Aalto University
School of Electrical
Engineering

School of Electrical Engineering
Degree Programme of Bioinformation Technology

Caroline Ingo

A COMPARATIVE STUDY BETWEEN THE MEDICINAL MUSHROOMS *INONOTUS OBLIQUUS* AND *GANODERMA LUCIDUM* – AN ANALYSIS BETWEEN POLYSACCHARIDES, TRITERPENES AND THEIR FUNCTIONS

Master's thesis for the degree of Master of Science in Technology submitted for inspection, Espoo, 25 November, 2013.

Supervisor **Professor Heikki Ojamo**

Instructor **Professor Heikki Ojamo**

M.Sc. Atte Lötjönen

Acknowledgements

This thesis was written for the company Four Sigma Foods as a part of my trainee period in Hong Kong. Collection of information started already in January 2013, but the work itself was made from June to November.

I would like to thank Professor Heikki Ojamo and my supervisor Atte Lötjönen for all good advices and comments. Huge thanks to Mikko Revonniemi, Tero Isokauppila and Lari Laurikkala for introducing the mushrooms for me and for all support and advices. Thanks to the whole FSF team for a fantastic period both in Hong Kong and Finland.

Finally I would like to thank my parents for the support I have got during both happy and hard times, you are always there to encourage and help. Thanks to my sister for answering my questions about the writing process and of course for everything else you have done for me. Thanks to friends for being friends and thanks to everyone I have met during my studies that made it to such a fantastic time.

Vaasa, 25 November 2013

Caroline Ingo

Aalto University
School of Electrical
Engineering

Aalto University, P.O. BOX 11000, 00076 AALTO
www.aalto.fi

Abstract of master's thesis

Author Caroline Ingo

Title of thesis A comparative study between the medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum* – An analysis between polysaccharides, triterpenes and their functions

Department Department of Biotechnology and Chemical Technology

Professorship Bioprocess engineering **Code of professorship** Kem-70

Thesis supervisor Professor Heikki Ojamo

Thesis advisor(s) / Thesis examiner(s) Professor Heikki Ojamo, M. Sc. Atte Lötjönen

Date 25.11.2013 **Number of pages** 70 **Language** English

Abstract

The object for this master's thesis was to compare the medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum*. The most important compounds for both of them are polysaccharides and triterpenes. Both the compounds and the properties of the mushrooms are similar, despite that they are different in several ways.

In the theoretical part are some basic facts about medicinal mushrooms presented together with the compounds. *Inonotus obliquus* and *Ganoderma lucidum* are introduced more extensively with their compounds and properties.

The analytical part compares the contents of the mushrooms, and the properties they have depending on if polysaccharides or triterpenes induce them. Both similarities and differences between the mushrooms are reviewed.

The analysis shows that *Inonotus obliquus* and *Ganoderma lucidum* contain β-glucans as the most important polysaccharides. There are some differences between the glucans, but in general they act in the same way by having immunostimulating and antioxidative activities, as well as lowering the blood glucose and cholesterol levels. *Inonotus obliquus* contains much more carbohydrates than *Ganoderma lucidum* that affects the amount of polysaccharides and makes it for instance a stronger immunostimulator. The triterpenes of both mushrooms are different, but most of them have the same base. They have antioxidative, anti-cancer, anti-virus and anti-fungal effects, as well as lowering cholesterol effects. Triterpenes from *Ganoderma lucidum* have also adaptogenic and anti-allergical effects, and can inhibit harmful cardiovascular activities. *Ganoderma lucidum* contains more lipids than *Inonotus obliquus*, which indicates that it has stronger properties induced by triterpenes.

The dissimilarity in the contents of the mushrooms gives differences in their otherwise similar properties.

Keywords Medicinal mushrooms, Inonotus obliquus, Ganoderma lucidum, polysaccharides, triterpenes

Aalto-universitetet
Högskolan för
elektroteknik

Aalto-universitetet, PB 11000, 00076 AALTO
www.aalto.fi

Sammandrag av diplomarbetet

Författare Caroline Ingo

Titel En jämförelsestudie mellan läkesvamparna *Inonotus obliquus* och *Ganoderma lucidum* – En analys mellan polysackarider, triterpener och deras funktioner

Institution Institutionen för bio- och kemiteknik

Professur Bioprocessteknik **Kod för professuren** Kem-70

Övervakare Professor Heikki Ojamo

Handledare / Granskare Professor Heikki Ojamo, M. Sc. Atte Lötjönen

Datum 25.11.2013 **Sidantal** 70 **Språk** Engelska

Sammandrag

Målet med det här diplomarbetet var att jämföra läkesvamparna *Inonotus obliquus* och *Ganoderma lucidum*. De viktigaste kemiska ämnena för dem båda är polysackarider och triterpener. Föreningarna är relativt likadana och deras egenskaper liknar varandra, trots att de skiljer sig på flera sätt.

I teoridelen presenteras basfakta om läkesvampar samt deras polysackarider och triterpener. *Inonotus obliquus* och *Ganoderma lucidum* introduceras lite mer omfattande samt deras kemiska föreningar och egenskaper.

Analysdelen jämför svamparnas uppbyggnadskomponenter samt de egenskaper svamparna har beroende på om det är polysackarider eller triterpener som har alstrat dem. Både likheter och olikheter mellan svamparna granskas.

Analysen visar att både *Inonotus obliquus* och *Ganoderma lucidum* innehåller β-glukaner som de viktigaste polysackariderna. Det finns vissa skillnad mellan deras glukaner, men de verkar långt på samma sätt genom att stimulera immunförsvaret, inneha antioxidanta effekter och sänka kolesterol nivån och blodsockret. *Inonotus obliquus* innehåller en betydligt större mängd kolhydrater jämfört med *Ganoderma lucidum*, detta pekar på att den även innehåller större mängd polysackarider och är således effektivare, speciellt som immunstimulerare. Triterpenerna hos de båda svamparna är olika, men de flesta har samma bas. Bådas triterpener har antioxidanta, anticancer, antivirala och antifungala effekter, samt kolesterol sänkande effekter. Utöver det har triterpener från *Ganoderma lucidum* även adaptogena och antiallergiska effekter, samt inhiberande effekter på skadliga kardiovaskulära aktiviteter. *Ganoderma lucidum* har visat sig innehålla mer lipider än *Inonotus obliquus*, vilket tyder på att den har starkare egenskaper alstrade av triterpener.

Olikheterna i svamparnas grundkomponenter ger skillnader i deras i övrigt likande egenskaper.

Nyckelord Läkesvamp, Inonotus obliquus, Ganoderma lucidum, polysackarider, triterpener

Contents

Nomenclature

AIF	Apoptosis inducing factor
ConA	Concanavalin A
CR3	Complement receptor 3
Da	Dalton, molecular weight
FDA	Food and Drug Administration
HMPC	Committee on Herbal Medicinal Products
IBD	Inflammatory bowel disease
IFN-γ	Interferon γ
IL	Interleukin
LD_{50}	Median lethal dose
LPS	Lipopolysaccharide
MDA	Malondialdehyde
Mn-SOD	Manganese superoxide dismutase
NF-κB	Nuclear factor-κB
NK cells	Natural killer cells
NO	Nitric oxide
ROS	Reactive oxygen species
SOD	Superoxide dismutase
STZ	Streptozotocin
TCM	Traditional Chinese Medicine
TNF-α	Tumor necrosis factor α
WHO	World Health Organization

1 Introduction

The introduction presents the motivation and objectives of the thesis, explains the most used terms and the methods used for the thesis, as well as looks at the structure of the work.

1.1 Motivation and objectives

The two medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum* both contain groups of active compounds such as polysaccharides and triterpenes. Some of the compounds differ in each species, but others are similar. In many cases, they are thought to have same properties, but seem to be acting in different ways. In this thesis I will analyze the differences and similarities between some of the main polysaccharides and triterpenes found in the mushrooms and their functions. Especially how it comes that the same compounds have different functions in the two mushrooms. Besides, there are no other studies to be found, where these two mushrooms and their active compounds have been compared before. The thesis is written for Four Sigma Foods, a company producing medicinal mushrooms, as a project to get more and deeper information about their two main products Instant Chaga, containing *Inonotus obliquus* and Instant Reishi, *Ganoderma lucidum*.

1.2 Definition of terms

The most typical terms that will be used in the thesis are medicinal mushrooms, polysaccharides and triterpenes, and their varieties. Here I give a brief explanation of the terms.

Medicinal mushrooms contain bioactive compounds, for instance polysaccharides and triterpenes that are used for medicinal applications. Usually their hard texture and bitter taste make them impossible to digest, which means they have to be prepared before any kind of application. Here, the medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum* are in focus.

Polysaccharides are long carbohydrate chains, containing monosaccharide units linked together with glycosidic bonds. They can be classified as biological macromolecules and they present the highest capacity of carrying biological information since they have the greatest potential for structural variability. The monosaccharide units can interconnect at several points to form a variety of branched or linear structures. A general formula for polysaccharide is $C_x(H_2O)y$ where *x* usually is a high number from 200 to 2500. Due to the fact that the monosaccharides that often are repeated in the chain contain six carbons, another formula would be $(C_6H_{10}O_5)_n$ where *n* can be anything from 40 up to 3000. [1] Several different polysaccharides can be found in medicinal mushrooms, but the most typical ones are β-glucans, like the one in figure 1. The linkages between the glucans are very important for the properties that glucans have. [2]

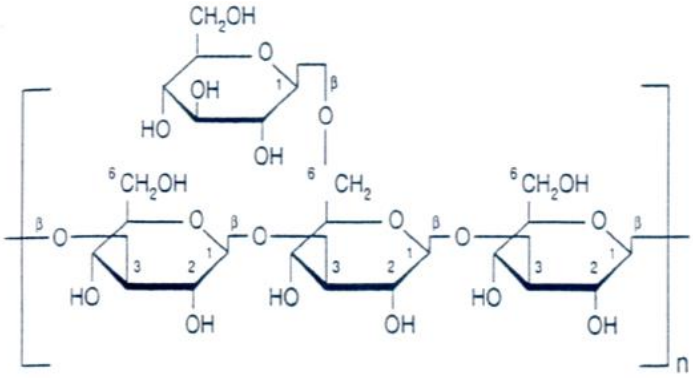


Figure 1. A typical diagram of a β-D-glucan. The main chain is β(1-3)-D-glucan with a β(1-6)-branch. [2]

Triterpenes belong to the versatile group of terpenes, consisting of isoprene C_5 units, see an example in figure 2. The name terpene comes originally from turpentine, which was made from resin of pine trees. [3, 4] Terpenes are divided into subgroups according to the number of isoprene units: mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), ses- (C_{25}), tri- (C_{30}), and tetraterpenes (C_{40}). The molecule is either acyclic or has a cyclic structure with one or several rings. Typical examples of terpenes are menthol (monoterpene) and β-carotene (tetraterpene). [5, 6] Both triterpenes and triterpenoids are usually used to describe the same C_{30}-terpenes. Triterpenoid is said to be a broader term, since it covers natural degradation products, beyond the natural terpenes that the expression triterpenes is used for. [5, 7] Many of the terpenes found in medicinal mushrooms are triterpenoids, even though the term triterpenes will be used in this work to cover all kind of terpenes.

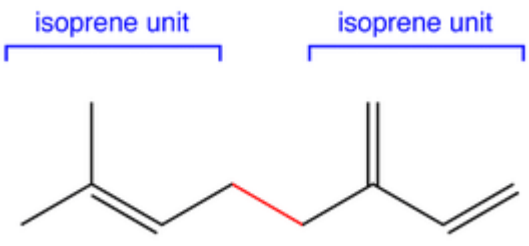


Figure 2. A simple terpene of two isoprene units.

