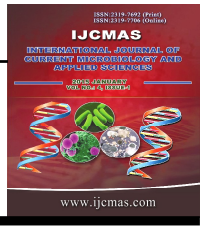
Int.J.Curr.Microbiol.App.Sci (2015) 4(3): 674-683

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 3 (2015) pp. 674-683**
http://www.ijcmas.com



Original Research Article

Investigations on antimicrobial activity in vitro of liquid cultures of *Cantharellus cibarius*

Teodora P. Popova*

University of Forestry, Faculty of Veterinary Medicine, 10 Kl. Ohridski Blvd., Sofia 1756, Bulgaria
**Corresponding author*

A B S T R A C T

The inhibitory effect of pure liquid cultures of the mycorrhizal fungus *Cantharellus cibarius* was tested *in vitro* against 16 pathogenic Gram-positive and Gram-negative bacterial strains, and 4 strains of oval fungi of the genus *Candida*. The classic agar-gel diffusion method and the method of determining the minimum inhibitory concentration (MIC) were used. Significant inhibitory effect of the culture fluids of *C. cibarius* against all tested microbial species was determined. MIC_{50} of the cultures were similar and lower than those of control broad-spectrum antibiotics thiamphenicol and gentamicin. Highest and fastest occurring antimicrobial activity showed the cultures with 2% bee honey - still at 10th day. The cultures with 2% fructose exhibited the highest antimicrobial activity about 24th day. At longer cultivation this effect began to weaken. Liquid cultures of *C. cibarius* can be used to produce effective antimicrobial drugs with broad spectrum activity against bacteria and fungi. This approach would be ecological and inexpensive because of the possibilities for cheaper production of such cultures in high amounts. The use of liquid cultures of mushrooms with such advantageous properties for the production of drugs would be significantly more efficient than use of collected wild mushrooms because of progressively reduced their numbers.

K e y w o r d s

Cantharellus cibarius, liquid cultures, antimicrobial activity

Introduction

Mushrooms are not only sources of nutrients, but also are useful for the prevention and therapy of diseases such as hypertension, hypercholesterolemia and cancer (Ah Kim et al., 2008). Chanterelle (*Cantharellus cibarius* Fr.) from class *Basidiomycetes* of regnum *Fungi* is one of the most appreciated and preferred edible mushrooms due to its specific taste and pleasant aroma.

One of its important properties known and appreciated in Bulgaria is its effects as an aphrodisiac. Some of the compounds produced by fungi already are isolated and identified and show and others quite significant medicinal properties, such as an immunomodulating, anti inflammatory, antioxidant, antiviral and antimicrobial (Valentao et al., 2005; Puttaraju et al., 2006). It has been shown that *Cantharellus*

cibarius contains many beneficial phytochemicals such as carotenoids, tocopherols, ascorbic acid, phenols and others (Barros et al., 2008; Barros et al., 2009). Teichmann et al. (2007) found that these mushrooms contain ergosterol in high concentrations and are better sources of vitamin D2 in comparison with the cultivated mushrooms. Palacios et al. (2011) found in them a significant content of phenols and flavonoids as demonstrated significant antioxidant properties of *C. cibarius*, excelling these in many others valuable edible mushrooms.

The combination of bioactive compounds and rich nutritional composition of this fungus makes it very special food (Barros et al., 2008). Unfortunately, its populations in Europe decrease in recent decades, which is largely due to anthropogenic factors. As it is mycorrhizal, the attempts to isolate the mycelium and creation of technologies for the artificial breeding date back nearly a century (Melin, 1922; Melin, 1925). It is difficult for the cultivation of land for afforestation of trees and mushroom production due to insufficient mycelial colonization of the substrate (Sharma et al., 2011).

The isolation of pure cultures and receiving of mycorrhizoids for afforestation of land is difficult also because of the symbiosis of *C cibarius* with some bacteria (primarily *Pseudomonas fluorescens*), contamination with molds, slow development and insufficient mycelium colonization of the substrate (Darnell, 1994; Sharma et al., 2011). On the other hand the requirements of *C cibarius* for *in vitro* cultivation are not fully explored. For these reasons, we also barely after repeated attempts achieved isolation of a pure cultures of several strains of *C. cibarius* from fresh fruiting bodies. Performed by us experiments to achieve fruitfulness (fruiting bodies formation) in laboratory and greenhouse conditions with the help of cultivation with different substrates, growth factors and growing plants were not successful. In the course of these studies, however, we found some beneficial effects of the liquid cultures of these fungi that can be easily maintained in the laboratory conditions and we continued our studies in this direction. One of these useful properties is the antimicrobial activity of the liquid cultures of *C cibarius*. By reason of ever increasing antibiotic resistance of pathogenic microorganisms in the world, the search for new antimicrobials, including from edible mushrooms, is particularly relevant.

