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## MEDICINAL MUSHROOM *GANODERMA LUCIDUM* IN THE PRODUCTION OF SPECIAL BEER TYPES

**ABSTRACT:** Mushrooms like *Ganoderma lucidum* have been used for thousands of years as a traditional medicine in the Far East. *Ganoderma* received wide popularity as an eating mushroom with high nutritive value, but even more as medical fungi. It has been used for the treatment of various diseases: hepatitis, hypertension, insomnia, and even cancer. Due to its extraordinary action, it is often called “Elixir of life”, “Food of gods” and “Mushroom of universe”. The intracellular and extracellular polysaccharides ( $\beta$ -glucane) inhibit the growth of several types of cancer. Mushroom produces triterpenes of which especially ganoderic acid showed cytotoxicity on primary tumor liver cells, inhibition of histamine release, hepatoprotective effect, stimulation of the immune system functions, inhibition of the aggregation of blood plates, etc. On the other hand, beer as a purely natural beverage obtained in the process of fermentation, contains a number of ingredients which are important for human organism, and in moderate usage has favourable reaction on the general health condition of the body. As such, beer is a very good basis for the development of a number of new products with defined pharmacodynamics influence.

In this work, we have investigated the possibilities of using extracts of mushroom *Ganoderma lucidum* in the production of special beer types. The composition of mushroom, properties of the most important active ingredients, extraction procedures, and sensory characteristics of the beers on the basis of such extracts were determined. The most important parameters of quality and possibility of adjustments using extracts of different medicinal herbs were investigated.

### INTRODUCTION

Mushrooms like *Ganoderma lucidum* (Fr.) Karst (*Ganodermataceae*) has been a focus of public and health interest in recent years. It has been used in traditional Chinese medicine for centuries, and it is well known as *Ganoderma* in China, Reishi in Japan, and Young Zhi in Korea. During a long history period, it was regarded as a „Herb of Deathlessness” or „Miraculous King of the Herbs”. Over the past decade, *Ganoderma* was extensively researched in medi-

cal area. It contains numerous bioactive natural components, polysaccharides, ganoderic acids, ergosterols, proteins, unsaturated fatty acids, vitamins and minerals, with properties conducive to normalizing and balancing the body. They can enhance health and help in relief of a multitude of diseases (Zhou et al., 2007). Numerous studies have proved that anti cancer properties of *Ganoderma* come from polysaccharides, mainly from  $\beta$ -glucans. Polysaccharides are extracted with hot water, salt solutions, alkali solutions and dimethyl sulfoxide solution. Extractions are preformed from mycelia to dry fruit body of fungi. Among those, neutral polysaccharides ( $\beta$ -1 $\rightarrow$ 3,  $\beta$ -1 $\rightarrow$ 6 homo D-glucan), acidic glucan and polyglycan are bioactive. Glucan consisting of (1 $\rightarrow$ 3)-, (1 $\rightarrow$ 4)-, and (1 $\rightarrow$ 6)- $\beta$ -D linkages has been characterized with novel antitumor activity against oncogenesis and tumor metastasis (Yuen and Gehl, 2005). The cancer cell cytotoxicity can be explained as the direct killing of cells or the inhibition of cell proliferation. Using the tetrazolium (MTT) method, inhibition of proliferation has been shown in various cancer cell lines, including murine lymphocytic leukemia L1210 and Lewis lung carcinoma (LLC), human hepatoma PLC/PRF/5 and KB, human breast cancer MDA-MB-123, human prostate cancer PC-3, human breast cancer MCF-7, human cervix uteri tumor HeLa, and low-grade bladder cancer MTC-11 (Yuen and Gehl, 2005).

The other main group of *Ganoderma* components are triterpenoids. Their pharmacological effects are known as antioxidative, immune-modulating and antitumor. Major triterpenoides isolated from *Ganoderma* mushroom are different types of ganoderic acids. There are A, B and C ganoderic acids. Now, there are more than 130 oxygenated triterpenes (mostly lanostane-type triterpenes) that have been isolated from the fruiting bodies, spores, mycelia and culture media. They are divided into C30, C27 and C24 compounds according to the number of carbon atoms and based on the structure and the functional groups (Hui et al., 2004; Gao et al., 2005; Luo and Lin, 2002). It has been demonstrated that ganoderic acids -R, -T, -U, -V, -W, -X, -Y, and -Z; lucidimol-A and -B; Ganodermanondiol; ganoderiol F; and Ganodermanontriol exert cytotoxic-based carcinostatic effects on cancer cells, and many of them also possess anti-angiogenesis activity (Silva et al., 2003; Min et al., 2000). When we consider proteins and peptides isolated from *Ganoderma*, several active proteins and bioactive peptides have been isolated. Protein LZ-8 isolated from *Ganoderma* has shown to be mitogenic toward mouse splenocytes *in vitro* and immune-modulating *in vivo* by reducing antigen-induced antibody formation and by completely preventing the incidence of autoimmune diabetes in non-obese diabetic mice (Zhou et al., 2007). Immune-modulating activities of phytochemicals (Ganopoly) from *G. lucidum* affect the body's immune system through the following pathways: (1) By activating macrophage, Ganopoly facilitates the T-lymphocytes transferring to cytotoxic T cells, enhances the number and activity of the B-lymphocytes and the natural killer cells. (2) Ganopoly can activate the reticuloendothelial system and the complement system; induce the various immune factors, such as INF, TNF and so on. (3) It may have influence on the "Nerve Endocrine Immune System." (4) Ganopoly can facilitate RNA, DNA and protein synthesis in cells, and enhance the contents of the cGMP and cAMP in cells as well (Habijani et al.,

