Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 1 pp. 135–138, 2012
ISSN 0001-6837
Polish Pharmaceutical Society

AN ANTIOXIDANT IN FRUITING BODIES AND IN MYCELIA FROM *IN VITRO* CULTURES OF *CALOCERA VISCOSA* (*BASIDIOMYCOTA*) – PRELIMINARY RESULTS

BOŻENA MUSZYŃSKA*, KATARZYNA SUŁKOWSKA-ZIAJA and HALINA EKIERT

Chair and Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 301-688 Kraków, Poland

Key words: antioxidant, *Calocera viscosa*, β,β-carotene, *in vitro* culture

Fruiting bodies of mushrooms belonging to the taxon *Basidiomycota* are a known source of a variety of biologically active compounds belonging to both primary and secondary metabolites (1, 2). The best known fungal polysaccharides are used for anticancer therapy (3, 4). Recently, Muszyńska (5–7) have hinted at the occurrence of numerous non-hallucinogenic indole compounds in edible, conditionally edible and inedible *Basidiomycota* species. A number of recent mycochemical papers have reported on the presence of many different terpene compounds (8). Studies of Barros (9, 10) indicated antioxidant activity of fruiting bodies extracts from several *Basidiomycota* species and attributed it to tetraterpenes, carotenoids. Under the influence of carotenes occurring mainly in the intestinal wall and the hepatocytes, β-carotene breaks down to vitamin A (retinol). Retinol is a part of the rhodopsin and is responsible for proper vision twilight. It is necessary for the proper functioning of the epithelium, participates in the divisions of the epithelium and protects cells from damage caused by solar radiation and free radicals (11). Quantitative analysis of carotenoids in fruiting bodies of one of the species investigated by that team, *Cantharellus cibarius*, has demonstrated that fungal fruiting bodies can be a valuable rich source of those antioxidants.

The aim of the present study was to initiate *Calocera viscose* culture *in vitro*, determine optimal conditions for mycelia growth and to evaluate the β,β-carotene contents in extracts of fruiting bodies and mycelia from *Calocera viscosa* (Pers.; Fr.) Fr. *in vitro* cultures. *Calocera viscosa* is a species common in Polish, European and Asiatic coniferous forests, which develops intensely yellow-orange branched, bush-like fruiting bodies. This species is a good source of free exo- and endogenous amino acids, indole compounds, sterols (especially ergosterol) and unsaturated fatty acids (12). The carotenoids content in *Calocera viscosa* fruiting bodies growing in Poland was investigated by Czeczuga (13) at the end of the seventies years of the 20th century.

In this study, for the first time we analyzed extracts from *in vitro* cultures that had been established earlier by Muszyńska (12). The cultures were maintained under different conditions to optimize biomass growth and to establish the most beneficial conditions for β,β-carotene accumulation.

EXPERIMENTAL

Origin of fruiting bodies

The studies were conducted on fruiting bodies of *Calocera viscosa* (Pers.; Fr.) Fr. collected at natural sites in mixed and coniferous forests in southern Poland (deposited in the Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Kraków, Poland).

Initial culture

Initial cultures were derived from explants originating from the top parts of branched, bushlike fruiting bodies of *Calocera viscosa*. These pieces of fruiting bodies were sterilized with 70% ethyl alcohol and placed on Petri dishes with solid Oddoux medium (14). Cultures were incubated at a tempera-