As the population of these especially useful fungi is constantly decreasing, it would be prospective a study of their culture fluids for useful biological properties and their use for the production of prophylactic and therapeutic medicines. That's why the purpose of these studies is testing of liquid cultures of *C cibarius* for antimicrobial activity against various microorganisms *in vitro.*

Materials and Methods

Liquid cultures. Tested was the the effect of pure cultures of *Cantharellus cibarius*, isolated from fresh fruiting bodies of the fungus, collected near Sofia and cultured in liquid medium with a pH of 6.6, prepared with distilled water, ammonia as a nitrogen source and different carbohydrate composition (Table 1). They are cultured under aerobic conditions at a temperature of 22^{o}C for 10-31 days depending on the time of mycelial growth.

Microorganisms: In the experiments were used pure cultures of 16 pathogenic Gram-positive and Gram-negative bacterial strains (by 4 strains of the species *Staphylococcus aureus, Streptococcus pyogenes, Esherichia*

coli and *Pseudomonas aeruginosa*), and 4 strains of the genus *Candida* (3 of *Candida albicans* and 1 of *Candida tropicalis)*. Microorganisms were isolated from animals with different infections, undergone long-term therapy with antimicrobial agents and show *in vitro* resistance to various antibiotics (mainly to streptomycin, penicillin, oxacillin, ampicillin, and some of them to amoxicillin and others).

Controls. In the determination of the antimicrobial activity of the cultures as controls were used the antibiotics thiamphenicol and gentamicin with a wide spectrum of activity, to which the tested bacterial strains did not show resistance.

Tests for antimicrobial activity. Studies were carried out by the classic *agar-diffusion* method of Bauer, Kirby et al. (1966). Microbial suspensions were inoculated at a dose of 2.10^6 cells/ml on Mueller-Hinton's agar with pH 7,2 – 7,4 and 4 mm layer thickness in Petri dishes with diameter 9 mm. Undiluted cultures were administered by instillation by 0,1 ml in 9-mm wells in the agar with a thin agar layer at the bottom. Results were recorded by measuring the diameters of inhibitory zones in mm, including the hole diameter. According the three-tier system of Bauer-Kirby, inhibitory effect of the cultures of *C. cibarius* and gentamicin was established at zones > 12 mm, and > 17 mm for thiamphenicol.

The minimum inhibitory concentrations (MICs) were determined by the method of two-fold serial dilutions on Mueller-Hinton's agar as per Ericsson and Sherris (1971). Calculated were the minimum concentrations of the culture liquids (milliliters liquid per milliliter of culture medium) causing a reduction in the number of colonies of the microorganisms by 50% in comparison with untreated controls (MIC_{50}).

Statistical analysis was performed by the standard method of Student – Fisher.

Result and Discussion

Successful isolation of spawn from fruiting bodies of the edible mushroom *C. cibarius* in pure cultures was performed. Isolates were adapted and cultured in liquid and on solid nutrient media. It was found that under such conditions the growth of mycelium in the medium with honey is best after 10-15 - day culturing, while using fructose - between 22th and 30th day.

Antimicrobial action of liquid cultures of *C. cibarius* against all tested microorganisms was established. The results obtained by the agar-gel diffusion method are shown in Table 2.

