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**ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF WILD
BASIDIOMYCETE MUSHROOM *GANODERMA APPLANATUM***

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ABSTRACT

Hot water extract (AN), partially purified polysaccharides (AP) and hot alkali extract (ANa) obtained from wild mushroom *G. applanatum* were examined for their antibacterial and antifungal activity. Five Gram negative (*Proteus hauseri*, *Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Shigella sonnei*, *Yersinia enterocolitica*), five Gram positive (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Geobacillus stearothermophilus*, *Enterococcus faecalis*) bacterial strains, as well as two fungal strains (*Candida albicans* and *Cryptococcus neoformans*), all belonging to the American Type Culture Collection (ATCC), were tested by broth microdilution assay. In general, Gram-positive bacteria were more sensitive in the presence of tested extracts than Gram-negative bacterial species. Almost all tested extracts demonstrated a high microbistatic potential, and in most cases the microbicidal effect also has been reached. The best antibacterial effect of hot water alkali extract, ANa, was observed in the case of *E. faecalis* (MIC 0.039 mg/mL, MBC 1.25 mg/mL), while the same extract was the most effective antifungal agent towards *C. neoformans* (MIC 0.078 mg/mL, MFC 2.5 mg/mL). This research indicates possibility to use selected extracts, obtained from the mushroom *G. applanatum*, in order to

prevent the development of some pathogenic microorganisms and food spoilage.

Ključne reči: *Ganoderma applanatum*, mushroom extract, bactericidal, bacteriostatic, fungicidal, fungistatic

INTRODUCTION

Ganoderma applanatum (Pers.) Pat., also known as artist's conk, artist's bracket or bear bread, is a very common, perennial, woody shelf fungus, widely distributed throughout the world, mostly being found in temperate and tropical zones. It usually appears individually or in clusters, on the sides of hardwood trees, which is their specific niche. Growing on logs, stumps or dead or dying trees, this basidiomycete is very important decomposer of wood material, contributing to the mineralization of organic waste.

Among numerous mushrooms, woody polyporaceae *G. applanatum* is unique in being consumed for its pharmaceutical value rather than as a food. It was used in traditional medicine of the Far East for treating many disorders in the body. Modern scientific research, which was carried out very intensively in recent decades, confirms a number of positive effects of this valuable mushroom on human health. A variety of bioactive compounds, such as polysaccharides, polyphenols, triterpenoids, amino acids, polypeptides are present in fruiting bodies and mycelium of *G. applanatum*. These active ingredients contribute to the biological activities, such as antitumor and immunomodulating [1, 2] or antiviral properties [3]. This, highly valuable mushroom also contain antioxidant compounds that have important ability to trap free radicals and thus inhibit the oxidative mechanisms which lead to the degenerative diseases [4, 5]. *G. applanatum* possesses liver protective activity by decreasing reactive oxygen species; its terpenes renewed activities of antioxidant enzymes and suppressed the inflammatory response [6]. A possible effectiveness of this mushroom as an anti-obesity agent because of the ability to inhibit the differentiation of preadipocytes into mature adipocytes, by reducing triglyceride accumulation, is proven [7].

During recent years many investigations confirmed antimicrobial activity of numerous edible and inedible mushrooms extracts [8, 9, 10, 11, 12, 13]. This could be of great importance, considering that there are a number of microorganisms which are able to cause different diseases, spoil food, cosmetic and pharmaceutical products whose use might cause intoxication. A common way to fight against microorganisms is the use of the appropriate antibiotics, but there are also significant problems caused by their long-term use, since the microorganisms

become resistant to them. Additionally, there are many side effects, especially during prolonged and improper use of antibiotics. Therefore, the search for new antimicrobial agents without adverse effects, such as various herbs and mushrooms, is of major importance. Regarding these facts, it would be very suitable to develop naturally occurring product which would be able to reduce or completely interrupt the proliferation of microorganisms.

The aim of this investigation was to determine the *in vitro* antibacterial and antifungal effect of hot water extract (AN), partially purified polysaccharides (AP) and hot alkali extract (ANa) obtained from wild mushroom *G. applanatum*.