* Corresponding author: e-mail: muchon@poczta.fm

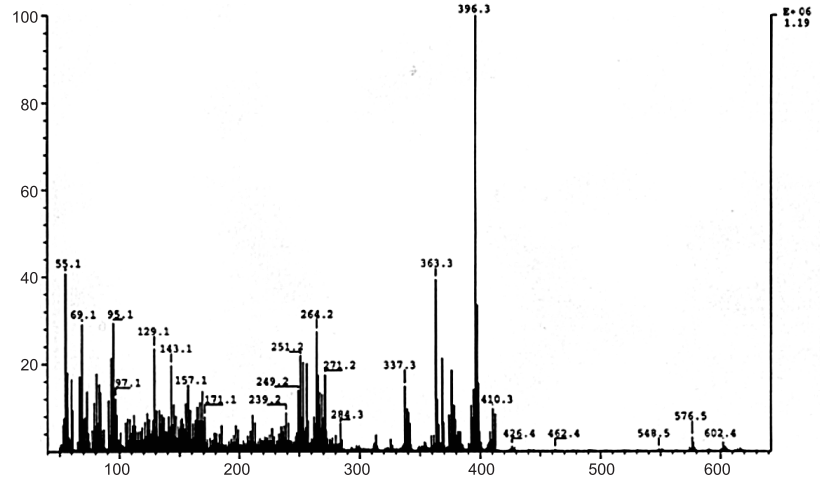

Figure 1. Spectrum EIMS of β,β-carotene from mycelium of *Calocera viscosa* (solid medium)

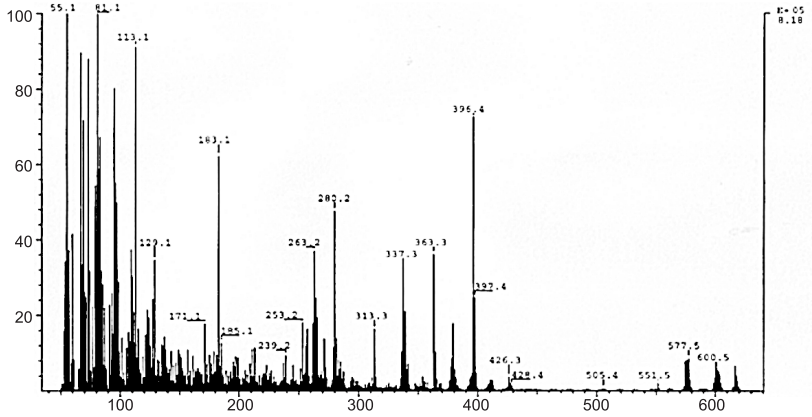

Figure 2. Spectrum EIMS of β,β-carotene from mycelium of *Calocera viscosa* (liquid medium)

ture 25±2°C under 12-h light (900 lx)/12 dark cycle and were subcultured every second week. For more details see Muszyńska (12).

Experimental *in vitro cultures*

Solid culture was maintained on Petri dishes, on Oddoux medium (14). Stationary liquid culture was established from the solid culture by transferring 0.1 g of mycelium into an Erlenmayer flask (500 mL) containing 250 mL of liquid Oddoux medium. Both types of experimental cultures were maintained under the same conditions as initial culture and were subcultured every two weeks.

Extraction

Lyophilized fruiting bodies and mycelia from *Calocera viscosa in vitro* cultures on solid and liquid media were extracted in a percolator with petroleum ether. Extracts were concentrated in a vacuum evaporator.

For the purification of the extracts, we used preparative PTLC method on aluminum silica 60 plates (Merck, Art. No 1.05554.0001), on which 2 mL of the extracts was loaded and chromatograms were developed in mobile faze: petroleum ether : ethyl acetate (20 : 1, v/v). The obtained fractions were concentrated to dry extracts and after dissolv-

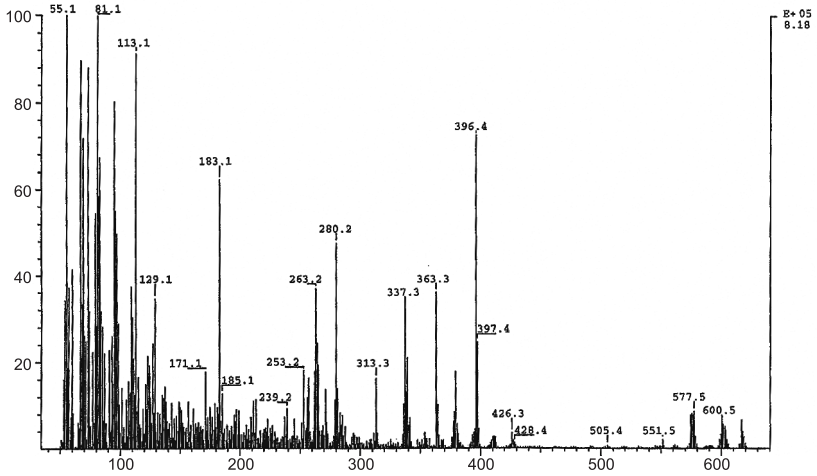

Figure 3. Spectrum EIMS of β,β-carotene from fruiting bodies of *Calocera viscosa*