2001). Apart from the previously mentioned pharmacological functions, people in China use *Ganoderma* in the treatment of fatigue, coughing, asthma, insomnia, indigestion, hypertension, high cholesterol and neurosis; it could also reduce the side effects and pain during chemotherapy and radiotherapy for cancer patients (Zhou et al., 2007).

In recent years, usage of natural substances, such as herbs and medicinal mushrooms, has significantly increased. Beer, as a fully natural product containing numerous health promoting ingredients, can serve as a very good basis for developing a wide variety of products with specific pharmacodynamic activity. The purpose of this study was to evaluate the potential effects of *Ganoderma lucidum* extract for the production of beer for special health use.

## MATERIAL AND METHODS

### *Raw materials*

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

Tissue of mushroom *Ganoderma lucidum* was cut into pieces and mixed with alcoholic solution 70% vol. of ethanol. Extraction was performed by daily mixing at a magnetic stirrer for 10 minutes and then leaving the solution to stand in a dry and dark place at room temperature. Period of the extraction was 21 days. After the extraction, the solution was filtered and concentrated in vacuum to 1/5 of the initial content.

The prepared extract was added aseptically to commercially produced bottled pills, taking into account the recommended daily doses and sensory acceptability. After injection the bottles were immediately closed and matured at 5°C for one day.

### *Analysis*

*Ganoderma* extract, starting beer, and beers enriched with extract were analyzed by LC/MS and <sup>1</sup>H-NMR methods. LC/MS analysis was performed on an Agilent MSD TOF coupled to an Agilent 1200 series HPLC, using Zorbax Eclipse XDB-C18 column (RR, 30 x 2.1 mm i.d., 3.5 mm). Mobile A phase was 0.2 % formic acid in water, and mobile phase B was acetonitrile. The injection volume was 5 µl, and elution was performed at 0.7 ml/min with gradient program (0—1.5 min 5% B, 1.5—10 min 5—95% B, 10—15 min 95%B, 15—16 min). Mass spectra were acquired using an Agilent ESI-MSD TOF. Capillary voltage 4000 V, Fragmentor voltage 140 V, Nebulizer pressure 45 psig, drying gas 12 l/min, gas temperature 350°C, mass range (100—1500) m/z; negative and positive ionization mode. The processing of data was done with the software Molecular Feature Extractor.

The <sup>1</sup>H-NMR (200 MHz) spectra were recorded on a Varian Gemini 2000 spectrometer in CDCl<sub>3</sub>. Chemical shifts are given on the δ scale relative to TMS as internal standard.

### Sensorial evaluation

The consumers' acceptance test was carried out by 100 untrained consumers with the following average profile: 95% were 20–25 years of age, 29.5% of female gender, 72.4% with the consumption frequency of one or more beers per week, and 27.6% were beer non-drinkers. The beer samples were evaluated using a 5 point scale. Consumers did not have any formal training or experience in the description of beer flavour. An average value of each sensorial attribute and given consumers profile was calculated and expressed as “radial” diagrams.

## RESULTS AND DISCUSSION

Relative contents of active constituents in *Ganoderma lucidum* extract are shown in Table 1.