MATERIAL AND METHODS

Sample collection

Fresh wild-growing fruiting bodies of mushroom *G. applanatum* were collected from the side of wild beech (*Fagus sylvatica* L.) trunk, from the forest park Košutnjak, Belgrade, Republic of Serbia, in the autumn 2014. Carpophores were identified by authors from an examination of macro- and micromorphology, in comparison to standard descriptions in the monographs and taxonomic treatments. Carpophores were brush-cleaned, air-dried at 40°C to constant mass and ground into fine particles, and stored in the dark prior to analysis. The mycelial culture which was stored at 4°C in the mushrooms culture collection, as well as representative voucher specimens were deposited at the herbarium of the Department for Industrial Microbiology (Faculty of Agriculture, University of Belgrade).

Preparation of extracts

AN, AP and ANa were obtained from wild mushroom *G. applanatum*, according to previously described procedures [14].

Antibacterial and antifungal activity testings with broth microdilution method

Five Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* *Geobacillus stearothermophilus*, *Enterococcus faecalis*) and five Gram-negative bacterial species (*Proteus hauseri*, *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Shigella sonnei*, *Yersinia enterocolitica*), were challenged in this study to ascertain the antibacterial properties of AN, AP and ANa from *G. applanatum*. Selected species of bacteria originate from ATCC (American Type Culture Collection, Rockville, Maryland). These microorganisms were chosen for the bioassay as well known food spoilage and pathogenic microorganisms.

Working concentrations of bacteria, as well as stock solutions and working solutions were prepared according to previously described method [9].

Two yeasts' ATCC strains (*Candida albicans*, *Cryptococcus neoformans*) were also used to determine antifungal potential of *G. applanatum* extracts. These pathogenic yeasts were maintained on malt agar and the same media was used to confirm the absence of contamination and the validity of the inocula. Before testing, each yeast species was recovered by sub-culturing in the malt broth, aerobically, for 24 h, at 37°C. Working concentrations of yeasts, as well as stock solutions and working solutions were prepared in the same manner as for bacteria.

Broth microdilution method employed to determine minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) or minimum fungicidal concentrations (MFC) is described previously [9, 15]. Concentrations of mushroom extracts ranged from 0.0097 to 20.0 mg/mL. Amoxicillin trihydrate was used as a positive control for bacterial strains, while fluconazole was used as a positive control for yeasts strains.

Statistical analysis

All measurements were done in triplicate. The experimental data were subjected to one-way analysis of variance (ANOVA) and Fisher's LSD was calculated to detect significant difference ($p \leq 0.05$) between the mean values.

RESULTS AND DISCUSSION

The broth microdilution method as a rapid quantitative determination of MIC, based on the color change caused by the enzymatic activity of viable bacteria, was applied. Well defined endpoints appeared as the results of the metabolic activity of bacteria, i.e. TTC reduction (Table 1).

In the presence of *G.applanatum* extracts, the growth inhibition of almost all tested microorganisms was observed. Gram-negative bacteria appeared to be more resistant than Gram-positive bacteria; exceptions were *S. enteritidis* (MIC 0.625 mg/mL, MBC 10.0 mg/mL) and *Y. enterocolitica* (MIC 0.3125 mg/mL, MBC 10.0 mg/mL) after exposure to ANa. Only in these two cases the influence of ANa resulted in MBC achieving.

In general, Gram-positive bacteria were more susceptible than Gram-negative bacterial species. Among tested extract, ANa is shown to be the most effective against Gram-positive bacteria (MIC 0.039-0.625 mg/mL; MBC 1.25-10.0 mg/mL); slightly weaker activity was observed in AP (MIC 0.156-1.25 mg/ml; MBC 2.5-10.0 mg/mL), while AN (MBC 0.3125-5.0 mg/mL; MBC 5.0-20.0 mg/mL) was the least active.

Table 1. Antimicrobial activity of AN, AP and ANa, and standard antibiotics amoxicillin (AMX) and fluconazole (FLU), expressed as MIC (mg/mL), MBC (mg/mL) and MFC (mg/mL), determined by the broth microdilution method. Data are expressed as mean of the three replicates. Standard deviations are not shown, as the results for all replications were equal.

Tabela 1. Antimikrobna aktivnost ekstrakata AN, AP i ANa, i standardnih antibiotika amoksicilina (AMX) i flukonazola (FLU), izražena kao MIC (mg/mL), MBC (mg/mL) i MFC (mg/mL), određena mikrodilucionom metodom u bujonu. Podaci su izraženi kao srednje vrednosti tri ponavljanja. Standardne devijacije nisu prikazane jer su rezultati svih ponavljanja jednaki.

