The Balkans Scientific Center of the Russian Academy of Natural Sciences

6th International Scientific Conference

Modern Trends in Agricultural Production, Rural Development and Environmental Protection

P R O C E E D I N G S

Vrnjačka Banja, Serbia

June 27–28, 2024

Modern Trends in Agricultural Production, Rural Development and Environmental Protection

Publisher
The Balkans Scientific Center of the Russian Academy of Natural Sciences, Belgrade

In cooperation
Faculty of Agronomy, Čačak
Fruit Research Institute, Čačak
Institute for Animal Husbandry, Zemun-Belgrade
Faculty of Agriculture, East Sarajevo
Soil Science Institute, Belgrade
Faculty of Hotel Management and Tourism, Vrnjačka Banja
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ISBN
978-86-6042-005-5

Circulation
80 exemplars

Printed by
SaTCIP d.o.o. Vrnjačka Banja

Belgrade, 2024

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DETERMINATION OF GLUCAN CONTENT AND ANTIBACTERIAL ACTIVITY OF CHANTERELLE MUSHROOM EXTRACTS

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ABSTRACT

Mushroom extracts have been found to have significant biological activity, as determined by biological, chemical, and pharmacological tests. The incorporation of mushroom extracts can enhance the quality and nutritional value of food, making it particularly relevant for the development of innovative food and functional products.The aim of this research was to determine the glucan content and antibacterial activity of chanterelle (Cantharelluscibarius) mushroom extracts. Aqueous extract was characterized with higher ($p<0.05$) totalglucan content compared to the ethanolic extract. In accordance, both α- and β-glucan had higher ($p<0.05$) values in the aqueous (2.68%; 24.35%, respectively) compared to theethanolic extract (1.92%; 19.57%, respectively). Moreover, both tested extracts from Cantharelluscibarius showed moderate to good antibacterial activity. It is important to notice that both extracts showed higher antibacterial activity against Enterococcus faecalis (12.7 mm; 12.8 mm), Shigellasonnei (16.6 mm; 15.7 mm) and Pseudomonas aeruginosa (11.3 mm; 10.05 mm) compared to gentamicin or neomycin, as a positive control.Therefore, it can be pointed that this mushroom extracts can be a substitute for some of the synthetic antibiotics used for industrial purposes.This opens up new possibilities for further use of the Cantharelluscibariusextracts in various industries.

Keywords: *Wild mushroom, extraction, antimicrobial potential.*

INTRODUCTION

According to the phyla Ascomycota and Basidiomycota, mushrooms are fungi that produce spongy fruiting bodies, particularly that possess a stalk and an envelope top. Mushrooms are composed of 90% water and 10% dry material. Additionally, it has a physicochemical composition that is important for nutrition.Mushrooms have long been considered to have medicinal value. The early herbalists were more interested in the medicinal properties of mushrooms than in their basic value as a source of food (Chang and Miles, 2004).

Several important compounds including bioactive polysaccharides (lentinan), dietary fiber, ergosterol, vitamins B_1, B_2, C, phenols, flavonoids and minerals have been

isolated from the fruiting body, mycelia, culture medium of the mushrooms, as well as their extracts (Gursoy et al., 2010).

The study of biologically active compounds (phenolic compounds, flavonoids, carbohydrates) that are part of the mushroom or plants composition occupies an increasingly important place in terms of the medical effect these compounds have on consumers' health (Stojanova et al. 2021; Stojanova et al., 2022).

In the process of obtaining bioactive compounds from raw materials, extraction is the first and crucial step. Many of the secondary plant metabolites are expensive or impossible to synthesize in labs, so extraction from natural sources is the best option for their separation and concentration. There are various techniques for extraction, ranging from traditional to modern, each with its own advantages and disadvantages. The extraction technique chosen affects the composition and isolation of the obtained extract as well as the effectiveness of the final product. Therefore, extraction is a vital step in obtaining different preparations (Stojanova et al., 2024).

Among the bioactive compounds in mushrooms, polysaccharides are those that show most antitumoral, antiviral and immunomodulatory activity. In particular, the polysaccharides that are found on the cellular wall are those that show most bioactivity. These polysaccharides are: chitin, cellulose and β-glucans (Mizuno and Nishitani, 2013).

The stimulation of the host's immune defence by bioactive polysaccharides derived from wild mushrooms has a significant effect on the maturation, differentiation and proliferation of many types of immune cells in the host (Wasser, 2011).