1.3 Research method

The research method is based on literary material. The material is found mostly from scientific databases like PubChem, SciFinder, and Wiley Online Library, by searching for words, terms and combinations of them, such as medicinal mushrooms, *Inonotus obliquus*, *Ganoderma lucidum*, polysaccharides, triterpenes, immunostimulating activity, anti-cancer, et cetera. Based on the written theory, the similarities and differences between the compounds and the two mushrooms are described and their functions analyzed.

1.4 Structure of the thesis

The theoretical part consist of chapter 2, 3 and 4. In chapter 2, some basic facts about medicinal mushrooms and their compounds are introduced. In chapter 3 and 4 *Inonotus obliquus* respectively *Ganoderma lucidum* are introduced separately and their active compounds and functions are presented more in detail. Chapter 5 gives the result by analyzing the similarities and differences with focus first on contents and compounds. Later the overall properties caused by polysaccharides and triterpenes are analyzed and closer connections and differences are tried to be found. In chapter 6, the result are discussed and summarized together with error analysis and suggestions for further work.

2 Medicinal mushrooms

Medicinal mushrooms belong to the kingdom fungi. Fungi represent one of the five kingdoms of life [8]. They differ from bacteria due to the fact that they are eukaryotes with well-defined membrane-surrounded nuclei, containing a definite number of chromosomes. Opposite to green plants, they are heterotrophic, and cannot produce their own energy with sunlight and inorganic compounds. Furthermore, most of the fungi have cell walls, through which the nutrients must pass in a soluble form, which distinguishes them from animal cells that don´t have cell walls. [2] The mammals separated from the fungus 460 million years ago, but we still share 30% of the same genes with the fungus. In the world there are estimated to be around 1.5 million different fungus species, but only 14-22,000 of them are known, even though there are reports arguing that the amount of fungi can vary from 500,000 to 9.9 million species. [9] The number of safe mushrooms are said to be around 700-2000, depending on what report you read, and 400-700 mushrooms with medicinal properties. [9, 10]

In this chapter you will get a better description of what medicinal mushrooms are, how they are classified, what the most typical therapeutic applications are, some of the regulations for them and some basic facts about their polysaccharides and triterpenes.

2.1 Classifications of mushrooms and their history

Fungus can be divided into classes, such as micro- and macrofungi, where microfungi are molds, rusts and mildews. Fungi and mushroom are not synonyms. The term mushroom may vary depending on who you ask, but the most used description is that “mushroom is a macrofungus with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand”.[11] Mushrooms are usually

divided into four different groups; edible, poisonous, hallucinogenic and medicinal mushrooms. [2, 3] Medicinal mushrooms contain therapeutic properties and not only nutritional value, as most mushrooms do. Medicinal mushrooms are usually not edible because of their coarse and hard texture or bitter taste. Compared with edible mushrooms, they have more fungal cell wall material and secondary metabolites that have a wide range of pharmacological activities. The fungal cell wall contains mainly β-glucan-chitin complexes and mannoproteins that cannot be digested in the human gastrointestinal tracts, due to the lack of enzymes that digest them. [2, 12] The fungi kingdom contains five phyla, a rank between kingdom and class: *Chytridiomycota*, *Zygomycota*, *Glomeromycota*, *Ascomycota* and *Basidiomycota*. Mushrooms are *Basidiomycota* and some are *Ascomycota*, while the rest of the phyla are different kinds of microfungi. Most of the medicinal mushrooms belong to the phylum *Basidiomycota* that is known as higher fungus. *Basidiomycota* means club-fungi and the spores are produced attached to the club-shaped structure. [2, 9]

The use of fungi as a medicine dates back to as long as 3000 BC, when macrofungi was used to treat diseases in traditional folk medicines. [13] Medicinal mushrooms are most known for being used in the Traditional Chinese Medicine (TCM). One of the earliest books found in China about mushrooms dates back to 100-200 BC. [2] Medicinal mushrooms have not only been used in the East, they have also been a part of the European medicine in the past. But as time has changed, folk medicine in the West was considered backward. In the East, Chinese never draw a distinction between the folk medicine and higher medicine, due to the evidence that the medicine cured illnesses. Other explanations why medicinal mushrooms still continue to live in the Eastern medicine are for instance Buddhist monks spreading the information around Asia during their walks and Taoist priests using the mushrooms for healing. [3] Nowadays the medicinal mushrooms have become more widely used, due to the increasing number of scientific researches done about them. They are especially popular in Japan, China and Korea, but seem to come more and more so even in the Western world. [2] After penicillin was discovered in 1929, fungi were regarded as rich sources of natural antibiotics and other bioactive compounds. [13]

2.2 Therapeutic effects, active compounds and different species

The therapeutic effects of medicinal mushrooms include antioxidant activity, effects to lower high blood pressure and cholesterol, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial effects. One of the strongest treatment areas coming up is medicinal mushrooms fighting against cancer. Although, mushrooms have been used for a long time in Asia and Russia, it is only four decades ago it became possible to isolate the relevant compounds for this purpose and start to use them in controlled experiments.[2] Medicinal mushrooms are considered as strong immunoregulators that strengthen the immune system when it is weak and weaken it when it is overactive. [3]

Medicinal mushrooms contain several bioactive compounds that give them their therapeutic properties. Polysaccharides are one of the best known groups and the most potent substances with anti-tumor and immunomodulatory effect. Moreover, triterpenes, sterols, amino acids, as well as nicotinic acid, riboflavin (vitamin B2), pantothenic acid, and vitamin B, C, and K are typical for medicinal mushrooms. [3, 13] Mushrooms are overall a good nutritional source with a high amount of proteins (10-40% of dry weight), low in carbohydrates (3-21%), and high on fiber (3-35% of dry weight). [2]

Typical species of medicinal mushrooms are *Inonotus obliquus*, *Ganoderma lucidum*, *Ophiocordyceps sinensis*, and *Trametes versicolor*. There are several edible mushrooms with therapeutic properties that can be classified as both medicinal and edible mushrooms, for instance *Lentinus edodes* (Shiitake) and *Grifola frondosa* (Maitake). [2] The most common medicinal mushrooms together with their main active compounds and therapeutical effects are collected in table 1.

Table 1. The most common medicinal mushrooms and their main active compounds and main effects.

Species	Common name	Active compound	Effects	References
Inonotus obliquus	Chaga	Polysaccharides, polyphenols, triterpenes	Anti-tumor, immuno-stimulating, antioxidative	[14-18]
Ganoderma lucidum	Reishi	Polysaccharides, triterpenes	Anti-tumor, anti-HIV, antioxidative, cholesterol lowering, immuno-modulative, adaptogenic	[19-22]
Ophiocordyceps sinensis	Cordyceps / Caterpillar fungus	Polysaccharides, cordycepin, sterol	Antioxidative, anti-fatigue, production of testosterone, anti-cancer	[23-26]
Hericium erinaceus	Lion's mane	Hericenones C to H, erinacines A to I	Immuno-modulative, improve the production of nerve growth factor, cognitive	[27-30]
Lentinus edodes	Shiitake	Lentinan, glucans	Anti-microbial, anti-bacterial, anti-tumor, immuno-modulative	[31]

Grifola frondosa	Maitake	Polysaccharides, D-fraction	Immuno-modulative, anti-cancer	[32-34][32, 33]
Trametes versicolor	Turkey tail	Polysaccharo-peptides (PSP), protein-bound polysaccharide K (PSK)	Anti-cancer, immuno-modulative	[35, 36]
Agaricus blazei	Agaricus	Protein-bound polysaccharides, polysaccharides	Anti-cancer, immuno-modulative	[37]

Medicinal mushrooms can be taken in three main ways; as a kind of fluid extract, capsule, or powder. Medicinal mushrooms in capsule form usually come from dried and powdered mycelium, and from fruiting body or dried extract. The powder is encapsulated or pressed into pills. In extract form, water and alcohol are used to extract the active compounds of the mushroom. Water extracts mainly polysaccharides and alcohol extracts mostly triterpenes and sterols. [2, 3] Intake of multiple mushrooms is considered more effective, since each mushroom appears to produce its own unique type of polysaccharides and triterpenes. One may for instance stimulate the production of T cells while another helps natural killer (NK) cells to do their job. [3]

2.3 Regulations and safety

Medicinal mushrooms are usually not classified as pharmaceuticals but rather as dietary supplements, functional food, nutriceuticals, phytochemicals, mycochemicals, biochemopreventives, or designer foods depending on the country. Terms, such as functional food refer to food that has special beneficial effects on the human health and is a part of the standard diet. The regulations for

these products vary from country to country. [11, 38] In 1991 the World Health Organization (WHO) published "Guidelines for the Assessment of Herbal Medicines". The objective for these guidelines is to "define basic criteria for the evaluation of quality, safety, and efficacy of herbal medicines and thereby to assist national regulatory authorities, scientific organizations, and manufacturers to undertake an assessment of the documentation/submission/dossiers in respect to such products." Medicinal mushrooms are included to herbal medicines. [39] In the United States, Food and Drug Administration (FDA) is one of the decision making bodies for the safety regulations of medicinal mushrooms. In Europe the Committee on Herbal Medicinal Products (HMPC) is the preparing committee at the European Medicines Agency that is responsible for preparing the Agency's opinions on herbal medicines. In 2004 simplified registration procedure was introduced to overcome difficulties encountered by member states in applying pharmaceutical legislation to traditional herbal medicinal products in a uniform manner. A list of herbal substances, preparations and combinations had been established on the scientific opinion of the HMPC. "The simplified procedure allows the registration of herbal medicinal products without requiring particulars and documents on tests and trials on safety and efficacy, provided that there is sufficient evidence of the medicinal use of the product throughout a period of 50 years, including at least 15 years in the Community." [40-42] Compared to the U.S., European countries do not wait for proof before they take actions against a product if they suspect that it may cause harm. [11]

All products that affect body functions, for instance blood pressure, immune response et cetera are called pharmacodynamics, which are said to lack safety at too high dosages. This means that medicinal mushrooms cannot have pharmacological action without toxicological action. However, it is commonly believed that certain mushrooms can be considered safe due to their long time of use. Though, the "safe" in historical times is different from the term in the modern time. The majority of medicinal mushrooms used as dietary supplements are cultivated commercially and are not from the wild. This guarantees proper identification and pure products, which means they can be considered safer in practice. [11] Usually when someone has a bad reaction after taking multiple mushrooms, the cause is a lack of an enzyme needed for digesting a particular

mushroom or compound. Very few allergic reactions have ever been recorded when taking medicinal mushrooms. This means that either taking the mushrooms alone or mixing them together can be considered safe. Mushrooms collect and neutralize toxins, toxic heavy metals and other by-products. Because of this, it is very important that the quality of the mushrooms that are used for dietary supplement is tested carefully and that they are cultivated in clean environments to guarantee non-toxic products. [3]

2.4 Polysaccharides

Bioactive polysaccharides in medicinal mushrooms can be found in mycelium, in the fruiting body, in spores and in sclerotium. Fungal cell walls contain mushroom polysaccharides that exist as structural components in the wall. Two major types of polysaccharides can be found in the wall; (1) rigid fibrillar of chitin or cellulose and (2) a matrix-like β-glucan, α-glucan and glucoproteins. Schizophyllan is a water-soluble (1-6)-branched β(1-3)-glucan that is attached to the outer layer of the fungal cell wall, and secreted to the extracellular matrix (exopolysaccharides). This makes the wall resisting the pressure from outside. [12]

One of the polysaccharides that have been identified as the major biologically active polysaccharide is β-D-glucan. The basic β-D-glucan is a repeating structure with the D-glucose units joined together in linear chains by beta-bonds. These can extend from carbon 1 of one saccharide ring to carbon 3 (β(1-3)), carbon 4 (β(1-4)) or carbon 6 (β(1-6)), see an example in figure 3. β-Glucan is a big molecule; those found in mushrooms have a molecular weight of 1.5 up to 2 million Dalton (Da). Glucans with high molecular weight have been reported to be more effective than those with lower molecular weight. Even solubility in water, size of the molecules, branching rate and form has been shown to correlate with activity of glucans. [3, 9, 43]

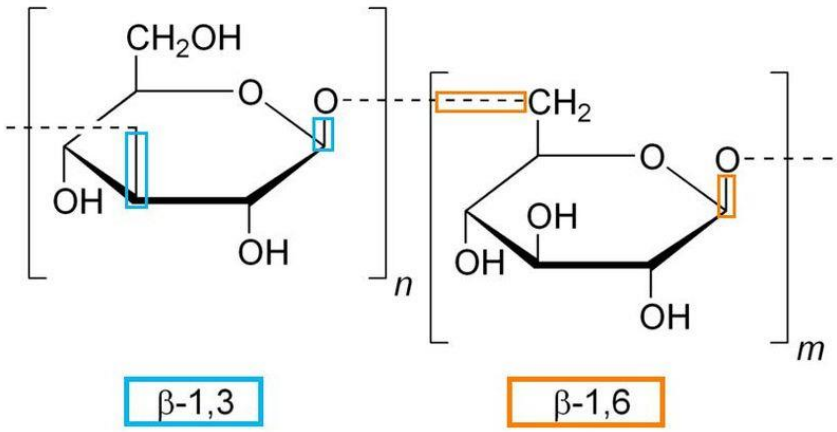


Figure 3. A schematic figure over β(1-3) and β(1-6)-glucan.