As can be seen from the table, at the bacteria tested the largest average diameters of the inhibitory zones are measured at the culture liquid № 4, as are commensurable with those of thiamphenicol (P>0,05) and gentamicin and were significantly higher than those of the culture fluid № 1 and 2 (P <0,01). For *E. coli*, the average diameter of the sterile areas of the liquid № 3 was significantly greater than that of № 1 and 2, P<0,05 and P<0,01 respectively, as well as than that of № 4 and of both control antibiotics. The difference between material № 4 and antibiotics was not significant (P>0,05). The lowest was mean value at № 2, significantly less than those of № 4 (P<0,01) and of gentamicin and thiamphenicol (P<0,001). For *P. aeruginosa* the average diameters of the inhibitory zones of liquids № 1 and 2 were significantly smaller than those of № 3, 4 and of both antibiotics (P<0,05). Differences between materials № 3, 4 and the antibiotics were not significant (P>0,05). For *Candida spp.*

differences between № 3, 4 and thiamphenicol also were not significant (P>0,05). Reliably greater were the average diameters of № 1 and 4 compared with those of № 2 and 3 (P<0,05). The data presented show that the liquid culture on 10 th day (№ 1) exhibited a stronger antimicrobial effect, unlike this on 17 th day (№ 2), at which this effect was reduced reliably. Cultures in a medium with fructose (№ 3 and 4), however, showed a better inhibiting effect than those with honey (№ 1 and 2). Best expressed antimicrobial activity was observed in the one-month culture in medium with fructose (№ 4). In the agar-gel diffusion method best antifungal effect against *Candida* spp. exhibited cultures № 1 and 4.

Table 3 presents MIC_{50} of liquid cultures of *C. cibarius* for the tested microorganisms from different groups. As can be seen from the summarized results, MIC_{50} of the four tested cultures for all used microorganisms were significantly lower than those of thiamphenicol (P<0,001) and not reliably lower than MIC_{50} of gentamicin (P>0,05), which is an indication for high antimicrobial activity of the culture fluids. The significant antibacterial effect of liquid cultures of the fungus better yet is seen from the graphical representation of their MIC_{50} in comparison of these of control antibiotics in Figure 1. The differences of MIC_{50} between the four cultures were not significant (P>0,05). MIC_{50} for thiamphenicol at all tested bacteria were significantly higher than those of gentamicin (P<0,01 and P<0,001). As it is seen from Table 3, the most pronounced was the antimicrobial activity of the 24-day cultures of *C. cibarius* (№3).

In the course of these studies we also found a very good growth of *C. cibarius* added in plates with cultures of the used microorganisms. The growth of the fungus was not inhibited in the presence of a variety of Gram-positive and Gram-negative bacteria such as *S. aureus*, streptococci and *E. coli*, but most strains of *P. aeruginosa* and fungi of the genus *Candida* inhibited the growth of *C. cibarius*.

After two weeks of cultivation at room temperature the mycelium expanded peripherally and grew on a large part of the microbial growth of all used bacterial strains (Figure 2) with the exception of *S. pyogenes* 1, *P. aeruginosa* 1, *P. aeruginosa* 177 and *P. aeruginosa* 357. The growth of mycelium, however, was weak and limited in the presence of *C. albicans* 1 and *C. tropicalis* 324. Very good development of the mycelium was observed and on agar saturated with an antibiotic (gentamicin or thiamphenicol), as well as at addition in the agar of potentiated sulphonamides - Sulfamethoxazole+Trimethoprim.

Results obtained in the cultivation of the isolates showed that the carbohydrate composition of the nutrient medium is important, both for the time of development of the liquid cultures of *C. cibarius*, and for their antimicrobial activity. In broth with honey the growth of the fungus was twice faster and was well pronounced even on the 10th day. These cultures showed and a significant antimicrobial activity, which was manifested most early - even on the 10th day. Cultures with fructose in the nutrient medium, however, showed even better antimicrobial activity, although the culture time was longer – 2 - 4 weeks. Their antimicrobial activity was best expressed 3 weeks after the start of the cultivation (about 24th day).

Regardless of the composition of the culture medium, the antimicrobial activity of liquid cultures was highest still in the emergence and early development of the fungal

mycelium in the logarithmic phase of growth. At continuation of the cultivation, during the stationary phase of growth is probably not achieved accumulation of a larger quantity of antimicrobial substances in the culture, but on the contrary - a small decrease in this activity was observed, probably due to inactivation or metabolization of some of these substances.

With respect to the nitrogen source in the nutrient medium our preliminary studies have shown that the best for *C. cibarius* is ammonia. With its addition in the culture medium an optimal development of the fungus is achieved. These our results are consistent with data of Rangel-Castro et al. (2002), which also found that the most appropriate source of nitrogen for *C. cibarius* is ammonia. According to these authors, one of the reasons for the decline of population of the beneficial mycorrhizal fungi is the increased deposition in the soil of hardly digestible by these fungi nitrogen compounds and the lack of the needed for them inorganic nitrogen.