Numerous studies have shown that regular consumption of certain mushroom species as either a regular food or as extracted compounds is effective in both preventing and treating specific diseases, mainly through immunopotentiation and antioxidant activity. Thus, the intake of mushrooms and their extractable bioactive compounds appears to be effective in cancer prevention and growth inhibition. Another important fact is the certainty that mushroom extracts, compared with other drugs, show a very low toxicity when regularly consumed, even in high dosages (Reis et al., 2014).

Mushrooms have been recognized as functional foods and as a source for the development of medicines and nutraceuticals (Lindequist et al., 2005; Poucheret et al., 2006). They could also be a source of natural antibiotics. Some mushroom extracts, including *Laetiporussulphureus* (Turkoglu et al., 2007), *Ganodermalucidum* (Gao et al., 2005) and *Lentinusedodes* (Hatvani, 2001) have already demonstrated antibacterial activity.

The golden chanterelle, *Cantharelluscibarius* Fr. (Cantarellaceae), is an edible mushroom, yellow to orange with fruity and mildly peppery taste. It grows in deciduous and coniferous forests, in groups. Previous studies revealed nutrients and nutraceutical composition like phenols including flavonoids and phenolic acids, vitamins, volatile compounds, indols, sterols, and minerals. Studied biological activities were antimicrobial, antioxidative, cytotoxic, and anti-inflammatory (Kolundjzic et al., 2017).

The aim of this research was to determine theglucan content and antibacterial activity of chanterelle (*Cantharelluscibarius*) mushroom extracts.

MATERIALS AND METHODS

In this research, as a work material *Cantharelluscibarius* Fr. mushroom was used, collected from the territory of the Republic of North Macedonia. The collected fresh mushrooms were chopped into thin slices. The mushroom pieces were dried in a chamber dryer with hot air at a temperature of 40 ºC for 6–7 h (Stojanova, 2017). Dried mushrooms

were first ground to a fine powder and then, extracted in two ways, with water and ethyl alcohol as extragens.

Preparation of aqueous extract

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with about 200 mL of distilled water, and after that was extracted on a boiling water bath for 1 h. To determine the yield of the extract, the mass of empty evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Preparation of ethanolic extract

Ethanol extract was prepared by Vidović et al. (2011) method. The measured mass of dried and finely powdered mushroom sample (10 g) was poured with 100 mL of 50% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45 °C. To determine the yield of the extract, the mass of the evaporation flask while it is empty was measured, and then with the evaporated sample. From the difference of these two values, the extract yield was obtained.

Determination of total, α and β-glucan content

The content of total glucan and α-glucan in aqueous and ethanolic extracts was determined using specific kits Mushroom and Yeast Beta-glucan Assay Procedure, K-YBGL 11, 2019 (Megazyme Co. Wicklow, Ireland) according to the manufacturer's instructions.

The β-glucan content was calculated as the difference between the total glucan content and the α-glucan content.

Determination of antibacterial potential

Disc diffusion analysis for determination of antimicrobial activity was performed by Klaus et al. (2015) method. Aqueous and ethanolic extracts of the tested mushroom were prepared at a concentration of 12 mg/mL.As positive controls were prepared discs soaked with gentamicin and neomycin (30 µg/mL). After incubation, the zone of inhibition (mm) was measured.

Statistical analysis

The obtained results were statistically processed using SPSS 20. To determine the statistical significant differences of the obtained results Independent Sample T-test (p = 0.05) as well as ANOVA (*post hoc* Tukey's test, p = 0.05) were used.

RESULTS AND DISCUSSION

Since antiquity, conventional medicine has valued edible fungus for its tremendous health benefits. A variety of ways that mushrooms can improve human well-being depend on their biological components. A growing number of people are interested in extracting bioactive components from mushrooms to create functional foods (Zhang et al., 2019).

Globally, antimicrobial resistance poses a severe threat to public health, particularly with the emergence of multidrug-resistant organisms that are now almost immune to all antibiotics. Finding bioactive compounds from plants and animals that can be utilized as alternatives to conventional antimicrobials is therefore becoming increasingly important.

Table 1. Glucan content in tested extracts (% dry matter extract)

Cantharellus cibarius	**n**	**Total glucan**	**α-glucan**	**β-glucan**
		$\bar{x}$ **± SD**	$\bar{x}$ **± SD**	$\bar{x}$ **± SD**
Aqueous extract	3	27.03 ± 0.07[a]	2.68 ± 0.02[a]	24.35 ± 0.11[a]
Ethanolic extract	3	21.49 ± 0.25[b]	1.92 ± 0.05[b]	19.57 ± 0.13[b]

[a, b] - values of the different extracts marked with different letters, have a statistically significant difference (p<0.05), T-test.