Most of the *Basidiomycota* mushrooms have been shown to contain polysaccharides that have anti-tumor and immunostimulative effects. Anti-tumor polysaccharides isolated from mushrooms are either water-soluble β-D-glucans with heterosaccharide chains of xylose, mannose, fructose, ribose, galactose and uronic acids or β-D-glucan-protein/-peptide complexes, so called proteoglycans. [3, 12] The structural features are important for anti-tumor action, for instance glucan with β(1-3) linkages in the main chain and additional β(1-6) branch points has high anti-tumor effect compared with β-glucans containing mainly (1-6) linkages. In general, β-glucans with more (1-6)-linkages have less anti-tumor activities. Beyond the linkages of the main chain and side branches, the degree of branching is another factor that affects the immunostimulating activity. Triple-helical tertiary conformation of β(1-3)-glucans from medicinal mushrooms is known to be important for the immunostimulative effects. [9, 43] In triple helical β-glucans, three β-glucan are chains linked together forming a helix. Figure 4 shows an illustration of this. [44] The big size of β-glucans is said to be connected with the immunostimulating activity. [3] There are though exceptions like α(1-3)-glucuronxylomannans (53-1000 Da) that are not strongly dependent on molecular weight, but is as effective as the polysaccharides with high molecular weight. [9, 12]

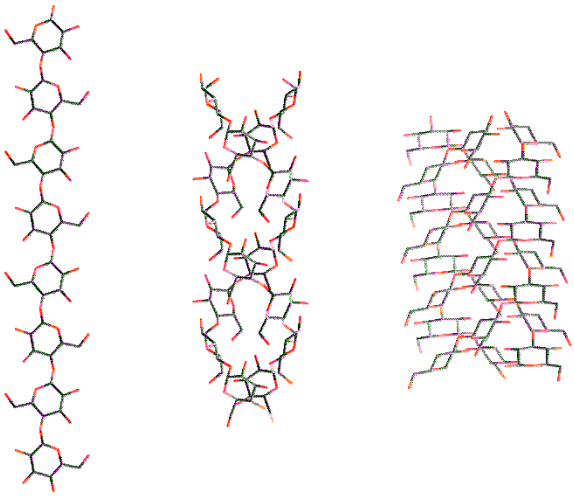


Figure 4. A single helix, a double helix and a triple helix of polysaccharides. The triple helix is a β(1-3)-glucan. [44]

Anti-tumor polysaccharides from medicinal mushrooms do not attack cancer cells directly, but activate the immune system in the host organism that fight against cancer cells [9, 43]. It has been shown that β-glucans bind to specific membrane receptor of phagocytic cells and NK cells, stimulating their germ-killing abilities. Each mushroom seems to have its own different type of β-glucan that means that each mushroom stimulate the immune system in different ways. This is also a reason why it is a good way to administrate different kinds of medicinal mushrooms. [3] Moreover, polysaccharides from mushrooms help the body to adapt to various environmental and biological stresses, as well as supporting nervous, hormonal, immune system, and regulatory functions. [9] β-glucans have also shown that they have effect against high blood cholesterol and asthma. [3]

2.5 Triterpenes

As mentioned in the introduction, triterpenoids are the broader term, but here we use the term triterpenes for them both. Triterpenes can also be classed under groups such as cholesterol and phytosterol. [45] Triterpenes are one of the most

important and diverse group of active compounds found in medicinal mushrooms [12]. They can be isolated both from the mycelium and the fruiting body, and sometimes even from the spores. In general, they have a molecular weight around 400-600 kDa and the chemical structure is complex and oxidized. [19, 46] Triterpenes are not only found in medicinal mushrooms, they are also important compounds in many other medicinal plants. [3, 47]

Triterpenes has been used as antiseptics since the ancient Greeks time and nowadays they are an important help for the immune system, since they are said to have immunomodulatory effects. Most triterpenes are also anti-inflammatory and have anti-microbial, anti-viral and anti-fungal effects. Moreover, triterpenes have hepatoprotective, painkilling and tonic effects. Tonic effects mean that the triterpenes help the body to tone and improve well-being. One of the most important effects of triterpenes is the anti-tumor effect that, contrary to polysaccharides, attack the tumors directly and not through the affect on the immune system. [3, 6, 19, 47]

The most known of the triterpenes found in medicinal mushrooms are ganoderic acid and lucidenic acid, which are found in *Ganoderma lucidum*, as well as, lanosterol and betulinic acid, found in *Inonotus obliquus*. [19, 48, 49]

3 *Inonotus obliquus*

The mushroom *Inonotus obliquus* is a white rot fungus that belongs to the *Hymenochaetacae* family of *Basidiomycetes*. In nature, it is parasitic and grows as a sterile conk (sclerotia) on the trunks of trees, usually *Betula* species, such as birches, at the latitude of 40° N-68° N in Russia, Korea, Northern and Eastern Europe, Northern China and Northern areas of the United States. Worldwide *Inonotus obliquus* is more known under the name Chaga, which comes from the Russian name for the mushroom, *Tsaga*. Another Latin name for the mushroom is *Fuscoporia obliqua.* [2, 10, 50] Figure 5 shows a several pictures of *Inonotus obliquus*.

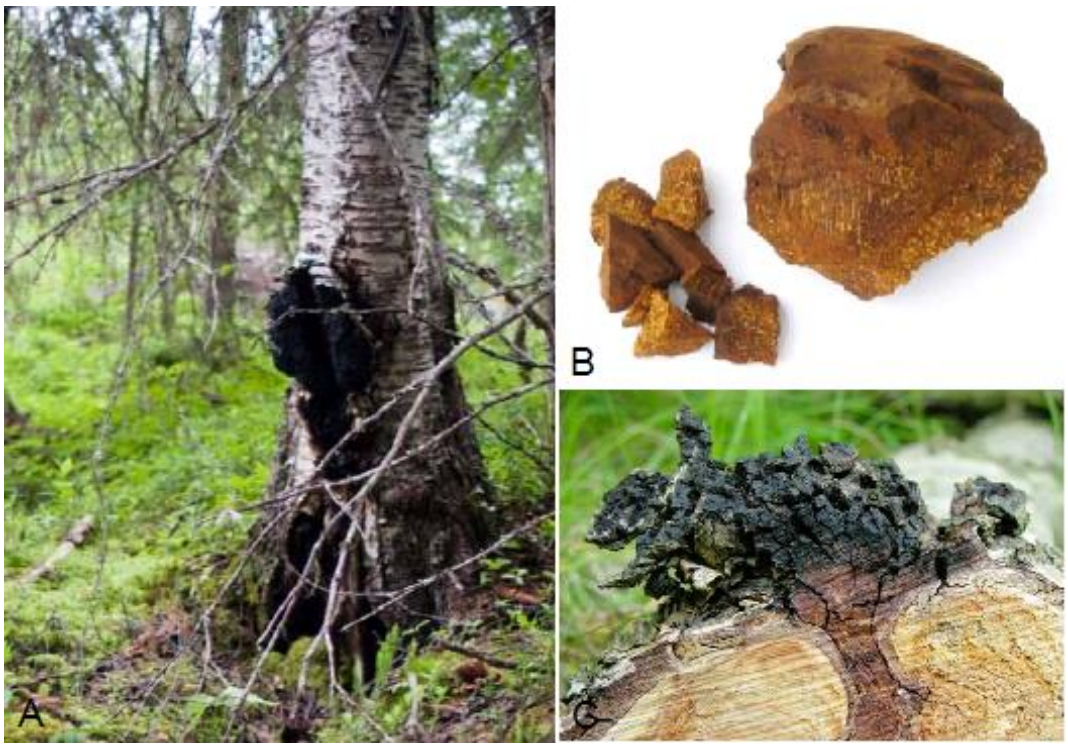


Figure 5. (A) A massive *Inonotus obliquus* in a Finnish forest, (B) the inner part of the mushroom when the surface is removed, and (C) a cross section of the mushroom. [10]

Spores of *Inonotus obliquus* are spread by air and fall into cracks or other damaged sites of a tree, where they start to grow into the wood and form a

mycelium that consume the juice of the tree. The mycelia start to penetrate into the wood and growing a wedge shaped fruiting body, see figure 5C. After around four years the fruiting body or sclerotia breaks through the bark of the tree and forms a large ball-like overgrowth structure, with a diameter of 5 cm to 40 cm, outside the tree. The surface of the conk is irregularly cracked with deep fissures that are dead fungal mass. Under the surface it consists of interwoven mycelia in a woody texture with rusty color, see figure 5B. In the nature it grows very slowly, less than 1 cm a year. The fungus is gradually destroying the tree by living of its energy and growing bigger, and usually the fungus has killed the tree in 7-12 years after the tree has been affected [10, 51]. When the fruiting body breaks through the bark, new spores are falling off and are spread by the wind. [48, 49]

Inonotus obliquus has to be collected only from living or freshly cut old trees. In fallen or dry trees, the fruiting body is destroyed and the useful substances found in it reduce rapidly without energy from the tree. When the mushroom is collecting, the overgrowth is cut off the trunk and the inner loose part, the real fruiting body, is removed. [10, 48]

In this chapter, the use of *Inonotus obliquus* is discussed together with its history. The most important polysaccharides and triterpenes are mentioned together with their pharmacological effects and in some cases, also how they work.