Table 1. Contents of β-carotene (mg/g d.w.) in wild, commercial mushrooms and in mycelia from cultures *in vitro*.

Species	Author	Contents of β-carotene µg/g d.w.
Agaricus bisporus *Agaricus silvaticus* *Agaricus silvicola* *Boletus edulis* *Calocybe gambosa* *Cantharellus cibarius* *Craterellus cornucopoides* *Marasmius oreades*	Barros, (9, 10)	1.95 ± 0.10 5.42 ± 0.10 3.02 ± 0.12 2.73 ± 0.32 6.41 ± 1.27 13.56 ± 0.51 12.77 ± 0.19 1.99 ± 0.14
Calocera viscosa	Czeczuga, (13)	2.46
Calocera viscosa (fruiting bodies) *Calocera viscosa* (mycelium from liquid cultures *in vitro*) *Calocera viscosa* (mycelium from solid cultures *in vitro*)	Muszyńska (this paper)	7.5 ± 0.15 3.5 ± 0.10 7.1 ± 0.32

ing in chloroform were analyzed by UV-Vis, EIMS and HPLC methods.

UV-VIS analysis of β,β-carotene

Purified by PTLC method extracts of fruiting bodies and mycelia from cultures *in vitro* on solid and liquid medium were analyzed for the presence of β,β-carotene by spectrophotometry using UV-Vis instrument – UV-Vis Cary-Varian Spectrophotometer. Absorption measurements were carried out at λ = 400–500 nm; solvent: chloroform AR; standard: β,β-carotene (Sigma-Aldrich). In all three extracts was found an increase of the growth of absorption maxima at λ_{max} = 415 and λ_{max} = 440 nm characteristic for β,β-carotene.

EIMS analysis of β,β-carotene

Electron Impact Mass Spectrometry Analysis (EIMS) was performed at the Regional Laboratory of Physicochemical Analyses and Structural Research. Apparatus: High Resolution Mass Spectrometer with options: EI, ESI, GC-MS, Finnigan MAT 95S. In the results of these studies, the spectra were obtained for the chloroform extracts from the fruiting bodies and mycelia from cultures on solid and liquid medium. The EIMS spectra for the chloroform extracts from the fruiting bodies and mycelia from *in vitro* cultures on solid and liquid medium containing peaks characteristic for β,β-carotene are shown in Figures 1–3.

Estimation of β,β-carotene by HPLC method

Contents of β,β-carotene in extracts from fruiting bodies and in mycelia maintained in *in vitro* cultures on solid and liquid medium were determined after preliminary separation with PTLC method. Qualitative and quantitive analyses were made by HPLC method according to the procedure described by Britton (15). Briefly, the analytical conditions were as follows:

HPLC apparatus: Merck/Hitachi; pump-L-7100; column-Purospher RP–18 (4 × 200 mm, 5 μm); solvent system: acetonitrile/methanol/water (76:6:1, v/v/v); flow rate 1 mL/min; detector L–7400, λ = 450 nm; standard: β,β-carotene (Sigma-Aldrich).

RESULTS AND DISCUSSION

A 20-fold fresh biomass growth in PTLC, UV Vis, EIMS and HPLC cultures on solid medium and a 15-fold growth in liquid cultures were obtained within a 14-day growth cycle. The identity of β,β-carotene was confirmed on the basis of its parameters PTLC, UV-Vis, EIMS and HPLC methods. The identity of β,β-carotene by EIMS method also was made and described by Lutneas (16). The HPLC method was used for quantitation of β,β-carotene in extracts from fruiting bodies and in mycelia from *in vitro* cultures on solid and liquid medium.

