Antioxidant Activity, Bioactive Compounds and Antimicrobial Effect of Mushrooms Extracts

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Bulletin UASVM Animal Science and Biotechnologies 72(1) / 2015

Print ISSN 1843-5262; Electronic ISSN 1843-536X

DOI:10.15835/buasvmcn-asb:10922

Abstract

Ganoderma applanatum and *Flammulina velutipes* are known for their nutritional value. In the present study was tested their antimicrobial and antioxidant capabilities. Due to the resistance of pathogens to synthetic agents, there is a real need for new natural products with practical utility. Alcoholic extracts of macromycetes' mycelial biomass, obtained in three different culture media were investigated for antimicrobial potential against gram-positive and gram-negative bacteria, namely *Escherichia coli*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas fluorescens*, and *Enterobacter cloacae*. Alcoholic extracts of mycelial biomass obtained in medium prepared with wine yeast had a higher inhibitory activity compared to the other two extracts, which had reduced inhibitory activity. Both antimicrobial and antioxidant capacity was dependent on the growth medium of mycelia biomass. The concentration of phenolic compounds directly contributed to the antioxidant action.

Keywords: *antimicrobial, antioxidant activity, bioactive compounds, mushrooms, DPPH*

INTRODUCTION

Due to the large diversity of pathogenic microorganisms and their acquired resistance to antibiotics, scientists have sought solutions in natural vegetal resources rich in bioactive compounds with antimicrobial properties having a wide area of practical applications, in human and veterinary medicine and nutrition, and plant protection, also (Rodino *et al.*, 2014). Among these vegetal resources, the macromycetes can be an alternative source of new antimicrobials and antioxidants for pharmaceutical use.

Macromycete mushrooms are well known for their nutritional and culinary properties, being used as natural medicines for people of all ages. Besides these qualities, they are also recognized as a source of biologically active compounds with medicinal value, possessing anticancer,

antiviral, hepatoprotective, hypocholesterolemic and immunoprotective properties (Rathee *et al.*, 2012).

There is a great number of references in the literature on some mushrooms such as *Trametes versicolor*, *Ganoderma applanatum* and *G. lucidum*, *Laetiporus sulphureus*, *Flammulina velutipes*, which proved to be an excellent source of natural products with therapeutic properties. These mushrooms can offer a wide variety of bioactive compounds that possess significant antimicrobial and antioxidant activities (Sławińska *et al.*, 2013; Siljegovic *et al.*, 2011). Therefore, mushrooms are rich sources of natural antibiotics and the extracts of the mycelium and fruiting bodies of different species were previously reported to present antimicrobial activity.

In this paper, is presented an overview of the antimicrobial and antioxidant properties of two mushroom extracts with special highlight on some of the bioactive compounds responsible for the antimicrobial properties.

MATERIALS AND METHODS

Macromycete species considered were commercial strains of *Flammulina velutipes* and *Ganoderma applanatum* isolated from natural sources. To obtain mycelial biomass, mycelial discs grown in Petri dishes were inoculated into 3 types of liquid medium (medium prepared according to Hwang *et al.* (2008), medium prepared with extract of sorghum deposit obtained after bioethanol production and medium prepared with yeast wine). After inoculation, the recipients were incubated at 25°C on the rotary shaker at 125 rpm for 10 days. Biomass obtained from each liquid medium was filtered and weighed. Alcoholic extracts were obtained by grinding and sonication of the mycelium biomass in alcohol 70° (ratio biomass : alcohol was 1:10), centrifuged for 15 minutes at 5000 rpm. The supernatant was used for the determination of the antimicrobial activity against *E. coli*, *B. cereus* and *B. subtilis*, *S. aureus*, *E. faecalis*, *P. fluorescens* and *E. cloacae* (belonging to the collection of Biotechnologies department of the National Institute of Research and Development for Biological Sciences and Biotechnologies Faculty, UASVM Bucharest). Antimicrobial activity testing was evaluated using disk diffusion method. The concentration of polyphenolic compounds in the mushrooms extracts, expressed as gallic acid equivalents (GAE) was determined using the Folin Ciocalteu reagent as described by de Oliveira *et al.* (2012) with some modifications, 100 µl extract + 1ml water + 100 µl Folin Ciocalteu. The mixture was added 300 ml of sodium carbonate of 20% concentration.

Antioxidant capacity was determined using DPPH solution 0.1 mmol/L as described in a previous study by Butu *et al.* (2014a), as follows: 0.5 ml of the extract was added over 1 mL of DPPH 0.1 mmol/L (solution prepared daily and protected from light). The mixture was vigorously stirred and incubated at room temperature for 30 minutes, and then the optical density was measured at 515 nm wavelength using methanol as a control. Control sample contained all components mentioned above except hydroalcoholic extract.

Optical density of DPPH in methanol solution has not undergone major changes during the entire analysis. DPPH radical destruction capacity was calculated using the following equation:

$$AA_{DPPH} (\%) = \frac{OD_{control} - OD_{sample}}{OD_{control}} \times 100 \quad (1)$$

where, $OD_{control}$ = the optical density of the control, and OD_{sample} = the optical density of the sample with extract.

