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INFLUENCE OF THE EXTRACTS ISOLATED FROM *GANODERMA LUCIDUM* MUSHROOM ON SOME MICROORGANISMS

ABSTRACT: *Ganoderma lucidum* (Leyss.: Fr.) Karst, a mushroom-like fungus is one of the most famous traditional Chinese medicinal herbs. It received wide popularity as a healthy food and medicine in the Far East for more than 2000 years, because of its high medicinal value. One of very interesting aspects of *G. lucidum*'s performance is antimicrobial effect due to the extracts derived from this mushroom, which contain bacteriolitic enzyme, lysozyme and acid protease. The effects of these extracts depend on their composition, extraction mode and refining. Bioactive components isolated from several *G. lucidum*'s strains showed different effects on the investigated microorganisms. In some cases, the influence was very intensive, with inhibitory or stimulating effect, while some of them did not show any influence on the investigated microorganisms.

KEY WORDS: antimicrobial effect, extraction, extracts, *Ganoderma lucidum*

INTRODUCTION

For more than 2000 years, *Ganoderma lucidum* has been regarded as a popular folk medicine in the Far East, used to treat various human diseases, such as hepatitis, hypertension, hypercholesterolemia, gastric cancer and many others. Due to its ability to cure many different diseases it received names like "Elixir of life", "Food of Gods", "Mushroom of the Universe". Its intracellular and extracellular polysaccharides showed inhibition of the growth of several types of cancer cells (Sone et al., 1985, Wang at al., 2002, Zhang et al., 1994). Besides, it also produces many oxygenated triterpenes, especially ganoderic acid, with various biological functions such as cytotoxicity to hepatoma cells, inhibition of histamine release, inhibition of cholesterol synthesis and absorption, stimulation of platelet aggregation, and inhibition of thrombin induced platelet aggregation (Shiao et al., 1994).

Antimicrobial drugs derived from different kinds of microorganisms have long been used for prophylactic and therapeutic purposes. However, using the same antibiotic for a long period may cause the resistance of microorganisms

to that antibiotic. So, the investigation of influences of different kinds of polysaccharides derived from mushrooms on microorganisms and their effects on host's immune system are very important today. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to the current antibiotics (S m i t h et al., 2002).

MATERIALS AND METHODS

Maintenance of microorganisms

Antimicrobial effects of the extracts of *G. lucidum* on certain strains of microorganisms were investigated:

1. *Escherichia coli* ATCC
2. *Bacillus cereus* ATCC
3. *Staphylococcus aureus* ATCC
4. *Salmonella enteritidis* — Faculty of Agriculture collection
5. *Proteus mirabilis* — Faculty of Agriculture collection
6. *Saccharomyces cerevisiae* ATCC
7. *Aspergillus niger* — Faculty of Agriculture collection

ATCC — American Type Culture Collection, Rockville, Maryland

Bacterial strains of *E. coli*, *B. cereus*, *S. aureus*, *S. enteritidis* and *P. mirabilis* were maintained on nutrient agar. The slant was inoculated and incubated at 37°C for 24 h, than stored at 4°C.

Investigated yeast *S. cerevisiae* and mould *A. niger* were maintained on malt agar. The slant was inoculated and incubated at 30°C, 48h for yeasts and at 25°C, 7 days for mold, than stored at 4°C.

Preparation of microorganisms

Bacterial strains of *E. coli*, *B. cereus*, *S. aureus*, *S. enteritidis* and *P. mirabilis* were inoculated in nutrient broth and incubated at 37°C for 24 h, to reach the concentration of 10^6 cells/ml.

Yeast *S. cerevisiae* and mould *A. niger* were inoculated in malt broth and incubated at 30°C for 24 h for yeast and at 25°C for 24 h for mold, to reach the concentration of 10^6 cells/ml.

Used strains of Ganoderma lucidum

For this experiment ten different extracts, derived from different strains of *G. lucidum* were used. Strains Gl-I, K1 were isolated naturally from Serbian woods, strains Gl-7 and Gl-349 were taken from the Research Plant International collection, Holland and strain Gl-K originated from China.

Hot extraction of bioactive compounds from dried Ganoderma lucidum *mushroom*

Powdered tissue (1—9 g) was washed with 96% ethanol (300 ml), than filtered and dried in vacuum (at 40°C for 60 min) up to getting powder. Dried filtercake was mixed with deionized water (300 ml) and glucans were extracted by autoclaving at 120°C for 20 min. Material was cooled down and centrifuged (10000 rpm, at 4—9°C for 10 min). Supernatant was mixed with 2 vol. 96% ethanol and left at 4°C untill precipitate was formed. After centrifuge (10000 rpm, at 4—9°C for 10 min) the collected pellet were dried in vacuum (at 40°C for 60 min) and the powder was dissolved in Tris buffer 0.01 M (50 ml). The suspension was dialyzed for 24 h at room temperature. Dialyze is necessary for refining because low-molecular weight molecules will pass through the membrane in solution, while high-molecular weight molecules, β-glucan will stay inside the membrane. After dialyzing the content was centrifuged (10000 rpm, at 4—9°C for 10 min) and 2 vol. 96% ethanol was added to supernatant and left at 4°C for a couple of hours. To remove supernatant centrifugation (10000 rpm, at 4—9°C for 10 min) was repeated and the pellets were dried in vacuum (at 40°C for 60 min). The dried pellets were dissolved in PBS and used for further examination on microorganisms.