Except the oval fungi *C. albicans* and *C. tropicalis,* in our researches only *P. aeruginosa* showed inhibitory effect on the growth of the mycelium of the mushroom *in vitro*. This is interesting because it is known that pseudomonads often are isolated from mycorrhizal fungi (Bianciotto et al., 1996). In these studies we also isolated *P. fluorescens* from the starting fruiting bodies of *C. cibarius.*

The antimicrobial activity of some fungi is long known. Several authors found such activity of extracts from fruiting bodies of *C. cibarius*. Cieniecka-Rosłonkiewicz et al. (2007) found a significant effect against bacteria, yeasts and molds, but also high insecticidal activity of such extracts against flies, cockroaches and the like, as well as action similar to the standard pesticides. Interestingly, the bactericidal activity of these extracts against plant pathogenic bacteria had been greater than against human bacterial strains. Santoyo et al. (2009) studied the antimicrobial properties of water and methanol extracts from edible mushroom species including *C. cibarius* against *Escherichia coli, Staphylococcus aureus* and *Candida albicans*. However, the authors emphasize that the oxidative enzymes might degrade potential antimicrobial compounds during the extraction procedure or interfere with their activity.

Tevfik et al. (2011) also established strong antimicrobial effect of methanol extracts from *C. cibarius* and some others wild edible mushrooms against Gram-negative and Gram-positive bacteria and yeasts, especially against *E. coli*. Kosanic et al. (2013) reported that acetone and methanol extracts of the mushrooms including *C. cibarius* have strong antimicrobial activity *in vitro*. They consider that mushrooms may be used as good sources of natural antioxidants and for pharmaceutical purposes in treating of various diseases. However, we believe that more efficient will be the use of liquid cultures of fungi with such advantageous properties for the production of drugs than of fungi collected from the nature because of continuous decline in their quantities.

Of great practical importance is the establishment of the chemical composition of compounds with antimicrobial and other beneficial properties produced by these fungi. Alves et al. (2013) investigated the antimicrobial activity of individual compounds present in extracts from *C. cibarius* and other mushroom species. They found antibacterial action against the majority of Gram-negative and Gram-

positive bacteria of different phenolic compounds in mushrooms, especially of 2,4-dihydroxybenzoic, protocatechuic, vanillic, syringic and *p*-coumaric acids. According to them, phenolic compounds could be used as antimicrobial agents, especially against some microorganisms resistant to commercial antibiotics.

In preparing drugs of fruiting bodies of fungi, however, the maximum extraction of active ingredients is important and requires specific treatment. According to us this is one of the reasons the use of liquid cultures of that fungus for production of antimicrobial drugs to be considerably more efficient and perspective than of extracts of fruit bodies. On the other hand, this approach would be significantly more environmentally friendly as it would protect natural populations of mushrooms from reducing their quantities. The cost of such products from liquid cultures would also be very low due to the possibilities for cheap production of such cultures in enough high quantities.

Mycorrhizal fungi also produce and a number of antioxidants and other compounds valuable for human health. Ribeiro et al. (2009) found in *C. cibarius* high content of beneficial polyunsaturated and monounsaturated fatty acids such as oleic, linoleic, palmitic and stearic. Ebrahimzadeh et al. (2010) found significant levels of phenols and flavonoids in ethyl acetate and methanol extracts of fruiting bodies of chanterelles and pronounced effect against free radicals. According to the authors, these fungi can ensure optimal delivery of antioxidant substances in food. Egwim et al. (2011) also established in *C. cibarius* the presence of varying quantities of alkaloids, flavonoids, saponins, tannins with absence of anthraquinones and steroids. These bioactive compounds with high antioxidant activity of mushrooms may be responsible for their therapeutic applications and also could be valuable in drug development. Some of these useful compounds likely are contained and in liquid cultures of these fungi. We believe that if these be established in them, their manufacturing by liquid cultures of these fungi would be especially promising, inexpensive and environmentally. Since the production of such cultures is cheap and available, in our opinion are necessary studies of liquid cultures of *C. cibarius* for content of useful components and for possibilities for production from these of appropriate dietary supplements and formulations, possibly including the properties of an aphrodisiac, as are inherent in the fruiting bodies of these mushrooms. Further studies are needed for the isolation and characterization of new compounds from liquid cultures of the mushrooms, which are responsible for the antimicrobial activity, as well as for other properties useful to humans.