Microorganism	Source		AN	AP	ANa	AMX	FLU
<i>Proteus hauseri</i>	ATCC 13315	MIC	10.0 ^{a1}	- ²	1.25 ^b	0.0027 ^c	nd ³
		MBC	-	-	-	0.0108	nd
<i>Escherichia coli</i> O157:H7	ATCC 35150	MIC	5.0 ^a	-	1.25 ^b	0.0054 ^c	nd
		MBC	-	-	-	0.0217	nd
<i>Salmonella</i> <i>enteritidis</i>	ATCC 13076	MIC	2.5 ^b	5.0 ^a	0.625 ^c	0.0007 ^d	nd
		MBC	-	-	10.0 ^a	0.0027 ^b	nd
<i>Shigella sonnei</i>	ATCC 29930	MIC	2.5 ^a	2.5 ^a	1.25 ^b	0.0054 ^c	nd
		MBC	-	-	-	0.0108	nd
<i>Yersinia</i> <i>enterocolitica</i>	ATCC 27729	MIC	1.25 ^a	1.25 ^a	0.3125 ^b	0.0108	nd
		MBC	-	-	10.0 ^a	0.0108 ^b	nd
<i>Listeria</i> <i>monocytogenes</i>	ATCC 19115	MIC	2.5 ^a	1.25 ^b	0.156 ^c	0.0003 ^d	nd
		MBC	20.0 ^a	10.0 ^b	5.0 ^c	0.0027 ^d	nd
<i>Staphylococcus</i> <i>aureus</i>	ATCC 6538	MIC	2.5 ^a	1.25 ^b	0.3125 ^c	0.0002 ^d	nd
		MBC	20.0 ^a	10.0 ^b	10.0 ^b	0.0014 ^c	nd
<i>Bacillus cereus</i> ATCC	ATCC 10876	MIC	2.5 ^a	1.25 ^b	0.625 ^c	0.0003 ^d	nd
		MBC	20.0 ^a	10.0 ^b	10.0 ^b	0.0054 ^c	nd
<i>Geobacillus</i> <i>stearothermophilus</i>	ATCC 7953	MIC	5.0 ^a	0.625 ^b	0.3125 ^c	0.0003 ^d	nd
		MBC	20.0 ^a	5.0 ^b	2.5 ^c	0.0027 ^d	nd
<i>Enterococcus</i> <i>faecalis</i>	ATCC 29212	MIC	0.3125 ^a	0.156 ^b	0.039 ^c	0.0003 ^d	nd
		MBC	5.0 ^a	2.5 ^b	1.25 ^c	0.0027 ^d	nd
<i>Candida albicans</i>	ATCC 24443	MIC	1.25 ^a	1.25 ^a	0.156 ^b	nd	0.0125 ^c
		MFC	20.0 ^a	10.0 ^b	10.0 ^b	nd	0.05 ^c
<i>Cryptococcus</i> <i>neoformans</i>	ATCC 76484	MIC	1.25 ^a	0.625 ^b	0.078 ^c	nd	0.0063 ^d
		MFC	10.0 ^a	5.0 ^b	2.5 ^c	nd	0.0063 ^d

¹Within the same row, means followed by different letters are significantly different at $\alpha=0.05$ (ANOVA, Tukey's HSD Test)

²- not achieved

³-not determined

The hot alkali extract, ANa, also was the most effective against fungal strains *C. neoformans* and *C. albicans* (MIC - 0.078 mg/mL and 0.156 mg/mL,

MBC - 2.5 mg/mL, and 10.0 mg/mL, respectively). The other two extracts, AP and AN, exhibited antifungal activity to a somewhat lesser extent (MIC - 0.625 - 1.25 mg/mL; MFC - 5.0-20.0 mg/mL).