3.1 Background and use

Inonotus obliquus has been used in folk medicine in Russia and Northern Europe since the sixteenth century. Traditionally, it has been used to treat diseases like different kinds of cancer, diabetes and cardiovascular diseases. Even liver diseases, tuberculosis and stomach ailment have been treated with *Inonotus obliquus*. [14, 18] In 1864, Dragendorff studied the chemical composition of this mushroom, though he did not find any active compounds and lost interest for the mushroom. About one hundred years ago, some Russian researchers analyzed

Inonotus obliquus and found active compounds. In the early 1970s the Nobel Prize winner in literature Aleksandr Solzhenitsyn, wrote in his book Cancer Ward about the fantastic effects that *Inonotus obliquus* had on cancer. After this writing, the mushroom became more known in the whole Western world. [10, 45, 48]

In Finland the mushroom has a strong history, especially since it has been used during the war time as a complement for tea or coffee, named 'woodpecker tea' (*tikka tee* in Finnish). [10] *Inonotus obliquus* is considered as a novel food in Finland, which means it has not been used for human consumption to a significant degree before year 1997, however, it is not considered to require the marketing authorization referred to in Novel Food Regulation (EC) 258/97 of the European Parliament according to the Finnish Food Safety Authority Evira. "The authorization is granted on an application containing a clarification of the safety of the product pursuant to the conditions approved by the Community." Nevertheless, *Inonotus obliquus* has required authorizations as dietary supplement; this means that *Inonotus obliquus* can only be sold in Finland and in the rest of the EU countries as a food supplement and in small-scale use, like tea, but not as a food product. [52-54] According to the Finnish freedom to roam it is allowed to pick mushrooms, however, it is not allowed to use equipment that can damage the tree that is usually the case with *Inonotus obliquus*. [55, 56]

3.2 Composition, extraction and safety

A typical composition of *Inonotus obliquus* found by x-ray fluorescence is 39% carbon, 9-10% potassium, 3.6% hydrogen, 0.4% nitrogen, 0.64% magnesium, 0.37% calcium, 0.33% chlorine, 0.23% phosphorus, 0.05% sodium, 0.04% rubidium, 0.02% sulfur, 0.02% manganese, some minor elements like iron, copper, zinc, vanadium, chromium and traces of nickel, selenium, iodine, barium, bromine, and strontium. This means that *Inonotus obliquus* consists of around 40-45% oxygen and that the compounds found in the mushroom are mainly oxygen-containing. [48] Divided into compounds, the weight ratio of some of the main parts are, 13.2% water, 2.4% proteins, 2.4% lipids, 10.1% ash, and 71.9% carbohydrates (12.0% β-glucan). [45]

Polysaccharides and triterpenes are the most important bioactive compounds found in *Inonotus obliquus*. Since the human cannot digest the cell walls found in the mushrooms, the compounds have to be extracted so they can be used, for instance as food supplement. The most common ways to extract the active compounds found in the mushrooms are hot water extraction and alcohol extraction. The hot water extraction draws out the water-soluble compounds, mainly different kinds of polysaccharides with a molecular weight from 10-1000 MDa. [43] *Inonotus obliquus* contains up to 40% of water-extractable substances per dry weight. [48] Depending on the temperature, it is possible to extract more substances; higher temperature gives a higher yield. [57] Alcohol extracts are usually based on methanol, ethyl acetate or ethanol and are mostly extracted after the hot water extract or separately to take out the in-soluble water compounds and low molecular weight substances, like different kinds of triterpenes and sterols, especially betulinic acid and betulin for *Inonotus obliquus*. [2, 57-59] Moreover, there is also a third extraction method that is becoming more common all the time, fermentation. This method cannot be used for wild harvested mushrooms. The bioactive compounds are received as a secondary metabolites produced by microorganism after microbial growth. The method is said to provide high quality extracts, even though large scale fermentation has high contamination risk. [14, 60] Usually, the liquid extract is dried to get a powder that can be used as a diet supplement, for instance as a drink. The extract can be vacuum dried, freeze dried or hot air dried. A study made by Ma *et al*.[61] showed that freeze dried polysaccharides of *Inonotus obliquus* had a higher polysaccharide yield and better antioxidant activity compared with the other drying methods. [61] Another method that has been found interesting is nanomill technology, where the fruiting body of *Inonotus obliquus* is ground into a superfine powder. The powder can increase bioavailability of insoluble or effective compounds. [15]

Inonotus obliquus is overall a very safe mushroom, without side effects. Some studies have showed that *Inonotus obliquus* is well tolerated in large doses, the deadly dose for mice (LD_{50}) was 6.5 g/kg body weight and doses up to 1.0 g/kg did not show any side effects of rabbits, dogs and cats. No toxicity of different *Inonotus obliquus* preparations, like tea, tincture, superfine powder and extract,

was presented in a 0.1-5-fold daily therapeutic dose of purified *Inonotus obliquus* in humans. [15, 48]

3.3 Polysaccharides

According to Wasser [9], 21 different polysaccharides have been found in the fruiting body of *Inonotus obliquus* and 8 different in the culture of *Inonotus obliquus* mycelium. There is a large difference in the sugar content of polysaccharides from sclerotia and mycelia. Xylogalactoglucan is a typical polysaccharide found in *Inonotus obliquus*, especially from the fruiting body. It is mentioned in most reviews about polysaccharides from medicinal mushrooms. It is composed of glucose, galactose, xylose, mannose, arabinose, and fucose with the molar contents of glucose, galactose, and xylose being the highest. The components from mycelia that have the highest effects are glucose and mannose, but even small amounts of xylose, galactose, and arabinose are essential. [12, 14]

3.3.1 Antioxidant activity

Inonotus obliquus has a very high antioxidant activity, it is for instance said to have the highest superoxide dismutases (SOD)-rate in the world [10]. SOD are enzymatic antioxidants that catalyzes superoxide (O_2^-) to oxygen and hydrogen peroxide. Free radicals in the body cause lipid peroxidation, decrease permeation and damage of membrane proteins, as well as, contribute to cellular interaction that can cause cancer. [61] Polysaccharides isolated by hot water-extract have in several studies been shown to possess these antioxidant activities. [49, 57, 61-63] The polysaccharides have both SOD activity and DPPH radical-scavenging activity. It is said that it is not the polysaccharides completely alone that have the activity, most likely they are dependent on the ratio of bound protein that makes a polysaccharide-protein complex, which is essential to the scavenging activity. Even melanin found in the outer part of the fruiting body, may be one of the

components together with polysaccharides that is responsible for the antioxidant potential. [57] Polysaccharides have the capacity to ameliorate the activity of enzymes that are antioxidants, to scavenge free radicals and to inhibit lipid peroxidation. For instance a study showed that polysaccharides from *Inonotus obliquus* can inhibit lipid peroxidation of mitochondria induced by Fe^{2+}-cystein with as much as 80 %. [49] The mechanisms for these antioxidant activities are for instance, prevention of chain inhibition, binding of transition metal ion oxidation catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging. Thermal and ultrasonic treatment of polysaccharides can give desired antioxidant ability and even physicochemical properties. [61, 63]

3.3.2 Immunostimulating effects and anti-tumor activity

Inonotus obliquus has shown both immunostimulating effects and anti-tumor activity that are connected to each other. Fan *et al.* [62] did a study, where they demonstrated that polysaccharides from *Inonotus obliquus* significantly enhanced the immune response in mice with tumors. The polysaccharides increase the proliferation of lymphocytes and improve the ability of the lectin Concanavalin A (ConA)-induced lymphocyte proliferation. Even the production of cytokines like Tumor necrosis factor α (TNF-α), Interferon γ (IFN-γ) and interleukin (IL)-4 increase with the help of polysaccharides, which can make them mediate phagocytosis. These reactions not only inhibit tumor and metastasis growth, but also ameliorate inflammatory bowel disease (IBD). [49, 62, 64]

Endo-polysaccharides from *Inonotus obliquus* are extracted from submerged culture of *Inonotus obliquus*. These endo-polysaccharides have shown a significant immunomodulatory activity. The chemical structure may vary, but usually they are small molecules with less weight than exopolysaccharides. They are closely related to humoral immunity or the antibody-mediated system, by activating B-cells and macrophages, but not T-cells, which is similar to the effects of lipopolysaccharides (LPS). Moreover, induced humoral immunity caused by

endo-polysaccharides is most likely the factor that inhibits tumor growth rather than direct cytotoxic effect against tumor cells. [14, 15] [14, 65]

It is demonstrated that there are differences in immunostimulating activity of purified and crude endo-polysaccharides, as well as endo-polysaccharides from sclerotia and mycelia. Production of nitrite is markedly increased by endo-polysaccharides purified with sodium phosphate buffer compared to crude endo-polysaccharides. This means that purified endo-polysaccharides enhance phagocytosis and nitric oxide (NO) production in macrophages, which is important for the immune system and inhibition of cancer tumors. [15] Sclerotial endo-polysaccharide inhibits cancer cells directly, while mycelial ones activate immune cells that indirectly have anti-cancer effects. Moreover, the production of endo-polysaccharides in sclerotia is much lower compared to the productivity of mycelia. [14] Overall, it is said that endo-polysaccharides from *Inonotus obliquus* have a significant inhibitory effect of melanoma tumor cells and that it most likely the endo-polysaccharides themselves that affect the activity, no combination with proteins for instance. [15]

3.3.3 β-Glucan

β-Glucans are some of the most effective polysaccharides found in *Inonotus obliquus*. There are between 8-15 g/100 g of β-glucans in *Inonotus obliquus*, depending on what extraction and analytical method is used. Since *Inonotus obliquus* has a high dietary fiber content, this may increase the amount of β-glucans. This polysaccharide is said to be extra effective in enhancing the immune function, but does not make it overactive, reduce cholesterol and blood glucose levels, prevent infections, and protect against the development of cancer. [45, 49, 66]

The mechanism behind the anti-cancer effect of β-glucans is probably due to the binding of complement receptor 3 (CR3) on macrophages that increases cytokines, H_2O_2, and O_2 production. Usually, cancer cells are not attacked by immune cells, since they cannot recognize tumors that are self-originating, but

together with CR3, β-glucans can locate the antigen. When neutrophils supplied with complement in form of CR3 come in contact with tumor cells, they can attack the tumors. [2, 45]

3.4 Triterpenes

Inonotus obliquus seem to have an abundance of triterpenes, where betulin, betulinic acid, ergosterol, lanosterol, and inotodiol are the most important ones. There are at least 20 different lanosterol triterpenes and together with different ergosterols, inotodiols and lupeols, one could count at least 50 different triterpenes in this mushroom. [16]; 418 Halmentoja, J. 2012}} Most of these substances are insoluble in water, but exhibit partial emulsification, which means that they can be isolated with hot water. [48]

3.4.1 Betulin and betulinic acid

Betulin (3β-lup-20(29)-en-3,28-diol) is a typical triterpene found in the bark of birch trees and one of the first natural products identified and isolated from plants in year 1788. Betulin can easily be converted to betulinic acid, which is much more active than betulin itself. In figure 6, it is easy to see the similarities between the two molecules. Betulinic acid (3β,hydroxyl-lup-20(29)-en-28-oic acid) is a pentacyclic triterpenoid that also can be find in the bark of birch trees, as well as in the bark of other hardwood trees. [67]

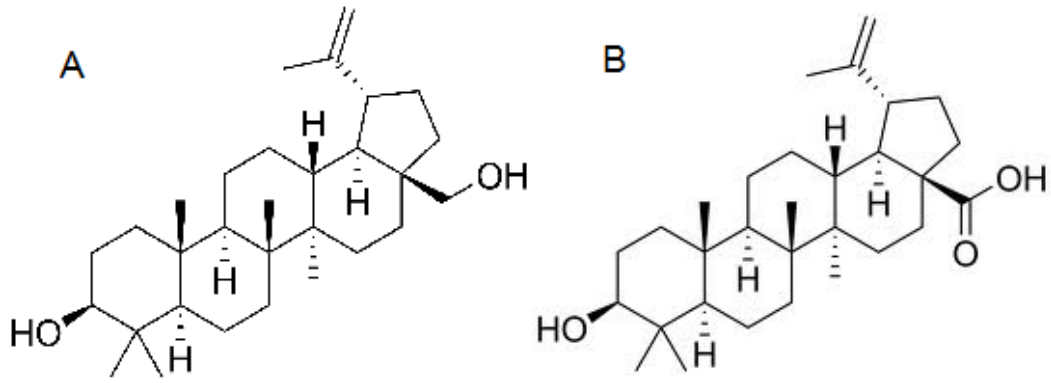


Figure 6. Betulin (A) and betulinic acid (B). It is easy to see that the betulinic acid can be converted from betulin.

