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DETERMINATION OF ANTIBACTERIAL POTENTIAL OF *AGARICUS MACROSPORUS* AND *RUSSULA VESCA* MUSHROOM EXTRACTS

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Abstract: The aim of this research was to determine the antibacterial potential ofaqueous and ethanolic extract of wild mushroom species: *Agaricus macrosporus* and *Russulavesca*. Extracts from *R.vesca* were characterized with higher values for total carbohydrates and total proteins. Both aqueous extracts had higher antibacterial activity compared to ethanolic extracts. Aqueous extract from *R.vesca* showed higher antibacterial activity against *B. cereus* (13.6mm), *E.faecalis* (12.1 mm), *E. coli*(16.7 mm) and *P.aeruginosa* (10.5 mm) compared to gentamicin or neomycin. This study represents a novel starting point for future researchin which mushroom extracts can be used in various industry fields.

Keywords: wild mushrooms, extracts, antibacterialactivity.

Introduction

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).Among the bioactive compounds in mushrooms, polysaccharides are those that show most antitumoral, antiviral and immunomodulatory activity (Mizuno andNishitani, 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).

Agaricusmacrosporus (F.H. Muller and Jul. Schäff.), commonly known as the white button mushroom, is one of the most economically important edible mushrooms. It is considered as a valuable health food with high contents of polyphenols, ergothioneine, vitamins, minerals and polysaccharides (Tian et al., 2012). Moreover, this mushroom has been demonstrated to possess various valuable biological properties including antimicrobial, immunomodulatory, anti-inflammatory as well as antioxidant activities. *Russulavesca*(Fr.) is a common and widespread edible mushroom on mainland Europe and North America. This mushroom appears in summer or autumn and grows primarily in deciduous forests. *Russulavesca* is considered edible and good, with a mild

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nutty flavour. In some countries, including Russia, Ukraine and Finland it is considered entirely edible even in the raw state (Dahlberg, 2019).

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 (Reis et al., 2014).

The aim of this research was to determinate the antibacterial potential of aqueous and ethanolic extracts of the wild mushroom species *Agaricus macrosporus* and *Russulavesca*.

Material and methods

In this research, as a work material two types of mushrooms collected from the territory of the Republic of North Macedonia were used: *Agaricusmacrosporus* and *Russulavesca*. 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 40ºC for 6–7 h. Dried mushrooms were first ground to a fine powder and then, extracted in two ways, with water and ethyl alcohol as extragens.

Aqueous extract was prepared by Sławińska et al. (2013) and Ribeiro et al. (2015) method. Ethanolic extract was prepared by Vidović et al. (2011) method.

The content of total carbohydrates in the extracts was determined by method of Masuko et al. (2005) in microtiter plates. Each mushroom extract was tested in triplicates and the resultsare shown in% of the dry weight of the extract.

The content of total proteins was determined by the method of Bradford (1976).Each mushroom extract was tested in triplicates and the resultsare shown in% of the dry weight of the extract.

Disc diffusion analysis for determination of antimicrobial activity was performed by Klaus et al. (2015) method. Aqueous and ethanolic extracts of the tested mushrooms 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.

The obtained results were statistically processed using SPSS 20. To determine the statistical significant differencesof 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

The content of carbohydrates and proteins is thought to greatly contribute to the antimicrobial activity of mushroom extracts. In line with that, some protein isolated form macromycetes have been reported against Gram-negative bacteria (Hleba et al., 2014). From data in Table 1, can be seen that both aqueous and ethanolic extracts from *R.vesca* were characterized with statistically significant (p<0.05) higher values for total carbohydrates (41.15± 0.07% for aqueous extract, i.e. 35.01 ± 0.31% for ethanolic extract) as well as total proteins (3.20 ± 0.58% for aqueous extract, i.e. 3.88 ± 0.23% for ethanolic extract) compared to the tested extracts from *A. macrosporus*.Nevertheless, it is reported that, generally, aqueous

extracts from both tested mushrooms were characterized with statistically significant (p<0.05) higher carbohydrates and proteins content, compared to both ethanolic extracts.

Tabela 1.Ukupnisadržajugljenihhidrata i proteina u ispitivanimekstraktima
Table 1. Total carbohydrates and proteins content in tested extracts

Vodeniekstrakti *Aqueous extracts*	n	Ukupni ugljenihidrati *Total carbohydrates* (%)	Ukupni proteini *Total proteins* (%)	Etanolni ekstrakti *Ethanolic extracts*	Ukupni ugljeni hidrati *Total carbohydrates* (%)	Ukupni proteini *Total proteins* (%)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD		$\bar{x}$ ± SD	$\bar{x}$ ± SD
Agaricusmacrosporus	3	39.26± 0.14^{aA}	4.61±1.01^{aA}	*Agaricusmacrosporus*	30.92± 0.17^{aB}	3.97±0.49^{aB}
Russulavesca	3	41.15± 0.07^{bA}	3.20±0.58^{bA}	*Russulavesca*	35.01± 0.31^{bB}	3.88±0.23^{aB}

$^{a, b}$- values of the same extract of different fungal species marked with different letters, have a statistically significant difference (p<0.05), T-test.
$^{A, B}$ - values of different extract of the same fungus, marked with different letters, have a statistically significant difference (p<0.05), T-test.

In addition, water as a polar extraction agent probably proved to be better due to the higher value of most of the chemical components (Stojanova et al., 2021) that are thought to be responsible for antimicrobial activity (e.g. total carbohydrates) than in ethanolic extracts of examined mushroom strains. Moreover, an exception was observed in the ethanolic extract from *R.vesca* that had statistically significant (p<0.05) higher protein content (3.88 ± 0.23%), compared to its aqueous extract and it is due to the chemical structure of this compounds.