Room temperature extraction of water-soluble bioactive compounds from dried mushroom Ganoderma lucidum

Powdered tissue (10 g) was mixed with water (300 ml) and steered on magnetic stirrer at room temperature for 24 h. After filtration, the supernatant was removed and $(NH_4)_2SO_4$ was added to 90% saturation. Centrifugation (10000 rpm, at 4—9°C for 10 min) was done and Tris 0.01 M was added to the pellets for dialyzing (at room temperature for 24 h). This is the way to obtain the lectins which will stay within the membrane and will be separated by the centrifugation (10000 rpm, at 4—9°C for 10 min). The obtained dry lectins were dissolved in PBS and used for the investigation of their influence on the observed microorganisms.

Influence of the extracts on microorganisms

Petri dishes were inoculated with 0.2 ml suspension of certain microorganism strains, and overlayed with 20 ml of medium. For bacterial growth, Mueller Hinton agar was used, and for yeast and mould, malt agar was used. Three filter disks (Schleicher & Schuell), 6 mm in diameter were placed on each agar, and 10 μl of appropriate mushroom extract was added. Blind probe contained only PBS, without any mushroom extract. The bacteria were incubated at 37°C for 24 h, the yeast was incubated at 30°C for 48 h, and the mould was incubated at 25°C for 7 days.

After incubation, inhibition zones around the filter disks were measured.

All experiments were performed in duplicate, for three times. The analysis of variance test ($P < 0.05$) was used to determine the statistical significance.

RESULTS AND DISCUSSION

Examination showed that the bioactive compounds derived from *G. lucidum* mushroom have had some influence on the observed microorganisms. PBS did not show any influence on the observed microorganisms.

The results of influence of ten different extracts derived from *G. lucidum* mushroom on the microorganisms are shown in the Table 1.

Tab. 1 — Influence of different extracts derived from *Ganoderma lucidum* on microorganisms

Microorganisms	Inhibition or stimulation zone in diameter (mm)									
	Mushroom extract									
	1	2	3	4	5	6	7	8	9	10
Salmonella enteritidis	2.66	2.66	—	1	2.66	3	—	2	3	2.66
Escherichia coli	2.33	1.33	—	—	2	2	—	—	3	2.5
Bacillus cereus	14.67*•	15*•	14.67*•	14*•	14.33*•	13.33*•	12*•	14*•	14*•	14*•
Proteus mirabilis	16*•	16*•	15*•	16*•	16*•	15.33*•	15*•	14*•	15*•	14.33*•
Staphylococcus aureus	—	—	—	—	—	—	—	—	—	—
Saccharomyces cerevisiae	—	—	—	—	—	—	—	—	—	—
Aspergillus niger	—	—	—	—	—	—	—	—	—	—

* stimulation of growth
— with no influence on growth
• significant difference was found between the treatment and the control ($p < 0.05$)

All investigated extract were derived by water extraction and alcohol precipitation, except the extract number 8 which was derived by hot water extraction.

The extracts derived from different strains and parts of fruitbodies of *G. lucidum* mushroom were used. These extracts were labeled with numbers 1—10:

1. Extract derived from the fruiting body of *G. lucidum*
2. Extract derived from the spore broken cell walls of *G. lucidum* Gl-K
3. Extract derived from the powdered fruiting body of *G. lucidum* K_1
4. Extract derived from the micro powdered fruiting body of *G. lucidum* Gl-K
5. Extract derived from the *G. lucidum* Gl-K hypha
6. Extract derived from the *G. lucidum* Gl-K powdered spores
7. Extract derived from the fruiting body of *G. lucidum* Gl-I by water extraction at room temperature

8. Extract derived from the fruiting body of *G. lucidum* Gl-I by hot water extraction

9. Extract derived from the fruiting body of *G. lucidum*-7

10. Extract derived from the fruiting body of *G. lucidum*-349

It was established that the extracts derived from *G. lucidum* Gl-K powdered spores (Fig. 4) and fruiting body of *G. lucidum*-7 had significantly (PT on bacteria *S. enteritidis*. The extracts derived from the fruiting body of *G. lucidum* Gl-I, the spore broken cell walls of *G. lucidum* Gl-K, the *G. lucidum* Gl-K hypha and the fruiting body of *G. lucidum*-349 (Fig. 2) showed reduced inhibition effects. The extracts derived from the powdered fruiting body of *G. lucidum* K_1 and from the fruiting body of *G. lucidum* Gl-I by water extraction at room temperature did not influence the growth of bacteria *S. enteritidis*.