Both betulin and betulinic acid have several biological activities. They both possess anti-inflammatory activity through inhibition of nitric oxide and prostaglandin E2 inhibition. [7, 67] Another enzyme that takes part in the inflammatory response is phospholipase A2. Betulin and betulinic acid bind to this enzyme and inhibit the activity. [6] In addition, betulinic acid inhibits inflammations and modulates the immune response by interfering with the activation of the transcription factor nuclear factor-κB (NF-κB), which is a key regulator of stress-induced transcriptional activation, and NF-κB-regulated gene expression. [67]

Because of the anti-inflammatory activity, betulinic acid has anti-tumor activity. One way this activity takes part is through apoptosis of cancer cells. Betulinic acid directly increases permeability of the mitochondrial membrane. This disturb the transmembrane potential, which trigger cytochrome c and apoptosis inducing factor (AIF) and finally activates caspase 3 and 8 that are important for apoptosis. [6, 67, 68]

Angiogenesis is the process when new blood-vessels are formed. This process is dangerous in cancer cells, since it gives nutrition and energy to tumors to keep on growing. Betulinic acid has shown effects to inhibit aminopeptidase N, which is an enzyme that is involved in the regulation of angiogenesis. [67]

The anti-tumor cytotoxicity of betulinic acid has been widely studied in several cancer cell lines, primary tumor samples and xenograft mouse models. Betulinic acid was first reported to have cytotoxic effect against melanoma cell lines, later anti-cancer activity was also reported against other cancer types, such as neuroblastoma, glioblastoma, medulloblastoma, Ewing tumor, leukemia as well as several carcinoma, for instance head and neck, colon, breast, hepatocellular, lung, prostate, renal cell, ovarian or cervix carcinoma. Likewise, betulinic acid demonstrated cytotoxic effect against cancer samples that have showed drug resistance, like standard chemotherapeutic agents. [67]

Betulinic acid has also shown inhibitory action against HIV replication. Betulin is said to be less effective even though it seems to have inhibitory effects as well. [6]

3.4.2 Ergosterol

Ergosterol (ergosta-5,7,22-trien-3β-ol) is a steroidal triterpene found in the cell membrane of *Inonotus obliquus*, see figure 7. The amount of ergosterol in cultured mycelia of the mushroom is as much as 82.2% of the total ratio of triterpenes according to Koyama [45] and much less in field-grown mycelia. Ergosterol is not present in animals and humans, which makes this compound to a useful compound for anti-fungal drugs. [45] In contrast to many other triterpenes found in *Inonotus obliquus*, ergosterol only has effect to delay tumorigenicity instead of inhibiting it. [16] Ergosterol has also shown antioxidant activity, likely together with other triterpenes. [18]

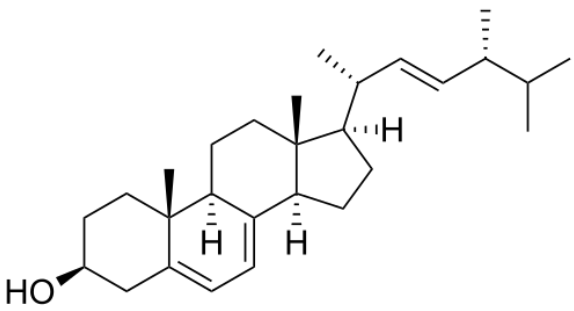


Figure 7. Ergosterol molecule, which can only be found in plants and mushrooms.

3.4.3 Inotodiol

Inotodiol (lanosta-8,24-diene-3β,22R-diol) is a triterpene alcohol and the most abundant triterpene in *Inonotus obliquus*. In field-grown mycelia of *Inonotus obliquus* the percentage of inotodiol is 25.5% and in cultured mycelia it is much less of the total triterpene amount. [45] Inotodiol is said to be responsible, at least partly, for the anti-mutagenic and antioxidant properties of the mushroom, as well as having direct anti-cancer effects. [16, 18] Inotodiol showed especially a significant activity against the cancer type blastoma. [48] Like betulinic acid, inotodiol has anti-proliferative effects through apoptosis, which becomes activated via caspase-3. It is believed that the OH-group substitution at carbon-22 in the molecule is one of the important reasons for the anti-proliferative effect against cancer cells, see figure 8. [69, 70]

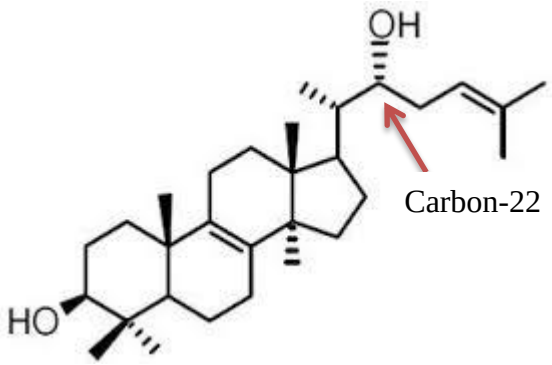


Figure 8. The OH-group substitution at carbon 22 in inotodiol is believed to be one of the important reasons for the anti-cancer activity that this molecule has.

3.4.4 Lanosterol

Lanosterol (lanosta-8,24-dien-3-ol) is a tetracyclic triterpene derivative, see figure 9. [48] In field-grown mycelia of *Inonotus obliquus* the percentage of lanosterol is 45.5% of the total triterpene ratio, while it is only 3.7% in cultured mycelia. [45] When triterpenes are synthesized in *Inonotus obliquus*, lanosterol is the basic substrate. It has been demonstrated that lanosterol has anti-viral effects. [45] Compared to many other triterpenes, lanosterol does not have direct anti-cancer effect, instead it has shown delayed tumorigenicity, like ergosterol has. [16] As

mentioned earlier, *Inonotus obliquus* has strong antioxidant activity and lanosterol is one of the active compounds that give this relatively strong activity. [18] It has also been reported that lanosterol analogues showed regulatory effects on the biosynthesis of cholesterol. [49]

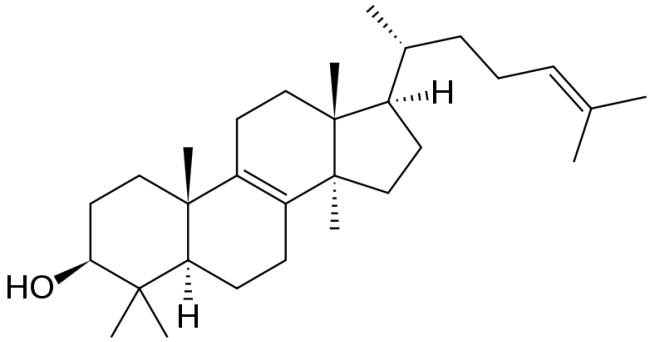


Figure 9. Lanosterol is one of the molecules that gives *Inonotus obliquus* its antioxidative activity.

In addition to all those triterpenes listed, 3β-hydroxylanosta-8,24,dien-21-al could also be mentioned. This triterpene has reported to have both anti-mutagenic effects as well as antioxidant properties. [16, 49, 70]

4 Ganoderma lucidum

Ganoderma lucidum is a white rot wood-degrading mushroom, belonging to the family *Ganodermaceae* of *Basidiomycota*. [19, 71] It can be found in dense, humid coastal provinces of China, in Japan, USA and in Korea. It grows on decaying stumps of chestnut, oak and other broad-leaf trees. Even though it grows wild, it is rare nowadays to come across in the nature. This is because the husk of the spores is very hard and they cannot sprout as readily as spores of other mushrooms can. Moreover, to sprout, the right combination of oxygen and moisture is needed. Fortunately, it is possible to cultivate it. [3] The Japanese name of the mushroom is Reishi, which also is the name that is most used in the western world and it means "10,000 year mushroom". The Chinese name is Lingzhi, literally meaning "supernatural mushroom" or "mushroom of immortality". [2, 3, 19, 72]

Ganoderma lucidum has a kidney-shaped cap and its most characteristic feature is its shiny lacquered look. Sometimes the spores appear on the cap and give it the look of a sandpaper. There are six different types of the mushroom depending on their color: red, white, black, blue, yellow and purple. The red *Ganoderma lucidum* is the one used for medicinal purposes, due to the highest amount of bioactive compounds. Figure 10 shows an old red cultivated *Ganoderma lucidum* together with younger black and purple mushrooms. The taste of *Ganoderma lucidum* is bitter and woody, due to its amount of triterpenoids. [3, 46]

Figure 10. A big cultivated red *Ganoderma lucidum*, with some smaller purple and black in Shangri-La in China.

Ganoderma lucidum can also be found in Finland. Most of them have been found around the coast line. It grows especially on the stem of alder trees and fallen stocks in groves and forests. According to the Finnish freedom to roam it is allowed to pick *Ganoderma lucidum* from trees. However, some texts mean that this mushroom is endangered in Finland, while the classification of endangered species, which started in 1999, means that *Ganoderma lucidum* is viable in some parts of Finland. [73, 74]

In this chapter, some history and application of the mushroom *Ganoderma lucidum* is presented. Its active compounds, polysaccharides and triterpenes are discussed some more and also how they work and what their applications are.

4.1 Background and use

Ganoderma lucidum is the medicinal mushroom that is said to historically been used the longest time for its medicinal properties, as long as 4000 years. [2] The mushroom has been used as a longevity- and vigor-promoting "magic herb" in China, Japan and Korea, but also for prevention and treatment of various kinds of diseases, such as hypertension, tumorigenic diseases, and immunological disorders. Nowadays it is frequently promoted as an effective agent against cancer. [20, 72]

The first time *Ganoderma lucidum* was mentioned in a book was in "Shen Nong Ben Cao Jing", which described herbs and their medicinal values in the Eastern Han dynasty in China during the years 25-220 AD. In 1552, the medicinal properties of *Ganoderma lucidum* were reported in one of the most well-known classical Chinese pharmacopoeia "Ben Cao Gang Mu". The first time this mushroom was recorded in modern scientific texts was in 1934. [43, 46, 75] Today it is even more known, considered as the premier medicinal mushroom and the number of publications about it increases every year, which indicates that the research accelerates all the time. In year 2003, the annual value of extracts sold of *Ganoderma lucidum* alone, was worldwide estimated to be over US$ 2.5 billion. [19, 75, 76] In the beginning of 2000, more than 90 brands of *Ganoderma lucidum* were registered internationally and the market is growing quickly, the worldwide consumption is estimated to be several thousands of tons at the moment. [46] Different kinds of formulations have been developed, patented and used as nutraceuticals, dietary supplements and pharmaceuticals. They are made from the fruiting body, spores, mycelium, and water or alcohol extracts of the mushroom, and can be found as syrup, injection, tablet, tincture, powder or in other additives. [19, 46] Like *Inonotus obliquus*, *Ganoderma lucidum* is classified as novel food according to the Novel Food Regulation (EC) 258/97 of the European Parliament, but considered as safe. [54]

In several studies, it has been shown that *Ganoderma lucidum* has anti-tumor and anti-inflammatory effects. For these biological activities it is mainly polysaccharides and triterpenes that are responsible. [3, 19, 20] Polysaccharides

stimulate the immune system, which results in increased production of cytokines, like INF-γ, IL and TNF-α, and activation of immune effectors, such as macrophages, NK cells and T cells. Triterpenes in the other hand suppress growth and invasive behavior of cancer cells. [3, 77, 78] *Ganoderma lucidum* can also lower the total cholesterol level, blood pressure, blood glucose, and reduce blood platelet binding, and if also having an anti-hepatotoxic activity. [3] Figure 11 lists different pharmacological effects and show if they are effects induced by polysaccharides or triterpenes (triterpenoids in the figure).

