-108.

Müllauer F., 2011. Betulinic acid induced tumor killing. https://ipfs.io, consulted at 21.01.2018.
Sanchez C., 2017. Reactive oxygen species and antioxidant properties from mushrooms. Synthetic and Systems Biotechnology, 2: 13-22.
Sun M.L., Zhao F., Shi M., Zhang X.Y., Zhou B.C., Zhang Y.Z., Chen X.L., 2015. Characterization and biotechnological potential analysis of a new exopolysaccharide from the arctic marine bacterium *Polaribacter* sp. SM1127. Scientific Report, 5: 18435.
Todd W., 2016. Chaga mushroom: Tinder fungus and pharmacy growing on a tree. https://survivalsherpa.wordpress.com, consulted at 20.01.2018.
Robinson E., Chaga - for powerful anti-aging benefits and a healthy immune system (www.lionheartherbs.com, consulted at 21.01.2018).
Singh V., Haque S, Niwas R., Srivastava, A., Pasupuleti M., Tripathi C.K.M., 2016. Strategies for fermentation medium optimization: An In-depth review. Frontiers in Microbiology, 7, 2087.
Vamanu E., 2013. Antioxidant properties and chemical compositions of various extracts of the edible commercial mushroom, *Pleurotus ostreatus*, in Romanian markets. Revista de Chimie, 64, 1: 49-54.
Vamanu E., 2014. Antioxidant properties of mushroom mycelia obtained by batch cultivation and tocopherol content affected by extraction procedures. Biomed Research International, 2014: 974804.
Vamanu E., Nita S., 2014. Bioactive compounds, antioxidant and anti-inflammatory activities of extracts from *Cantharellus cibarius*. Revista de Chimie, 65, 3: 372-379.
Vamanu E., Pelinescu D., Avram I., 2018. Antioxidative effects of phenolic compounds of mushroom mycelia in simulated regions of the human colon, *in vitro* study. Polish Journal of Food Nutrition and Science, 68,1: 83–90.
Vasile D., Dincă L., Enescu C.M., 2017. Impact of collecting mushrooms from the spontaneous flora on forest ecosystems in Romania. AgroLife Scientific Journal, 6, 1: 268-275.