The strongest inhibition effect on the growth of bacteria *E. coli* was observed in the case of the extract derived from the fruiting body of *G. lucidum*-349 (Fig. 1), and the extract derived from the fruiting body of *G. lucidum* Gl-I, while the extracts derived from the powdered fruiting body of *G. lucidum* K_1, the micro powdered fruiting body of *G. lucidum* Gl-K, the fruiting body of *G. lucidum* Gl-I by water extraction at room temperature and the fruiting body of *G. lucidum* Gl-I by hot water extraction, did not influence the growth of bacteria *E. coli*.

All investigated extracts derived from different kinds of *G. lucidum* mushroom showed significantly strong stimulating effects ($P < 0.05$) on the growth of bacteria *B. cereus* (fig. 5 and 6). The strongest effect showed the extract derived from the spore broken cell walls og *G. lucidum* Gl-K. The most reduced stimulating effect showed the extract derived from the fruiting body of *G. lucidum* Gl-I by water extraction at room temperature.

All investigated extracts had significantly strong stimulating effects ($P < 0.05$) on the growth of bacteria *Proteus mirabili*. The extracts derived from the fruiting body of *G. lucidum* Gl-I, the spore broken cell walls of *G. lucidum* Gl-K, the micro powdered fruiting body of *G. lucidum* Gl-K and *G. lucidum* Gl-K hypha (Fig. 3) were the strongest. The lowest stimulating effect showed

Fig. 1 — Inhibitory effect of extract derived from the fruiting body of *Ganoderma lucidum*-349 on the growth of bacteria *Escherichia coli*

Fig. 2 — Inhibitory effect of extract derived from the fruiting body of *Ganoderma lucidum*-349 on the growth of bacteria *Salmonella enteritidis*

Fig. 3 — Stimulating effect of extract derived from *Ganoderma lucidum* Gl-K hypha on the bacteria *Proteus mirabilis*

Fig. 4 — Inhibitory effect of extract derived from *Ganoderma lucidum* Gl-K powdered spores on the bacteria *Salmonella enteritidis*

Fig. 5 — Stimulating effect of extract derived from *Ganoderma lucidum* on the bacteria *Bacillus cereus*

Fig. 6 — Stimulating effect of extract derived from *Ganoderma lucidum* Gl-K, by hot water extraction, on the bacteria *Bacillus cereus*

the extract, derived by hot water extraction, from the fruiting body of *G. lucidum* Gl-I.

The investigated extracts did not show any effect on the growth of bacteria *S. aureus*, yeast *S. cerevisiae*, and mould *A. niger*.

CONCLUSIONS

G. lucidum, one of the oldest salutary remedy known for more than 3000 years, became a subject of interest in many contemporary science researching papers. Numerous experiments showed different possibilities of using of extracts derived from this mushroom in various disease treatments, improving immune system function which results in improving the general condition of an organism. One of the possible ways of utilising these extracts is their action on microorganisms which endanger human and animal health very often. Standard procedures in repression of harmful microorganisms are applications of antibiotics, but too much usage of antibiotics, and the ability of microorga-

nisms to develop resistance to them, result in their decreased effects on microorganisms. On the other hand polysaccharides derived from mushrooms became very interesting due to their influence on immune system and on microorganisms, that can be used in a struggle against them. In this work, the influence of some polysaccharides derived from *G. lucidum* mushroom on several microorganisms was investigated. In some cases the examined extracts showed very intensive influence, inhibitory or stimulating, while some of them did not show any influence on the examined microorganisms.

We believe that this investigation is of a current interest and should be continued with more microorganisms and numerous chemically defined fractions derived from the extracts of *G. lucidum* mushroom.

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УТИЦАЈ ЕКСТРАКАТА ИЗОЛОВАНИХ ИЗ ГЉИВЕ *GANODERMA LUCIDUM* НА НЕКЕ МИКРООРГАНИЗМЕ

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Резиме

Ganoderma lucidum (Leyss.: Fr.) Karst је једна од најважнијих традиционалних кинеских гљива. Према писаним подацима старим више од 2000 година користи се на Далеком истоку као здрава храна и лековита гљива. Један од врло интересантних аспеката коришћења гљиве *Ganoderma lucidum* је и употреба ове гљиве као антимикробног средства, захваљујући изолованим екстрактима који садрже бактериолитичке ензиме, лизозиме и киселе протеазе. Ефекти ових екстраката зависе од њиховог састава, начина екстракције и пречишћавања. Биоактивне компоненте изоловане из неколико сојева гљиве *Ganoderma lucidum* показале су различите ефекте на испитиване микроорганизме. У неким случајевима утицај екстраката је био врло интензиван, инхибишући или стимулишући, док неки од њих нису показали никакав утицај на испитиване микроорганизме.