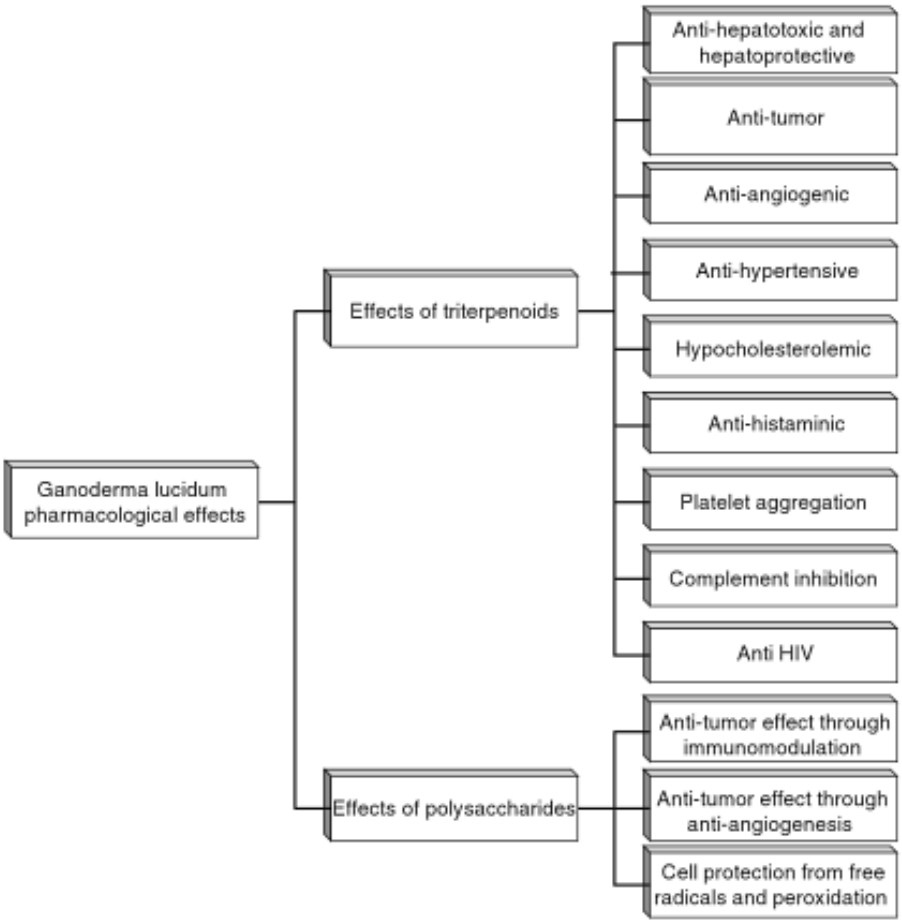


Figure 11. Pharmacological effects of *Ganoderma lucidum* from triterpenes and polysaccharides. [19]

4.2 Constitution, cultivation and safety

Ganoderma lucidum contains 59% fiber, 26-28% carbohydrate, 7-8% protein, 3-5% fat, and 1.8% ash. Minerals like phosphorus, silica, sulfur, potassium, calcium, and magnesium are also found, as well as lower amounts of iron, sodium, zinc, copper, manganese, and strontium. Heavy metals like cadmium and mercury were found in very small amounts. The bioactive molecules found are for instance triterpenes, steroids, phenols, nucleotides, glycoproteins and polysaccharides. Polysaccharides and triterpenes are the most important bioactive compounds. The percentage of these compounds can vary as much as from 0-11% for triterpenes and 1.1-15.8% for polysaccharides. These variations can depend on, for example, differences in production methods and species or strain used for the production. In table 2 eleven different *Ganoderma lucidum* products are listed and compared according to the percentage of triterpenes and polysaccharides, all products are found on the market. [19, 46]

Table 2. Percentage of triterpenes and polysaccharides in different *Ganoderma lucidum* products found on the market. [46]

Nature of product	Triterpenes [%]	Polysaccharides [%]
A (fruiting body extract)	1.36	4.48
B (fruiting body extract)	2.36	5.32
C (fruiting body extract)	1.88	15.70
D (fruiting body extract)	1.0	10.97
E (fruiting body extract)	0.44	7.51
F (fruiting body extract)	1.78	6.18
G (fruiting body extract)	1.44	13.30
H (fruiting body extract)	0.50	15.80
I (fruiting body extract)	7.82	7.66
J (fruiting body extract)	0.46	1.10
K (mycelium powder)	Undetectable	12.78

This table shows well how much the amount of polysaccharides and triterpenes can vary between different products. It does not automatically mean that a product with a high amount of triterpenes has a high amount of polysaccharides, or the other way.

The best way to cultivate fruiting body of *Ganoderma lucidum* is on natural logs, even though sawdust cultivation in bags and bottles is possible. It takes several months to cultivate fruiting body of the mushroom, so mycelia-based and culture broth-based products have become more popular all the time. Oxygen, humidity, and temperature are very important factors in the cultivation of *Ganoderma lucidum*. Mycelium grows at a temperature of 10-38°C, moisture of 65-70% and a pH value of 4.5-5.3. It does not necessarily need light. Fruiting bodies have their optimal cultivation temperature at 27-32°C, moisture at 90%, 70-80% or 30-40% depending on which stage they are in and a light strength at 50-450 lux. [19, 46]

Usually medicinal mushrooms are considered to be safe due to their long time of usage, but this criterion is not enough. *Ganoderma lucidum* has not shown any kind of toxicity in both acute and sub-acute studies. Even at such high doses as 5 g/kg of dry mushroom or 220 g/kg of fresh fruiting body in test animals, no toxic effect was observed. *Ganoderma lucidum* is considered safe for therapeutic use. LD_{50} of an intraperitoneal injection in mice has been shown to be around 38.3 g/kg of dry weight. [79, 80]

4.3 Polysaccharides

More than 150 types of polysaccharides have been isolated from *Ganoderma lucidum*. The main bioactive polysaccharides are β(1-3)- and β(1-6)-D-glucans. [81] They can be extracted from the fruiting body, spores and mycelia. The extraction is normally obtained with hot water and can be followed by precipitation with alcohol, but can also be extracted with water and alkali. Since polysaccharides from *Ganoderma lucidum* usually are heteropolymers, they also contain, in addition to glucans, xylose, mannose, galactose, and fructose. [19, 46]

4.3.1 β-Glucans

β-Glucans are one of the most bioactive polysaccharides found in *Ganoderma lucidum*. There are several different β-glucans, but two of the most known in this mushroom are ganodelan A and B. [3, 19]

β-Glucans have been shown to mainly be effective by stimulating the immune system. Some of them bind to CR3 on macrophages and other immune effectors, which cause phagocytosis of foreign cells. [2] Others stimulate T cells, which makes them release cytokines, such as IL-1β, IFN-γ, TNF-α, IL-2 and IL-6, as well as NO. [22, 46] The structure of β-glucan is important for the immunoregulation. Relatively small side chains and an organized helical structure, especial triple helical, of the glucan-molecules have been shown to be powerful. [19] Single helical conformer of β-glucan enhances NO synthesis, while triple helical does not. However, triple helical β-glucans, haves shown better results to stimulate IL-1, IL-6 and TNF-α production, compared with single helical β-glucans. It is most likely that the structure and compositions of β-glucans in mycelia and fruiting body is different, which might result in improving the natural immune system through different receptors and pathways. NK-κB activation is one of the most important signal pathways. β-Glucans are a good activator of NK-κB, which in turn activates proinflammatory cytokines. [22]

β-Glucans are also active in other ways than stimulating the immune system. It has been reported that ganodelan A and B can help release insulin, by favoring the influx of calcium in the pancreas β cells, which helps lowering high blood sugar. [3] Polysaccharides also decreased levels of lipid peroxidation in a trial with diabetic rats. [20]

4.3.2 Ganopoly

Ganopoly is a water-soluble polysaccharide fraction from *Ganoderma lucidum*. It has been shown to have anti-tumor activity through the stimulation of the immune system. It activates macrophages, T cells and NK cells. [78, 82, 83] Several clinical studies have also been done about the effect of ganopoly. [2] In a study made by Gao *et al*. [84], patients with advanced-stage cancer were treated with ganopoly orally for 12 weeks. The study indicated that ganopoly enhanced the immune system in patients with advanced-stage cancer. [85]

In clinical trials with patients suffering from type 2 diabetes mellitus, ganopoly has been shown to lower blood glucose after food intake. [83, 85]

Extracts from *Ganoderma lucidum* that does not contain triterpenes, but most likely ganopoly and β-glucans, have been reported to prolong sleep time as well as enhance sleep quality. It is not that clear what causes this action, but it is believed that it is associated with the immune system, especially with the TNF-α pathway, which level increases significantly when the extract is administrated. [86, 87]

4.3.3 Polysaccharides from spores

It is said that spores have a higher bioactivity than fruiting body of *Ganoderma lucidum*, but only a few different polysaccharides have been isolated from the spores. Water-soluble glucan from the spores has been shown to have immunostimulating and anti-tumor activities, like β-glucans from the fruiting body have. Most of all the polysaccharides isolated from *Ganoderma lucidum* seem to be different kinds of glucans, but the linkages are different, which could be a very important factor for their immunological activities. In contrast to β-glucans from fruiting body, they normally have a low molecular weight. The bioactive components in spores are suggested to be complex, compared with polysaccharides from fruiting body that one knows much more about. [81, 85]

4.3.4 Antioxidant activity

Polysaccharides from *Ganoderma lucidum* have shown antioxidant activity. They have for instance a prominent effect on free radical scavenging and abilities to protect organelles, like mitochondria against injury by peroxide. Moreover, some polysaccharides from this mushroom have also been reported to reduce reactive oxygen species (ROS) production and malondialdehyde content (MDA) and increase the activity of manganese superoxide dismutase (Mn-SOD). Polysaccharide peptide was described to enhance the scavenging abilities on reactive oxygen. [85]

In diabetic rats induced with streptozotocin (STZ), polysaccharides taken orally showed effects of normalizing impaired oxidative stress in the plasma and liver by dose-dependently increasing nonenzymatic and enzymatic antioxidants and reducing lipid peroxidation. [85]

One of the reasons for skin aging is H_2O_2 induced apoptosis of keratinocyte in the skin. Polysaccharides from *Ganoderma lucidum* may inhibit this reaction and regulate expression of genes which are related to skin cellular metabolism. These genes increase reparation of damaged DNA, dynamics of cells and the function of both differentiation and proliferation of cells. Furthermore, polysaccharides induce the expression of growth factors, which improves the mitosis and growth of cells. Extracts of Ganoderma lucidum is suggested to be a possible treatment to enhance the cellular immunological activity and slow down the ageing process in ageing people. [3, 88]

Polysaccharides from *Ganoderma lucidum* possess strong hypolipidemic effects. They can noticeably reduce the total cholesterol and triglyceride both in STZ-induced diabetic rats and in healthy animals. [85]