RESULTS AND DISCUSSION

Antimicrobial activity. This study shows that basidiomycetes possess antimicrobial activity. It is known that both fruiting bodies and mycelia of several species, such as *Lactarius sp.* (Bergendorf *et al.*, 1988; Anke *et al.*, 1989), *Boletus sp.* and *Cortinarius sp.* (Lee *et al.*, 1999; Nicholas *et al.*, 2001) and *Laetiporus sulphureus* (Fidler *et al.*, 2013) are containing antimicrobial substances and some of the results obtained in the present study are consistent with previous studies reported in literature (Rosa *et al.*, 2003; Suay *et al.*, 2000).

The results of the present research indicated that alcoholic extracts obtained from mycelium biomass, developed on each tested liquid medium, have the ability to inhibit strains of gram-positive and gram-negative bacteria, *Escherichia coli*, *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas fluorescens*, and *Enterobacter cloacae*. The extracts' activity was compared to the negative control one (70° alcohol).

In most cases, the alcoholic extracts of mycelial biomass obtained in medium prepared with wine yeast had a mean inhibitory activity tested compared with extracts of mycelial biomass obtained in other medium, where they had a reduced inhibitory activity or almost inexistent (Tab. 1).

Antioxidant activity. Other previous studies also concluded that mushrooms present powerful antioxidants. For the estimation of antioxidant activity, one of the commonly used mechanisms of inhibition of lipid oxidation is capturing free radicals (Butu *et al.*, 2014b) Radical scavenging activity (RSA) of the mushroom extracts was tested against 1,1-Diphenyl-2-picryl-hydrazyl; (DPPH) which is a stable free radical with a

Tab. 1. Antimicrobial activity of alcoholic extracts

	<i>G. applanatum</i> (Hwang medium)	<i>G. applanatum</i> (sorghum deposit medium)	<i>G. applanatum</i> (wine yeast medium)	<i>F. velutipes</i> (Hwang medium)	<i>F. velutipes</i> (sorghum deposit medium)	<i>F. velutipes</i> (wine yeast medium)
<i>E. coli</i>	+	+	++	-	-	++
<i>S. aureus</i>	-	+	-	-	++	-
<i>E. fecalis</i>	-	-	-	-	-	++
<i>P. fluorescens</i>	-	+	++	-	-	+
<i>E. cloacae</i>	-	++	+	-	+	++
<i>B. cereus</i>	+	++	++	-	-	-
<i>B. subtilis</i>	-	++	++	-	+	++

characteristic sorption at 515 nm. Keles *et al.* (2011) demonstrated that extracts of *F. velutipes* could present antioxidant activity through several mechanisms of free radical scavenging and reducing power, mechanisms that contributed to existence of total phenolic compounds in extracts. The results of the present study indicated DPPH radicals were reduced in all cases, but in different proportions. Comparing the values obtained in evaluating the antioxidant activity was observed that the antioxidant activity of hydroalcoholic extract obtained from mycelial biomass resulting in medium prepared with wine yeast is higher than that prepared with sorghum deposit. The concentration of polyphenols and antioxidant capacity was dependent on the growth medium of mycelia biomass (Tab. 2).

This study suggests that the antioxidant activity of ethanolic extracts from mushrooms is due to phenolic compounds content. In research conducted by Slawinska *et al.* (2013) was reported that both the solvent and the extraction method influence total phenolic content and antioxidant

activity of the extracts. In our case, this was due to the mycelia growth medium composition.

CONCLUSION

Mushrooms present a valuable source of antimicrobial and antioxidant agents, but require further studies to optimize the growth conditions and extraction methods to recover larger amounts of biologically active compounds. In conclusion, the results are promising, alcoholic extracts tested against pathogens had inhibitory effects depending on the culture media used for obtaining the mycelium biomass. The results of this study confirm the potential of macromycetes as an antimicrobial and an antioxidant agent.

Potential therapeutic implications of mushrooms are enormous, but detailed mechanisms of the various health benefits still require investigation. Exploring antioxidant and antimicrobial activity of mushrooms and isolation of active compounds with therapeutic value remains a challenge and still much work is to be done in this area of research.

Tab. 2. The concentration of polyphenols and antioxidant capacity of alcoholic extracts from mushrooms

	<i>G. applanatum</i> (Hwang medium)	<i>G. applanatum</i> (sorghum deposit medium)	<i>G. applanatum</i> (wine yeast medium)	<i>F. velutipes</i> (Hwang medium)	<i>F. velutipes</i> (sorghum deposit medium)	<i>F. velutipes</i> (wine yeast medium)
Antioxidant activity DPPH %	81.466 ± 1.022	69.141 ± 0.623	74.134 ± 0.206	51.552 ± 0.539	41.374 ± 1.297	74.044 ± 0.608
Polifenols mg.GAE /g	68.348 ± 1.525	41.209 ± 0.445	67.331 ± 0.560	33.350 ± 0.711	61.805 ± 0.566	125.121 ± 1.456

Acknowledgements. This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/13.

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