Based on data presented in Table 2, it can be seen that all of the tested extracts from both mushroom species showed moderate to good antibacterial activity. Generally, both aqueous extracts had statistically significant (p<0.05) higher antibacterial activity compared to both ethanolic extracts. On the other hand, both extracts from *R.vesca*were characterized with statistically significant (p<0.05) higher antibacterial activity compared to extracts from *A.macrosporus*. Nonetheless, it is important to notice that aqueous extract from *R.vesca* showed higher antibacterial activity against *B. cereus* (13.6 ± 0.06 mm), *E.faecalis* (12.1 ± 0.03 mm), *E. coli*(16.7 ± 0.05 mm) and *P.aeruginosa* (10.5 ± 0.03 mm) compared to gentamicin or neomycin, as a positive controls. These results are in accordance with the total carbohydrates and proteins content, while the highest antibacterial activity was determined in the aqueous extract from *R.vesca* which had the highest content of these bioactive compounds. However, ethanolic extract from *R. vesca* showed highest antibacterial activity against *L. monocytogenes*as well as *E. coli*, compared to the positive controls, which is due to the highest presence of total proteins in this extract, i.e. due to the structure of the extracted proteins that are correlated with the antibacterial activity. In their study, Nwachukwu and Uzoeto(2010) pointed out that hot water as well as ethanolic extracts of *P.*

squarrosolus and *R. vesca* inhibited both Gram-negative (*E. coli, P.aeruginosa, S. typhi*), Gram-positive bacteria (*B. cereus, S. aureus, S. pneumoniae*) and yeast (*C. albicans*).However, neither of them showed higher potential compared to gentamicin (Iwalokun et al., 2007).

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

Mikroorganizam *Microorganism*	n	*Agaricusmacrosporus*		*Russula vesca*		Gentamicin (30 µg/disc)	Neomycin (30 µg/disc)
		Aq*	EtOH*	Aq	EtOH		
		$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD	$\bar{x}$± SD
Staphylococcus aureus ATCC 25923	3	15.1± 0.02^{a}	13.8± 0.01^{b}	20.5± 0.09^{c}	20.1± 0.02^{d}	20,6± 0.03^{c}	17.0± 0.03^{e}
Bacillus cereus ATCC 10876	3	9.6± 0.02^{a}	9.0± 0.01^{b}	13.6± 0.06^{c}	10.9± 0.05^{d}	14.2± 0.01^{e}	13.5± 0.03^{c}
Listeria monocytogenes ATCC 19115	3	12.7± 0.07^{a}	12.3± 0.01^{b}	13.8± 0.06^{c}	14.1± 0.01^{d}	14.7± 0.03^{e}	14.0± 0.01^{d}
Enterococcus faecalis ATCC 29212	3	11.4± 0.05^{a}	9.6± 0.09^{b}	12.1± 0.03^{c}	12.5± 0.01^{d}	12.0± 0.03^{c}	1.9± 0.01^{e}
Escherichia coli ATCC 11230	3	15.9± 0.09^{a}	12.1± 0.03^{b}	16.7± 0.05^{c}	13.2± 0.03^{d}	18.4± 0.05^{e}	16.3± 0.03^{f}
Yersinia enterocolitica ATCC 27729	3	13.6± 0.01^{a}	13.0± 0.04^{b}	20.1± 0.03^{c}	13.3± 0.08^{d}	30.8± 0.01^{e}	27.1± 0.02^{f}
Shigella sonnei ATCC 29930	3	15.0± 0.03^{a}	13.4± 0.06^{b}	14.9± 0.03^{c}	13.6± 0.06^{d}	16.5± 0.02^{e}	14.9± 0.02^{c}
Proteus vulgaris ATCC 8427	3	19.7± 0.03^{a}	17.5± 0.05^{b}	21.8± 0,02^{c}	17.9± 0.03^{d}	23.9± 0.02^{e}	22.6±0.02^{f}
Pseudomonas aeruginosa ATCC 35554	3	10.5± 0.05^{a}	8.7± 0.01^{b}	10.5± 0.03^{a}	9.0± 0.01^{c}	17.7± 0.01^{d}	10.2± 0.02^{e}

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

On the other hand, many studies proved the antimicrobial potential of Agaricus sp. extracts on strains of *E. faecalis, S. aureus*and *P. aeruginosa* (Suntaxi and Loja, 2021). In line with that, the extracts of *A. bisporus*were used against *B.subtilis*, and of *A. silvicolae-similis* against *B. cereus, B. subtilis*or*S. aureus* (Barros et al, 2008). Likewise, the activity of *A. bisporus* against *S. aureus, B. subtilis, K. pneumoniae*and *P. aeruginosa* was reported (Jagadish et al, 2009).Similar observations were previously noted (Santoyo et al., 2009; Loganathan et al., 2009).The new components from natural sources that show good antibacterial potential may be used as a partial replacement of synthetic antibiotics or can be a basis for obtaining new functional food products. In line with this, further identification and isolation of different active compounds from mushrooms and commercializing their products is necessary.

Conclusion

Based on the presented data, it can be concluded that both tested extracts of *A.macrosporus*, as well as *R.vesca*, showed moderate to good antibacterial activity. Slightly higher(p<0.05) values were obtained in the aqueous extracts compared to the ethanolic extracts. Both analysed extracts of *R.vesca* had higher (p<0.05)carbohydrates and proteins content compared to both *A.macrosporus*extracts.

Therefore, it can be pointed that the aqueous extracts of both tested mushrooms showed good antibacterialproperties 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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