Tab. 1 — Relative content of constituents of *Ganoderma lucidum* extract

Constituents	RT/MS	Bruto molecular formulae	RRI*
Ganoderic acid E, d	4.575	C <sub>37</sub> H <sub>36</sub> O <sub>2</sub> ; C <sub>30</sub> H <sub>40</sub> O <sub>7</sub>	512.2
Ganoderic acid C 6	4.595	C <sub>30</sub> H <sub>42</sub> O <sub>8</sub>	530.2
Ganoderic acid G	4.721	C <sub>30</sub> H <sub>44</sub> O <sub>8</sub>	532.3
Ganoderic acid B, A	4.821	C <sub>30</sub> H <sub>44</sub> O <sub>7</sub>	516.3
Ganoderic acid C 2, C	4.951	C <sub>30</sub> H <sub>46</sub> O <sub>7</sub>	518.3
Ganoderic acid C, D, J	5.209	C <sub>30</sub> H <sub>42</sub> O <sub>7</sub>	514.2
Ganoderic acid B, A	5.521	C <sub>30</sub> H <sub>44</sub> O <sub>7</sub>	516.3
Ganoderic acid H	5.549	C <sub>32</sub> H <sub>44</sub> O <sub>9</sub>	572.2
Ganoderic acid E,d	6.004	C <sub>30</sub> H <sub>40</sub> O <sub>7</sub>	512.2
Ganoderic acid F	6.163	C <sub>32</sub> H <sub>42</sub> O <sub>9</sub>	570.2
Ganoderic acid C, D, J	6.180	C <sub>30</sub> H <sub>42</sub> O <sub>7</sub>	514.2
Ganodermanontriol, lucidumol A	7.389	C <sub>30</sub> H <sub>48</sub> O <sub>4</sub>	472.3

\* RRI-relative retention index

The presence of ganoderic acids A, B and Y was identified in the investigating extract. The isolated terpenoids have biological effect on cholesterol synthesis in human hepatic cell line *in vitro*. Ganoderic acids A and B inhibit hypoglycemic effects in several test systems and ameliorate the symptoms of diabetes. Ganoderic acids A and C inhibit farnesyl protein transferase, which catalyzes posttranslational farnesylation of Ras oncoprotein and is essential for the cell-transforming activity of Ras. Isolated ganoderic acids F contribute to atherosclerosis protection by the inhibition of angiotensin converting enzyme or platelet aggregation. Ganoderiol F, lucidimol A and ganodermanontriol identified from the extract have cytotoxic effect on LLC and Meth-A cancer cells. Ganodermanontriol founded in the extract inhibit HIV-1 protease (Zhou et al., 2007). This means that the usage of *Ganoderma lucidum* extract could potentially have positive effect on individuals with poor health.

The results of the sensory acceptance test of the samples compared with the starting beer are given in Figures 1 to 4.

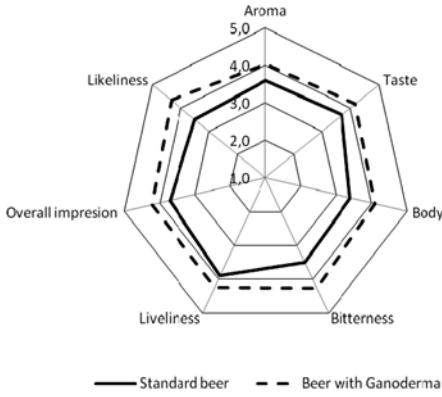


Fig. 1 — Sensory evaluation of standard beer samples and beer with *Ganoderma* by male beer drinkers

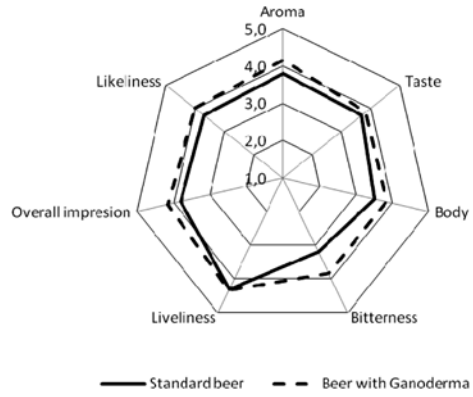


Fig. 2 — Sensory evaluation of standard beer samples and beer with *Ganoderma* by male beer abstinent

The consumers' acceptance test of male beer drinkers has shown that beer enriched with *Ganoderma* extract is absolutely compatible with standard beer. It is shown that in all analyzed parameters the enriched beer was even superior. The consumers have pointed out that fullness and bitterness were enhanced and enjoyable (Figure 1). The beer abstinent had almost similar opinions as the beer consumers, and the only exception was the freshness which was ranked as that of the standard beer (Figure 2). Both male beer drinkers and the abstinent have agreed that the obtained beer had full taste and pleasant bitterness. They have emphasized that bitterness was enhanced and the beer was strong.