This investigation revealed a great antimicrobial potential of different extracts obtained from *G. applanatum*. Of particular interest is extract ANa which proved to be a highly effective against most of tested microorganisms, especially *E. faecalis* and *C. neoformans*. These microorganisms could be triggers for very serious health disorders. Although *E. faecalis* is normal gut commensals, under suitable circumstances it can cause urinary tract or wound infections, endocarditis and bacteremia. *E. faecalis* is responsible for approximately 110.000 cases of urinary tract infections yearly [16]. Further, *C. neoformans*, an encapsulated yeast is a major cause of illness in persons with a weakened immune system (e.g. HIV patients), with an estimated 1.000.000 cases of cryptococcal meningitis occurring worldwide each year [17].

In recent years, several reports concerning antimicrobial power of different *G. applanatum* extracts have been published [18, 19, 20, 21]. These investigations suggest that the antimicrobial effect depend on the method of extraction, i.e. on the type of the extract, microorganism strain, exposure time. Karaman et al., 2009 reported that 60% methanol and 55% chloroform extracts (among others was also *G. applanatum*) reached a significant antibacterial activity against one or more targeted bacteria [22]. Regarding the fact that macrofungi are commonly collected either randomly or by locals in forests, it would be necessary to perform initial screening for possible antimicrobial activities by using crude aqueous or alcohol extractions. Proper selection of the extraction procedure directly determines the antimicrobial activity of the extract [23].

G. applanatum is one of the medicinally important mushroom which is used for the production of mushroom-based health care commercial biotech-products with preventive and curative effects. Nowadays, these kinds of products are available and highly sought in the world market. They could be find in dried forms as healthy food (“nutraceuticals”), as well as functional food additives (“pharmaceuticals” or “nutriceuticals”). Due to synergistic action of present bioactive molecules, the majority of mushroom products possess beneficial health effects and could be used without adverse effects [24].

CONCLUSIONS

Our investigation showed that *G. applanatum* is extremely valuable mushroom, with strong antimicrobial potential. Depending on the method of extraction it is possible to obtain different kinds of extracts that could act as microbistatic or microbicidal agents against certain species and strains of

microorganisms. The most prominent extract found to be ANa, obtained in very intense chemical processes of degradation, transformation and formation of new molecules, all of which did not diminished its biological activity. On the contrary, hot alkali extract ANa, exhibited microbicidal effect on the majority of tested strains. Since the food can be contaminated by various pathogenic and spoiling microorganisms, supplementation with targeted *G. applanatum* extracts could contribute to the reduction of food poisoning.

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ANTIBAKTERIJSKI I ANTIFUNGALNI POTENCIJAL GLJIVE *GANODERMA APPLANATUM* BAZIDIOMICETE IZ PRIRODE

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REZIME

Ispitivana je antibakterijska i antifungalna aktivnost vrelog vodenog ekstrakta (AN), parcijalno prečišćenog polisaharidnog ekstrakta (AP) i vrelog alkalnog ekstrakta, dobijenih iz *G. applanatum*, gljive iz prirode. Pet Gram negativnih (*Proteus hauseri*, *Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Shigella sonnei*, *Yersinia enterocolitica*), pet Gram pozitivnih (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Geobacillus stearothermophilus*, *Enterococcus faecalis*) bakterijskih vrsta, kao i dva soja gljiva (*Candida albicans* and *Cryptococcus neoformans*), od kojih svi pripadaju Američkoj tipskoj kolekciji kultura (ATCC), testirani su mikrodilucionom metodom u bujonu. Generelno, Gram pozitivne bakterije su bile mnogo osjetljivije u prisustvu testiranih ekstrakata nego Gram negativne bakterijske vrste. Skoro svi testirani ekstrakti pokazali su visok mikrobistatički potencijal, a u najvećem broju slučajeva dostignut je i mikrobicidni efekat. Najbolji antibakterijski efekat vrelog alkalnog ekstrakta, ANa, uočen je u slučaju *E. faecalis* (MIC 0.039 mg/mL, MBC 1.25 mg/mL), dok je ovaj ekstrakt bio i najefikasniji antifungalni agens prema *C. neoformans* (MIC 0.078 mg/mL, MFC 2.5 mg/mL). Ovo istraživanje ukazuje na mogućnost korišćenja odabranih ekstrakata dobijenih iz gljive *G. applanatum* u cilju prevencije razvoja nekih patogenih mikroorganizama i kvarenja hrane.

Key words: *Ganoderma applanatum*, ekstrakti gljiva, baktericidno, bakteriostatičko, fungicidno, fungistatičko