From the performed researches can draw the following conclusions: The liquid cultures of *C. cibarius* exhibit high antimicrobial activity *in vitro* against multi-resistant strains of Gram-positive and Gram-negative bacteria, and against oval fungi of the genus *Candida*. In nutrient medium with 2% honey the fungus growth is twice as faster and is well developed some more on the 10th day. These cultures exhibit and the most significant antimicrobial effect, which occurs earliest - even on the 10th day. The cultures with 2% fructose exhibit the highest antimicrobial activity about 24th day. The highest is the antimicrobial effect of the liquid cultures in the logarithmic phase of growth of the fungus. With longer cultivation this activity starts to weaken. The presence in the culture medium of antibiotics (thiamphenicol or gentamicin), of

potentiated sulphonamides, and of various Gram-positive and Gram-negative bacteria (*S. aureus*, streptococci, *E. coli*) does not affect negatively the development of *C. cibarius in vitro*. Solely *P. aeruginosa* inhibits the growth of the mycelium of the fungus, as well as *Candida albicans* and *Candida tropicalis*.

Table.1 Cultural characteristics of pure cultures of *C. cibarius* in liquid nutrient mediua

Culture №	Medium (carbohydrate composition)	Duration of cultivation
1	2 % bee honey	10 days
2	2 % bee honey	17 days
3	2 % fructose	24 days
4	2 % fructose	31 days

Table.2 Inhibitory effect of the tested culture liquids of *C. cibarius* on pathogenic bacteria and fungi in the agar-diffusion method

Microorganisms	No of strains tested	Inhibitory zones in mm					
		1	2	3	4	Th	G
S. aureus	4	**14,0**±0,2	**11,5**±0,0	**14,0**±0,4	**16,8**±1,6	**16,3**±2,3	**20,5**±2,7
S. pyogenes	4	**11,0**±0,5	**12,0**±0,3	**13,0**±0,0	**16,2**±1,4	**16,5**±0,9	**20,8**±0,8
E. coli	4	**18,0**±0,2	**10,0**±0,5	**22,0**±0,2	**18,2**±1,9	**20,3**±0,9	**21,8**±1,5
P. aeruginosa	4	**11,0**±0,0	**11,0**±0,3	**15,0**±0,3	**13,8**±1,4	**16,3**±0,9	**21,6**±0,9
Candida spp.	4	**17,0**±0,3	**11,0**±0,0	**12,0**±0,3	**14,5**±1,9	**12,8**±1,3	-
Total bacteria	16	**13,5**±1,6	**11,1**±0,4	**16,0**±2,0	**16,2**±0,9	**17,4**±0,9	**21,2**±0,5
Total all	20	**14,2**±1,5	**11,1**±0,3	**15,2**±1,8	**15,9**±0,8	**16,4**±1,2	**21,2**±0,5

1 – 4 – liquid cultures of *C. cibarius; Th - Thiamphenicol; G - Gentamicin*

Table.3 Minimum inhibitory concentration of culture liquids of four liquid cultures of *C. cibarius* against pathogenic Gram-positive and Gram-negative microorganisms

Microorganisms	No of strains tested	MIC_{50}					
		1	2	3	4	Th	G
S. aureus	4	**0,053** ±0,018	**0,163**±0,038	**0,044** ±0,006	**0,125** ±0,025	**21,3** ±6,2	**0,324**±0,246
S. pyogenes	4	**0,044** ±0,006	**0,020** ±0,00	**0,050** ±0,0	**0,138** ±0,038	**20,0** ±9,9	**0,133**±0,020
E. coli	4	**0,017** ±0,005	**0,094** ±0,039	**0,028** ±0,008	**0,075** ±0,042	**12,0** ±2,3	**0,371**±0,236
P. aeruginosa	4	**0,019** ±0,004	**0,113** ±0,051	**0,017** ±0,011	**0,127** ±0,047	**20,5** ±6,8	**5,125**±2,815
Candida spp.	4	**0,063** ±0,013	**0,200** ±0,00	**0,050** ±0,00	**0,200** ±0,00	**14,0** ±2,0	-
Total bacteria	16	**0,033** ±0,009	**0,098** ±0,029	**0,035** ±0,007	**0,116** ±0,014	**18,4** ±2,2	**1,488**±1,213
Total microorganisms	20	**0,039** ±0,092	**0,154** ±0,022	**0,038** ±0,007	**0,133** ±0,020	**17,6** ±1,9	**1,488** 1,213