According to data presented in Table 1, can be seen that aqueous extract of chanterelle mushroom was characterized by higher (p<0.05) α-glucan (2.68%) and β-glucan (24.35%) content, compared to the ethanolic extracts (1.92%; 19.57%, respectively).

The major structural feature of mushroom beta-glucans is a beta-1,3-d-glucan main chain with single d-glucosyl residues linked beta-1,3 along this main chain. Some of this glucan can be extracted from the fruiting body of the mushroom, and soluble beta-glucans are also produced by cultured mycelia (Chang and Wasser, 2012).

In their research, Sari et al. (2017) found that the popular culinary mushroom *Cantharelluscibarius* (Chanterelle) contains high values of beta-glucans (23.59 g/100 g dm in cap, 26.93 g/100 g dm in the stipe). These values can be compared to a study from Barros and co-workers (Kalac, 2009): all carbohydrates were 31.9% (with consideration of other carbohydrates such as chitin or sugars and oligosaccharides). Even that, some species such as *Cortinarius violaceus* (L. ex Fr.) Gray, *Leucocybeconnata*(Schumach.) or *Laccariaamethystina* (Cooke) show very similar or even higher beta-glucan values than well-known species with bioactive properties (*Lentinula edodes, Pleurotus* spp.). Among the wild grown mushrooms, *Boletus edulis* (which is also known as a common culinary mushroom) shows extreme differences in beta-glucan contents in its stipes (57.9 g/100 g dm) and caps (16.89 g/100 g dm).

According to a literature review, the three main polysaccharides in *A. bisporus* are alpha-glucan, beta-glucan, as well as galactomannan, with galactomannan making up 55.8% of the total. The health and immunity of the mucosa can be enhanced by *A. bisporus*. Consuming *A. bisporus* in the diet considerably increases secretory immunoglobulin-A secretion. The study found that both in vivo and in vitro, the polysaccharide from *A. bisporus* exhibits strong immunostimulatory and anticancer bioactivity (Smiderle et al., 2012).

Many studies from recent decades prove the bioactive effects of beta-glucans in well-known species such *as Lentinula edodes, Cantharelluscibarius*, *Grifolafrondosa* or *Pleurotus* spp. The non-edible mushrooms may also have great potential for medical purposes using beta glucan extracts, however, the toxic properties of some of these species needs to be remembered (Sari et al., 2017).

According on data presented in Table 2, it can be seen that both tested extracts from *Cantharelluscibarius*showed moderate to good antibacterial activity. Generally, aqueous extract was characterized with higher (p<0.05) values for most of the tested microorganisms compared to ethanolic one. Nonetheless, it is important to notice that both extracts showed higher antibacterial activity against *Enterococcus faecalis*(12.7 mm; 12.8 mm), *Shigellasonnei* (16.6 mm; 15.7 mm) and *Pseudomonas aeruginosa* (11.3 mm; 10.05 mm) compared to gentamicin or neomycin, as a positive control.

Table 2. Antibacterial activity of tested mushroom extracts (mm)

Microorganism	n	*Cantharelluscibarius*		Gentamicin (30 µg/disc)	Neomycin (30 µg/disc)
		Aq*	EtOH*		
		$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD
Staphylococcus aureus ATCC 25923	3	13.0 ± 0.09[a]	12.2 ± 0.06[b]	20,6 ± 0.03[c]	17.0± 0.03[d]
Bacillus cereus ATCC 10876	3	11.8 ± 0.02[a]	11.0 ± 0.09[b]	14.2 ± 0.01[c]	13.5± 0.03[d]
Listeria monocytogenes ATCC 19115	3	9.3 ± 0.11[a]	8.5 ± 0.14[b]	14.7 ± 0.03[c]	14.0± 0.01[d]
Enterococcus faecalis ATCC 29212	3	12.7 ± 0.09[a]	12.8 ± 0.02[a]	12.0 ± 0.03[b]	1.9± 0.01[c]
Escherichia coli ATCC 11230	3	14.2 ± 0.11[a]	13.4 ± 0.02[b]	18.4 ± 0.05[c]	16.3± 0.03[d]
Yersinia enterocolitica ATCC 27729	3	14.7 ± 0.05[a]	15.3 ± 0.10[b]	30.8± 0.01[c]	27.1± 0.02[d]
Shigella sonnei ATCC 29930	3	16.6 ± 0.01[a]	15.7 ± 0.13[b]	16.5± 0.02[c]	14.9± 0.02[d]
Proteus vulgaris ATCC 8427	3	19.3 ± 0.12[a]	18.6 ± 0.09[b]	23.9± 0.02[c]	22.6±0.02[d]
Pseudomonas aeruginosa ATCC 35554	3	11.3 ± 0.01[a]	10.5 ± 0.11[b]	17.7 ± 0.01[c]	10.2 ± 0.02[d]

[a, b, c, d] – values marked with different letters have statistically significant difference ($p<0.05$), ANOVA, *post hoc* Tukey's test; * Aq – aqueous extract; * EtOH – ethanolic extract

These results are in accordance with the glucan content, while higher antibacterial potential was determined in the aqueous extract, where higher glucan content was determined.