β,β-Carotene content in biomass from solid cultures was comparable with that found in fruiting bodies (7.1 and 7.5 mg/g d.w., respectively). Mycelia from liquid cultures contained half of that β,β-carotene amount which equaled 3.5 mg/g d.w. (Tab. 1).

These contents are higher than those determined by Czeczuga (13), in *Calocera viscosa* fruiting bodies collected in Poland, which were estimated spectrophotometrically as 2.46 mg/g d.w.. Barros demonstrated that of six *Basidiomycota* species collected from natural sites in the northwestern Portugal, *Cantharellus cibarius* (Chantarelle) contained the greatest amounts of β,β-carotene (13.56 mg/g d.w.), whereas the contents of this metabolite in the remaining species ranged from 1.95 to 12.77 mg/g d.w. (9). β,β-Carotene from *Cantharellus cibarius* is used to color eggs and broilers skins. In recent years, there was a systematic decline of this species and their commercial cultures were not obtained till now. So, *Calocera viscose* is the new, potential source of β,β-carotene. The similar contents of β,β-carotene in mycelia from *in vitro* culture on solid medium and fruiting bodies collected from natural conditions indicates that it may be possible to use this culture as a model for studies on the biosynthesis and accumulation of this compound. The contents of β,β-carotene obtained in the present study in *Calocera viscose* mycelium indicate that after optimization of culture conditions to facilitate the antioxidant accumulation, our *in vitro* cultures may become its competitive source in the future. This is the first report of the quantification of β,β-carotene in mycelia of *Calocera viscosa* cultured *in vitro*.

REFERENCES

1. Bernaś E., Jaworska G., Lisiewska Z.: Acta Scientiarum Polonorum Technologia Alimentaria 5, 5 (2006).
2. Pochanavanich P., Suntornsuk W.: Lett. Appl. Microbiol. 35, 17 (2002).
3. Ohstuka S., Ueno S., Yoshikumi C., Hirose F., Ohmura Y., Wada T., Fujii T., Takahashi E.: UK Patent 1331513, 26 September (1973).
4. Sułkowska-Ziaja K., Muszyńska B., Końska G.: Acta Pol. Pharm. Drug Res. 62, 153 (2005).
5. Muszyńska B., Maślanka A., Sułkowska-Ziaja K., Krzek J.: J. Planar Chromat. 20, 55 (2007).
6. Muszyńska B., Ziaja K., Ekiert H.: Pharmazie 64, 479 (2009).
7. Muszyńska B., Sułkowska-Ziaja K., Ekiert H. : Food Chem. 125, 1306 (2011).
8. Solomon P., Wasser S. P., Weis A.: Crit. Rev. Immunol. 19, 65 (1999).
9. Barros L., Cruz T., Baptista P., Esterinho L. M., Ferreira I. C.: Food Chem. Toxicol. 46, 2742 (2008).
10. Barros L., Venturini B.A., Esterinho C.M., Ferreira L.C.: J. Agric. Food Chem. 28, 3856 (2008).
11. Kostowski W., Herman Z.S.: Pharmacology. The principles of pharmacotherapy (Polish). pp. 127, 214, 686, PZWL, Warszawa 2007.
12. Muszyńska B.: Chemical and biological studies of fruiting bodies and mycelium from solid and liquid cultures of *Calocera viscosa* (Pers.: Fr.) Fr. Doctor's thesis, Pharmaceutical Faculty, Jagiellonian University 1999.
13. Czeczuga B.: Acta Mycol. 16, 115 (1980).
14. Oddoux L.: Activité antibiotique des myceliums d'homobasidien culture pure. Lyon (1960).
15. Britton G.: HPLC and Other Chromatographic Methods For Purification and Analysis of Carotenoids. Department of Biochemistry, University of Liverpool (1995).
16. Lutneas B.F., Bruas L., Kildahl-Andersen G., Krane J., Liaaen-Jensen S.: Org. Biomol. Chem. 1, 4046 (2003).

Received: 26. 10. 2010