MIC_{50} – 50% inhibition; 1 – 4 – liquid cultures of *C. cibarius; Th - Thiamphenicol; G – Gentamicin*

Figure.1 Minimum inhibitory concentration of four liquid cultures of *C. cibarius* (1 – 4) and the control antibiotics Thiamphenicol (Th) and Gentamicin (G) against pathogenic microorganisms

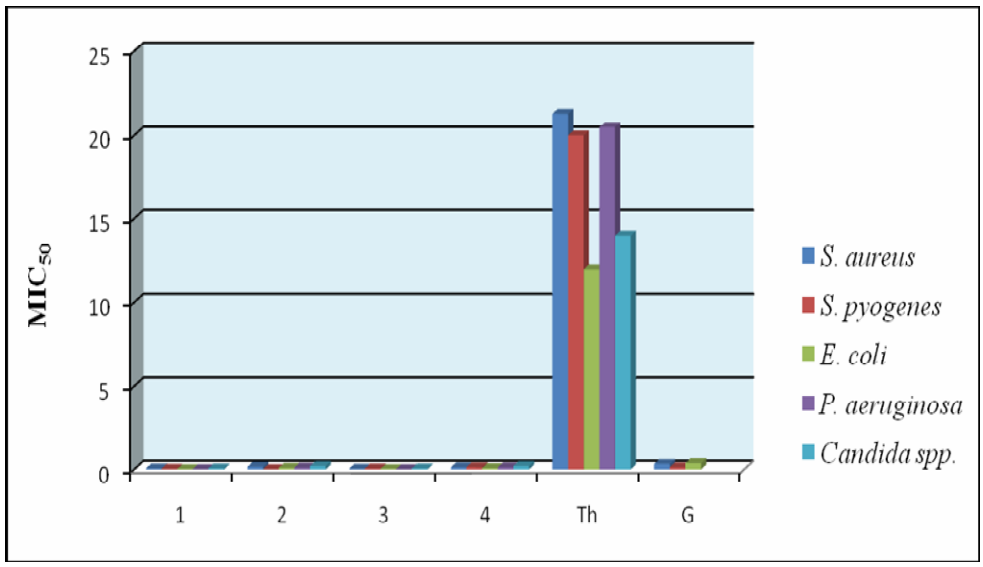


Figure.2 Mycelium of C. cibarius on solid nutrient medium

Acknowledgment

Heartfelt thanks to Valentin Atanasov for support in implementing research.

References

Ah Kim, J., D. Tay and de Blanco, E. C. 2008. NF-κB inhibitory activity of compounds isolated from *Cantharellus cibarius*. Phytotherapy Research. 22 (8): 1104–1106.

Alves, M. J., I. C. F. R. Ferreira, H. J. C. Froufe, R. M. V. Abreu, A. Martins and Pintado, M. 2013. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. Journal of Applied Microbiology. 115 (2): 346–357.

Barros, L., B. A. Venturini , P. Baptista, L. M. Estevinho and Ferreira, I. C. F. R. 2008. Chemical Composition and Biological Properties of Portuguese Wild Mushrooms: A Comprehensive Study. J. Agric. Food Chem. 56 (10): 3856–3862.

Barros, L., M. Dueñas, I. C.F.R. Ferreira, P. Baptista and Santos-Buelga, C. 2009. Phenolic acids determination by HPLC–DAD–ESI/MS in sixteen different Portuguese wild mushrooms species. Food and Chemical Toxicology. 47 (6): 1076–1079.

Bauer, A. W., W. M. Kirby, J. C. Cherris and Truck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. The Am. J. of Clin. Pathol. 45 (4): 493 – 496.

Bianciotto, V., C. Bandi, D. Minerdi, M. Sironi, H. V. Tichy and Bonfante, P. 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl. Environ. Microbiol. 62 (8): 3005-3010.