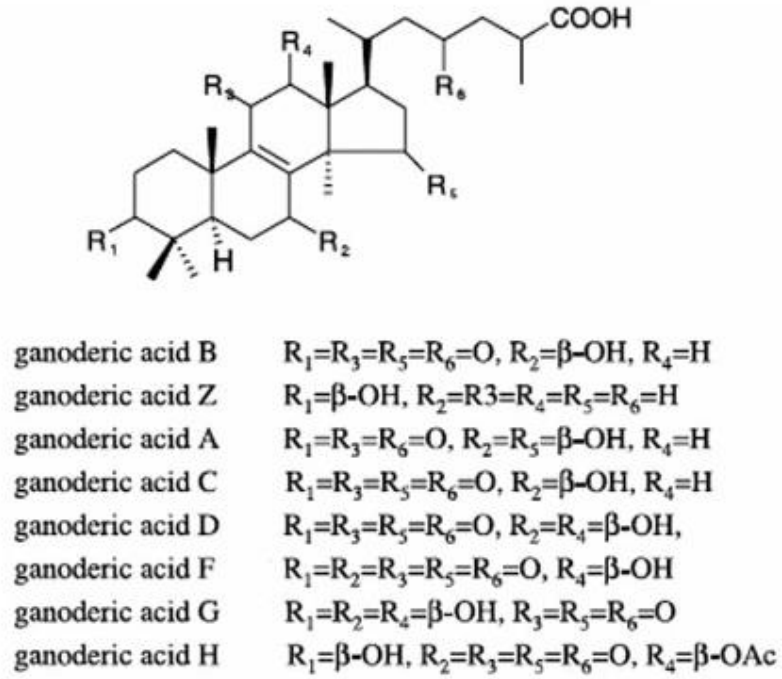
4.4 Triterpenes

Studies have reported from 100 up to 150 triterpenes being isolated from the fruiting body, mycelia and spores of *Ganoderma lucidum*. [2, 12, 19, 83] The chemical structure of triterpenes in *Ganoderma lucidum* is based on a metabolite of lanosterol, called lanostane. The triterpenes are extracted with ethanol, acetone, chloroform, ether, or a mixture of these. There are at least 40 different ganoderic acids (ganoderic acid A, B etc.), 14 ganoderiols, 5 ganolucidic acids, and 15 lucidenic acids, as well as several ganodermic acids, ganoderenic acids, and lucidones. [2, 19, 46, 77] The quality of the triterpenes depends on the age of different parts of the fruiting body. Boh *et al.* [19] reported that the highest amount of triterpenes were found in the tubes under the cap of the mushroom and the younger dark context layer of the cap of the mushroom, while the lowest were found in the older white context layer and the upper surface of the fruiting body. The triterpene content can be used as quality control of different ganoderma species. The triterpenes are also the reason to the bitter taste of *Ganoderma lucidum*. Some of the health benefits found from the isolated triterpenes are lipid-lowering and antioxidant activity. [46]

4.4.1 Ganoderic acids

In addition to the immunostimulating and anti-tumor effect that *Ganoderma lucidum* has, the adaptogenic and calming effect is startling. Adaptogens help to adapt to the surroundings and situations around you. When you are stressed it gives an anti-stress effect, while it can boost the energy if you are suffering from fatigue. The adaptogenic effect is mainly related to ganoderic acids, which are some of the most important compounds found in *Ganoderma lucidum*. Ganoderic acid F is reported to contribute to atherosclerosis protection by inhibition of angiotensin converting enzyme and also giving a calming effect on the nervous system. [3, 19] Ganoderic acid C is the most active compound with anti-allergic potency, but even ganoderic acid A and D have potency, since they have been shown to inhibit histamine release. Ganoderic acids B and D have been described

as strong anti-hypertensive agents and ganoderic acids T to Z show anti-tumor activity against hepatoma cells. Even ganoderic acids R and S have strong anti-hepatotoxic activity. [19, 82] Several ganoderic acids are presented in figure 12.



ganoderic acid B	R_1=R_3=R_5=R_6=O, R_2=β-OH, R_4=H
ganoderic acid Z	R_1=β-OH, R_2=R3=R_4=R_5=R_6=H
ganoderic acid A	R_1=R_3=R_6=O, R_2=R_5=β-OH, R_4=H
ganoderic acid C	R_1=R_3=R_5=R_6=O, R_2=β-OH, R_4=H
ganoderic acid D	R_1=R_3=R_5=R_6=O, R_2=R_4=β-OH,
ganoderic acid F	R_1=R_2=R_3=R_5=R_6=O, R_4=β-OH
ganoderic acid G	R_1=R_2=R_4=β-OH, R_3=R_5=R_6=O
ganoderic acid H	R_1=β-OH, R_2=R_3=R_5=R_6=O, R_4=β-OAc

Figure 12. Ganoderic acids are the most common triterpenes found in *Ganoderma lucidum*, there are around 40 different of them. [89]

Ganoderic acid T has also been shown to have proapoptotic effect on lung tumors, by causing mitochondrial dysfunction and to be cytotoxic against various carcinoma cell lines in a dose-dependent manner and less toxic to normal cell lines. [89] Ganoderic acid Me, inhibits tumor growth and metastasis of lung carcinoma. [46] Furthermore, ganoderic acids A, B, C, and D together with other triterpenes are reported to have antioxidant activity and ganoderic acid Mf, as well as ganoderic acid T-O have inhibitory effects on cholesterol synthesis. [19, 21]

Ganoderic acids are usually found in the fruiting body, but can also be found in spores. Together with other triterpenes from spores, ganoderic acids may have anti-HIV-1 protease activity. Ganoderic acids might also have inhibitory effects on hepatitis B virus. [12, 46, 77, 83]

4.4.2 Anti-cancer and cytotoxic effect

Even though it is mainly polysaccharides from *Ganoderma lucidum* that have immunostimulating effects, some triterpenes have shown similar activities. [2] But the anti-cancer effects that triterpenes have been shown to possess do not only depend on the immunostimulating activity, instead triterpenes from *Ganoderma lucidum* have direct cytotoxic effect against tumor cells. Especially inhibition of growth of hepatoma cells is typical for triterpenes from *Ganoderma lucidum*. [77] The cytotoxic effects are likely cell specific and depend on the chemical structure. Some studies suggest that triterpenes can have a significant chemopreventive potential. [90] Triterpenes that are fermented have shown differences in types, quality and relative proportion, and especially exhibit different effects in inhibition of tumor cells. [19]

4.4.3 Anti-HIV activity and effects on the cardiovascular system

It is not only ganoderic acids that have anti-HIV activity; also ganoderiol F and ganodermanontriol from *Ganoderma lucidum* have shown activity as anti-HIV-1 agents. Moreover, ganodermanontriol, ganolucidic acid A and lucidumol B showed strong anti-HIV-1 protease and HIV-2 protease activity. [19, 77] It seems like it is especially the carbon atoms C3, C24 and C25 in triterpenes from *Ganoderma lucidum* that are the most vital for the anti-HIV activity. [89]

Many different effects on the cardiovascular system have been reported. For instance, ganodermic acid S is able to inhibit platelet aggregation by stimulating the hydrolysis of phosphatidylinositol 4,5-bisphosphate. [3, 77] Several triterpenes have lowering effects on hypertension and cholesterol, and have hepatoprotection ability. These effects depend on inhibition of enzymes that affect a special process, like cholesterol synthase. [77]

Some antioxidative activities of triterpenes have also been examined. [77]

Other effects that have been reported are for instance prevention of tooth decay. The triterpene oleanolic acid has been found to be preventive against tooth decay. This activity is connected to inhibition of glucosyltransferase in *Streptococcus mutans*, a primary cariogenic bacterium. [6]

5 Analysis between *Inonotus obliquus* and *Ganoderma lucidum*

The analysis between the two medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum* is totally based on the theoretical part in the earlier chapters. First of all, the compositions and compounds found in the mushrooms are compared to each other. Later, the properties of the mushrooms are compared depending on if they are caused by polysaccharides or triterpenes found in the mushrooms.

5.1 Contents and compounds found in the two mushrooms

Like it has been mentioned several times already, the main compounds found in both *Inonotus obliquus* and *Ganoderma lucidum* are polysaccharides and triterpenes. In table 3 the overall compositions of the two mushrooms are compared.

Table 3. Contents of *Inonotus obliquus* and *Ganoderma lucidum* [2, 9, 10, 12, 16, 19, 45, 46, 63, 81, 83]

Components	***Inonotus obliquus***	***Ganoderma lucidum***
Carbohydrates	71.9%	26-28%
Proteins	2.4%	7-8%
Lipids	2.4%	3-5%
Ash	10.1%	1.8%
Amount of different polysaccharides	21 + 8 pcs	~150 pcs
Amount of different triterpenes	~50 pcs	~150 pcs

Inonotus obliquus contains 21 different polysaccharides in the fruiting body and 8 in the mycelium. Compared with *Ganoderma lucidum* that has as many as 150 different polysaccharides, the amount found in *Inonotus obliquus* is low. It is though, interesting that *Inonotus obliquus* contains almost 72% carbohydrates, which is a synonym to saccharides in biochemistry, while *Ganoderma lucidum* only contains 26-28% carbohydrates, even though there are so many different polysaccharides found in this mushroom. This probably means that the percentage amount of the polysaccharides in *Inonotus obliquus* is much higher than in *Ganoderma lucidum*. As mentioned earlier, medicinal mushrooms are usually low in carbohydrates, a typical amount is 3-21%. *Inonotus obliquus* has thus an extraordinary amount of carbohydrates. The most important polysaccharides for both mushrooms are β-glucans. Most likely *Inonotus obliquus* contains more of these compounds according to the carbohydrate percentage. The literature points out that *Inonotus obliquus* contain 8-15% β-glucans. Most likely *Ganoderma lucidum* has a small proportion of each polysaccharide even though there is a large amount of different polysaccharides. *Inonotus obliquus* is said to have a high amount of fiber, which also may explain a high amount of β-glucans. Interestingly *Ganoderma lucidum* contains as much as 59% fiber, while a typical amount is 3-35% in medicinal mushrooms in general. This maybe explains the large number of polysaccharides found in *Ganoderma lucidum*.

The total amount of different triterpenes found in *Ganoderma lucidum* is also 150, while the amount in *Inonotus obliquus* is around 50. *Ganoderma lucidum* contains more lipids, 3-5%, compared with *Inonotus obliquus* that has less with about 2%. Lipids, such as different kinds of sterols and even phenols could sometimes be classified as a lipid, have isoprenes as a typical building material that also are the building blocks in triterpenes. The higher percentage of lipids in *Ganoderma lucidum* could explain the higher amount of triterpenes. In contrast to polysaccharides, the triterpenes found in the two mushrooms are different from each other. The most important triterpenes found in *Inonotus obliquus* are betulin, betulinic acids, lanosterol and ergosterol, while the most important ones in *Ganoderma lucidum* are different kinds of ganoderic acids, ganoderiols and lucidenic acids. But looking at their molecular formulas they all have the same lanostane base with the same cyclic structure and side chains, even though betulin

and betulinic acids differ a bit more from the rest. From table 2, it is pretty clear that *Ganoderma lucidum* usually contains a higher amount of polysaccharides than triterpenes, which of course can also be understood from the amount of carbohydrates and lipids in the mushroom.

The percentage of proteins in *Ganoderma lucidum* is 7-8% and around 2% in *Inonotus obliquus*. Typically for medicinal mushrooms the amount is 10-14%, which means that *Inonotus obliquus* has very low protein content. The ash amount is around 10% in *Inonotus obliquus* and only 2% in *Ganoderma lucidum*. The ash content tells what is left after a sample of a mushroom is burned, which means inorganic compounds like salts and minerals. *Inonotus obliquus* seems to have a much higher amount of minerals, especially extremely high amounts of potassium (9-10%) and other minerals like magnesium (0.64%) and calcium (0.37%) are important.

Depending on where the compounds come from, they are more or less bioactive or have different structures. It has already been mentioned several times that the structure of the compounds is very important for their functions, as is the molecular weight. Each mushroom is said to have their own specific β-glucans, where for instance the heavy ones with β(1-3) chains are said to be the most effective compared with β(1-6). A typical weight for polysaccharides in medicinal mushrooms is 1.5-2 MDa. In *Inonotus obliquus* the typical molecular weight for polysaccharides is 10-100 MDa. This means that polysaccharides from *Inonotus obliquus* could be classified as more effective than polysaccharides from medicinal mushrooms overall based on the molecular weight. Even though a bigger molecule weight does not directly mean a better effect. For triterpenes, a normal molecular weight is 0.4-0.6 MDa, but they are much smaller than polysaccharides and function in a different way. In *Inonotus obliquus* there are more polysaccharides are found in the fruiting body than in the mycelium and even the sugar compositions differ in them. The same observations have been done for *Ganoderma lucidum*. Interestingly, compounds from spores of *Ganoderma lucidum* have been reported to be more bioactive than compounds from the fruiting body, even though glucans from spores have less weight than the ones from fruiting body. When the mushrooms are cultivated, there is also a big difference if they are grown in cultured or field-gown mycelia for instance for

triterpenes from *Inonotus obliquus*. Even the produced amount of polysaccharides is different in fruiting body and mycelia, as well as, if they are treated, such as fermented.