Recent study describes methanolic extracts obtained from *Agaricusbisporus* and *Cantharelluscibarius* (from black sea region of Turkey) as having high antibacterial activity against E. coli, assessed by disc diffusion method. The extraction solvent or even the mushrooms origin, as well as the bacterial strain could explain the differences in the antimicrobial activity reported by different authors for the same species (Ozen et al., 2011).

Several investigations have suggested that *F. velutipes* mushrooms contain antimicrobials as well. Researchers looked into the antimicrobial properties of extracts from various *F. velutipes* parts and found that mature *F. velutipes* mushroom extracts from both methanol and chloroform demonstrated great antimicrobial properties, especially for staphylococcal infections and *Bacillus subtilis*. Also discovered to have antimicrobial properties against Gram-positive and Gram-negative bacteria such as *B. subtilis, Bacillus pumilus, Staphylococcus aureus*, and *Pseudomonas aeruginosa* was a methanol extract of natural *F. velutipes* fruiting body (Kashina et al., 2016).

According to Ozen et al. (2011) *C. cibarius* showed antimicrobial activity but weaker than several sesquiterpenoids isolated from *Lactarius* sp. The most susceptible bacterium was *E. coli*. In literature, it seems that this mushroom is more effective against gram-negative than gram-positive bacteria.

Kolundžićet al. (2017) tested the antimicrobial potential of cyclohexane, dichloromethane, methanol and aqueous extracts of *C. cibarius*. Broth microdilution assay was performed against 10 bacterial, with emphasis on *Helicobacter pylori*. Methanol

extract was the most active against *H. pylori* strains with minimal inhibitory concentration values between 4 and 32 μg/mL.

Alves et al. (2012) reported that *Cantharelluscibarius, Lepistanuda* and *Ramaria botrytis* extracts did not show any activity against *S. aureus* and MRSA, at the tested concentrations (up to 20 mg/ml). Nevertheless, a previous report described high antimicrobial activity of those species against *S. aureus* isolated from pus (MIC 5 mg/ml) (Barros et al. 2008). It should be highlighted that the authors used a different extraction solvent (methanol), a different antimicrobial activity assay (agar streak dilution method based on radial diffusion), they dissolved the extracts in DMSO and not in water as in the present study, and specially they used a different strain, probably with a different antibiotic resistance profile (Alves et al., 2012).

Despite some similarities in the composition of mushroom samples, it is known that the chemical compositions of mushrooms are affected by a number of factors, namely, mushroom strain/type, composition of growth media, time of harvest, management techniques, handling conditions, and preparation of the substrates. Therefore, chemical contents and antimicrobial substances of species of mushrooms naturally grown in different geographic locations of world must be analysed (Ozen et al., 2011).

People are exerting more effort to find substances from nature that provide health benefits as they become more conscious of the potential negative effects of synthetic medicines and health supplements. *In-vivo* and *in-vitro* research have shown that mushrooms offer amazing medicinal potential and excellent nutritional value. All kinds of mushrooms provide a good supply of carbohydrates, protein, unsaturated fatty acids, and some vitamins, which are all nutritionally similar to vegetables. Although edible mushrooms are well known for their culinary and nutritional benefits, they are still less known about their medicinal potential.

CONCLUSION

Based on the presented data, it can be concluded that both tested extracts of *Cantharelluscibarius*mushroom, showed moderate to good antibacterial activity. Slightly higher ($p<0.05$) values were obtained in the aqueous extract compared to the ethanolic extract. In line with that, aqueous extract was characterized by higher total, alpha- and beta-glucan content, compared to ethanolic extract.

Therefore, it can be pointed that the aqueous extracts of both tested mushrooms showed good antibacterial properties that can be a substitute for some of the synthetic antibiotics used for industrial purposes. According to that, this study represents a novel starting point for future studies in which mushroom extracts can be used in various fields such as food industry, pharmaceutics, medicine or cosmetics.

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