Cieniecka-Rosłonkiewicz, A., A. Sas, E. Przybysz, B. Morytz, A. Syguda and Pernak, J. 2007. Ionic Liquids for the Production of Insecticidal and Microbicidal Extracts of the Fungus *Cantharellus cibarius*. Chemistry & Biodiversity. 4 (9): 2218–2224

Darnell, E. 1994. Formation and growth of ectomycorrhiza of *Cantharellus cibarius*. Mycorrhizae. 5: 89 – 97.

Ebrahimzadeh, M. A., S. M. Nabavi, S. F. Nabavi and Eslami, S. 2010. Antioxidant and free radical scavenging activities of culinary-medicinal mushrooms, Golden Chanterelle *Cantharellus cibarius* and Angel's Wings *Pleurotus porrigens***.** International Journal of Medicinal Mushrooms. 12 (3): 265-272.
DOI: 10.1615/IntJMedMushr.v12.i3

Egwim, E. C, R. C. Elem and Egwuche, R. U. 2011. Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms. American Journal of Food and Nutrition.
Doi:10.5251/ajfn.2011.1.2.89.94 © 2011, ScienceHuβ, http://www.scihub.org/AJFN.

Ericsson, H. M. and Sherris, J. S. 1971 Antibiotic sensitivity testing. Acta Path. Microb. Scand. Suppl. 217: 3 – 86.

Kosanic, M., B. Rankovic and Dasic, M. 2013. Antioxidant and antimicrobial activity of mushrooms. Bulgarian Journal of Agricultural Science. 19 (5): 1040-1046.

Melin, E. 1922. Untersuchungen fiber die Larix-Mykorrhiza. I. Synthese der Mykorrhiza in Reinkulture. Sven Bot Tidskr. 16: 161-196.

Melin, E. 1925. Untersuchungen über die Bedeutung der Baummykorrhiza. Gustav Fischer, Jena, Germany.

Palacios, I., M. Lozano, C. Moro, M. D'Arrigo, M.A. Rostagno, J.A. Martínez, A. García-Lafuente, E. Guillamón and Villares, A. 2011. Food Chemistry. Antioxidant properties of phenolic compounds occurring in edible mushrooms. 128 (3): 674–678.

Puttaraju, N. G., S. U, Venkateshaiah, S. M. Dharmesh, S. M. Urs and Somasundaram, R. 2006. Antioxidant activity of indigenous edible mushrooms. J. Agric. Food Chem. 54 (26): 9764–9772.

Rangel-Castro, J. I., E. Darnell and Taylor, A. F. S. 2002. Use of different nitrogen sources by the edible

ectomycorrhizal mushroom *Cantharellus cibarius*. Mycorrhizae. 12: 131 – 137.

Ribeiro, B., P. Guedes de Pinho, P. B. Andrade, P. Baptista and Valentão, P. 2009. Fatty acid composition of wild edible mushrooms species: A comparative study. Microchemical Journal. 93 (1): 29–35.

Santoyo, S., A. C. Ramírez-Anguiano, G. Reglero and Soler-Rivas, C. 2009. Improvement of the antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes. International Journal of Food Science & Technology. 44 (5): 1057–1064.

Sharma, R., R. C. Rajak and Pandey, A. K. 2011. Mass Multiplication of Ectomycorrhizal *Cantharellus* Inoculum for Large Scale Tailoring Nursery Inoculations of Bamboo Seedlings. Asian Journal of Scientific Research. 4: 84-89.

Teichmann, A., P. C. Dutta, A. Staffas and Jägerstad, M. 2007. Sterol and vitamin D_2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. LWT - Food Science and Technology. 40 (5): 815–822.

Tevfik, O., D. Cihan, A. Oznur and Ibrahim, T. 2011. Screening of Antioxidant, Antimicrobial Activities and Chemical Contents of Edible Mushrooms Wildly Grown in the Black Sea Region of Turkey. Combinatorial Chemistry & High Throughput Screening. 14 (2): 72-84(13).

Valentao P., P. B. Andrade, J. Rangel, B. Ribeiro, B. M. Silva, P. Baptista and Seabra, R. M. 2005. Effect of the conservation Procedure on the Contents of Phenolic Compunds and Organic Acids in Chanterelle (*Cantharellus cibarius*) Mushroom. Journal of Agricultural and Food Chemistry. 53: 4925–4931.