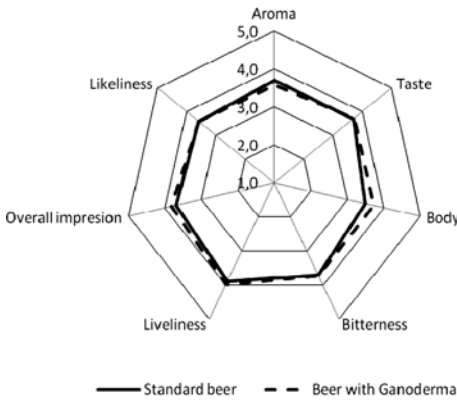


Fig. 3 — Sensory evaluation of standard beer samples and beer with *Ganoderma* by female beer drinkers

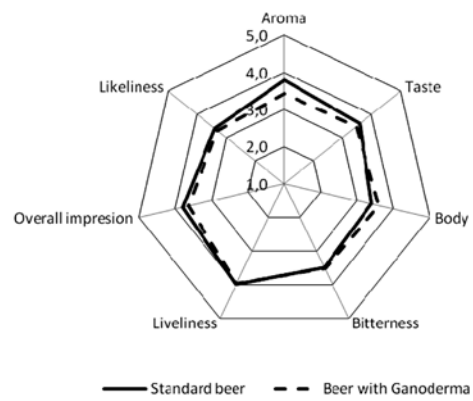


Fig. 4 — Sensory evaluation of standard beer samples and beer with *Ganoderma* by female beer abstinent

On the other hand, female beer consumers evaluated the starting standard beer with slightly higher grades than male drinkers, and found no big difference between the standard beer and beer enriched with *Ganoderma* (Figure

3). According to their opinion, the beer with *Ganoderma* was almost similar to the standard beer with slightly enhanced fullness and overall impression. As it could be expected, the female beer abstinenters graded the starting standard beer a bit worse than the beer drinkers, but also gave priority to the enriched beer. The only flaw they found was related to aroma, which was considered as rather poor (Figure 4). There was immense difference between male and female testers. Both male beer consumers and the abstinenters preferred full taste and strong bitterness, while female consumers normally preferred beer with lighter taste and aroma.

## CONCLUSION

The beer supplemented with *Ganoderma lucidum* extract as a natural source of nutritional supplements is very pleasant and even acceptable to beer abstinenters. Male beer drinkers and abstinenters conciliate in that the obtained beer is even superior in comparison to the starting standard beer in all tested sensory parameters. The obtained results indicate that standard beer can be successfully enriched with *Ganoderma* extracts as a source of natural nutritional supplements. Developed beer is superior not only in pharmacodynamic properties, but also in sensorial impression. Considering the above mentioned, it is obvious that the combination of beer and medicinal mushrooms and herbal extracts in recommended daily doses can give products with satisfactory sensorial properties. Numerous experiments showed different possibilities of using extracts derived from *Ganoderma lucidum* mushroom in various disease treatments, by improving immune system function which results in better general condition of organism. Such special beer base products should have predictable pharmacodynamic properties and can potentially be recognised as „traditional herbal medicinal products”. Their base is beer, fully natural product with tradition longer than 6000 years, with evident positive effect on the overall health condition and with the presence of alcohol as the only limiting factor. By eliminating alcohol, using alcohol-free beer as a base product and/or implying to adequate „suggested use”, this problem may be avoided.

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## МЕДИЦИНСКА ГЉИВА *GANODERMA LUCIDUM* У ПРОИЗВОДЊИ СПЕЦИЈАЛНИХ ПИВА

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### Резиме

Гљиве попут *Ganoderma lucidum* коришћене су хиљадама година у традиционалној народној медицини Далеког истока. Своју велику популарност *Ganoderma* је стекла као јестива гљива са високом нутритивном вредношћу, али још више као медицинска гљива. Коришћена је за лечење разних болести, између осталих и хепатитиса, хипертензије, инсомније, па и карцинома. Управо због свог изванредног деловања називана је „Еликсир живота”, „Храна богова”, „Гљива универзума”. Њени интрацелуларни и екстрацелуларни полисахариди ( $\beta$ -глюкани) доказано инхибирају раст неколико врста карцинома, а тритерпени које продукује, посебно ганодеринска киселина, показују цитотоксичност на примарне туморне ћелије јетре, инхибицију ослобађања хистамина, хепатопротективни ефекат, стимулацију функције имуног система, инхибицију агрегације крвних плочица итд. Са друге стране, пиво као потпуно природно пиће добијено ферментацијом, садржи многобројне састојке од значаја за организам и у умереној количини показује повољно деловање на опште здравствено стање организма. Као такво, пиво је веома добра подлога за развој низа нових производа дефинисаног фармакодинамичког деловања.

У раду је испитана могућност коришћења екстраката гљиве *Ganoderma lucidum* у производњи специјалних пива. Дати су састав гљиве, својства најважнијих активних састојака, поступци екстракције и сензорне карактеристике пива добијених на бази датих екстраката. Испитани су најважнији параметри квалитета и могућност кориговања употребом екстраката различитог лековитог биља.