5.2 Properties of *Inonotus obliquus* and *Ganoderma lucidum*

Both *Inonotus obliquus* and *Ganoderma lucidum* have similar properties. Immunostimulating effects and anti-cancer activities are two of the most important properties for both. Based on the theoretical part, the most relevant properties are listed in table 4 with the compounds that cause them.

Table 4. Effects of polysaccharides and triterpenes from the *Inonotus obliquus* and *Ganoderma lucidum* based on the theoretical part.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Antioxidant activity	●	●	●	●
Immunostimulating effects[1]	●	●	●	●
Anti-cancer activity[2]	●	●	●	●
Cardiovascular activities	●	●	●	●
Anti-HIV effects		●		●
Anti-fungal effects		●		
Glucose lowering effects	●		●	
Adaptogenic effects				●
Anti-allergic effects				●

[1] Immunostimulating activity means here all kinds of activities that enhance the immune system
[2] Anti-cancer activity means here also anti-mutagenic, anti-tumor activities etc.

The different properties will be discussed more in detail in the subtitles. Even differences and similarities between the two mushrooms as well as how their compounds act are reported.

5.2.1 Antioxidant activity

The antioxidant activities found in *Inonotus obliquus* and *Ganoderma lucidum* are listed in table 5, depending on which compounds and mushrooms cause them.

Table 5. Antioxidant activity found in *Inonotus obliquus* and *Ganoderma lucidum.*

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Antioxidant activity	•	•		•
Activate enzymatic antioxidants	•		•	
Reduce lipid peroxidation	•		•	
Enhance scavenging activity	•		•	
Reduce ROS and MDA			•	

The first row in table 5, antioxidant activity, is here because any further information about what kind of antioxidant activity is taking part is not specified. This line is marked for those compounds that only are said to have antioxidant activity. Polysaccharides from *Inonotus obliquus*, especially hot-water extract containing polysaccharides and the combination of polysaccharides and melanin have shown significant antioxidant activity. Most of the triterpenes found in *Inonotus obliquus*, ergosterol, lanosterol, inotodiol and 3β-hydroxylanosta-8,24,dien-21-al, all have antioxidant activity. As established, triterpenes in

Ganoderma lucidum are quite similar to the ones in *Inonotus obliquus*, so also them have been reported to have antioxidant activity. The ganoderic acids A, B, C and D were stated as activators. By treating the polysaccharides thermally and with ultrasound it is possible to get specific antioxidant activities.

It is though a bit too unspecified to talk about antioxidant activity, so what really happens is more interesting. A typical activation of these mushrooms is the activation of enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants are for instance SOD and Mn-SOD that have direct antioxidant activity by catalyzing superoxide (O_2^-) conversion to oxygen and H_2O_2. Non-specified polysaccharides from both *Inonotus obliquus* and *Ganoderma lucidum* have showed this kind of activation.

Another important antioxidant activity is the reduction of lipid peroxidation. This means that the antioxidants, whether they are an enzyme or another antioxidant, reduce the oxidation of lipids. Especially lipids found in the cell membrane and organelles like mitochondria are exposed to oxidation. These organelles become degraded by the oxidation and cell damage in caused, which can lead to a chain reaction. Both polysaccharides from *Inonotus obliquus* and *Ganoderma lucidum* have shown reductive activation.

Scavenging activity is a third way how antioxidants function. Here they enhance the scavenging abilities to reduce oxygen so the oxidation risk decreases. Also here, it is the polysaccharides from both mushrooms that are responsible for this enhancement, but together with protein or peptide complexes the effects are more significant.

The fourth method of antioxidant activity is the reduction of ROS and MDA. ROS (reactive oxygen species) are reactive molecules that include oxygen. They are very natural but in too high amounts, they can be harmful for the body. MDA (malondialdehyde) is a reactive molecule which is formed by ROS degrading lipids. MDA is usually a sign for oxidative stress in the body. According to the theory, it is only polysaccharides found in *Ganoderma lucidum* that have the activity to reduce MDA and ROS, but most likely also polysaccharides found in *Inonotus obliquus* have the same activity.

As can be seen in the table 5, it is mainly the polysaccharides from *Inonotus obliquus* and *Ganoderma lucidum* that have antioxidant activities that are specified on how they function. The antioxidant activity from triterpenes in the two mushrooms could though not be specified, since so many different triterpenes showed activity. Most likely polysaccharides have effects by enhancing different kinds of antioxidant enzymes, reduce lipid peroxidation et cetera, while triterpenes directly inhibit the oxidation in cells.

5.2.2 Immunostimulating effects

Table 6 lists different kinds of immunostimulating effects depending on which compounds are found in the two mushrooms causing them.

Table 6. The immunostimulating effect of *Inonotus obliquus* and *Ganoderma lucidum*.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Overall immunostimulating activity	•		•	
Increased production of cytokines	•		•	
Increased production of lymphocytes and macrophages	•		•	
Anti-inflammatory effect		•	(•)	

If no further explanation is given how the immunostimulating effect functions, it is named here as overall immunostimulating activity. This effect has been shown by polysaccharides from both *Inonotus obliquus* and *Ganoderma lucidum*. In

Inonotus obliquus, especially endo-polysaccharides that are smaller and lighter than a "normal" polysaccharide have been featured as immunomodulating compounds, while β-glucans, ganopoly and polysaccharides from spores of *Ganoderma lucidum* are the typical ones. It should be pointed out that the immune system not only is enhanced and becomes more stimulated, but also should not become overactive.

A very typical method of enhancement of the immune system is an increased production of cytokines. Cytokines are different kinds of proteins that work with cell signaling. TNF-α, IFN-γ and different IL are the most known ones and these activate macrophages, regulate immune cells and are pro- or anti-inflammatory. Even NO could be classified as a signaling molecule, although it is produced by macrophages. Moreover, NO is also toxic for bacteria. Endo-polysaccharides, β-glucans and probably other polysaccharides found in *Inonotus obliquus* have been reported to increase production of cytokines. Also β-glucans in *Ganoderma lucidum* have the same effect. One way to increase this production is for β-glucans to bind to CR3 on macrophages that increase the cytokine production. Moreover, β-glucans can together with CR3 find antigens bound to pathogens. The structure is important, it has for instance been shown that triple helical β-glucans have a better result in producing cytokines compared with single helical.

Lymphocytes and macrophages are probably the most known parts of the immune system. Endo-polysaccharides and other polysaccharides from *Inonotus obliquus* have effects to activate lymphocytes like B cells and NK cells as well as macrophages. β-Glucans and ganopoly from *Ganoderma lucidum* have mainly the same effect, but activate T cells rather than B cells. B cells are more connected to antibodies, while T cells are not. It could be concluded that polysaccharides from *Inonotus obliquus* are more connected to the immune memory than polysaccharides from *Ganoderma lucidum*. Both B and T cells on the other hand, can be distinguished from each other and other lymphocytes, this means that it is not possible to make this conclusion straight off, but it may be possible.

Anti-inflammatory effect could also be classified as an own subtitle or under the subtitle of anti-cancer effect. Betulin and betulinic acids have strong anti-inflammatory activity since they can inhibit the substance prostaglandin E2 that

for instance induces fever, as well as NO and the enzyme phospholipase A2, which have inflammatory effect. NO is not only good because of its toxic effects on bacteria, it can also have harmful effects. It activates NF-κB that is important in a gene expression responsible for inflammations. NF-κB is a multifunctional protein complex. In contrast to this, β-glucans in *Ganoderma lucidum* activates NF-κB that regulates proinflammatory cytokines, like TNF-α, which both induce and inhibit inflammations but also inhibit tumor growth. *Ganoderma lucidum* is reported to have more effective anti-inflammatory effects as well, which probably are caused by triterpenes.

The immunostimulating effects of *Inonotus obliquus* and *Ganoderma lucidum* depend only on polysaccharides, if the anti-inflammatory effect is not counted to this class. *Inonotus obliquus* probably has more impact on the humoral immunity that is connected to antibodies, while *Ganoderma lucidum* is closer to the cell-mediated immunity and is more cytotoxic. Anti-inflammatory effects are more related to triterpenes. As it has been mentioned several times before, it is better to activate different kinds of immunoregulating processes to get a better result by for example taking several different medicinal mushrooms.

5.2.3 Anti-cancer activity

Anti-cancer activity is often a direct result of the immunomodulating activity, but even direct anti-cancer effects are common. In table 7, different ways of anti-cancer effects are listed as well as the compounds causing them.

Table 7. Anti-cancer activities of *Inonotus obliquus* and *Ganoderma lucidum*.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Direct anti-cancer activity	•	•		
Cancer inhibition through apoptosis		•		•
Cancer inhibition through angiogenesis		•		
Cytotoxic effects		•		•
Inhibition of tumors and metastases		•		•

Direct anti-cancer activity means that a compound attacks the tumor, metastasis or cells directly trying to kill them. According to the theoretical part the triterpene inotodiol found in *Inonotus obliquus* is one effector of this. Surprisingly, even endo-polysaccharides from fruiting body of the same mushroom have direct anti-cancer effects, even though polysaccharides usually enhance the immune system instead of direct attack on metastasis or tumors.

Apoptosis is programmed cell death, where cells make a suicide in a controlled way without causing harm to the surroundings. Anti-proliferative effects of cancer through apoptosis, is possible caused by both betulinic acid and inotodiol from *Inonotus obliquus*. They increase the permeability for subjects through the mitochondrial membrane, which release the proteins cytochrome c and AIF. This activates the proteins caspase 3 and 8 that have an essential role in the apoptosis. In inotodiol the substitution of the hydroxyl group at carbon 22 is believed to be for the important mechanism for the activation. Betulinic acid does not contain anything similar as inotodiol, which means they most likely act in different ways. The anti-inflammatory effect of betulinic acid is supposed to be one reason for a later activation. Ganoderic acid T from *Ganoderma lucidum* has shown

proapoptotic effect by also affecting the permeability of the mitochondrial membrane. This has been shown especially in lung tumors.

Another cancer inhibition method is through inhibition of angiogenesis. Angiogenesis is the process where new blood vessels are formed. Betulinic acid inhibits the enzyme aminopeptidase N that participates in the angiogenesis regulation. This means that tumors cannot get the nutrition and energy they need to survive. Based on figure 11, also triterpenes in *Ganoderma lucidum* should have this effect.

Cytotoxic effects are directly toxic to cells. The cytotoxic effects can though be by immune cells, or reactions that cause necrosis or apoptosis. Betulinic acid and different triterpenes from *Ganoderma lucidum* have shown cytotoxic effects against several cancer types and even against types that seem to be drug resistant. *Inonotus obliquus* and *Ganoderma lucidum* can be important factors in cancer treatment when chemotherapy does not help or as an addition to medications.

Another way to inhibit cancer to proliferate or sometimes even to start, is to inhibit tumors and metastasis. Metastasis means that cancer is spreading, for instance from one organ to another. Triterpenes from both mushrooms have shown ability to inhibit tumors and metastasis, especially different ganoderic acids, betulinic acid, and inotodiol. Some of them are specific for a special cancer line. Ergosterol and lanosterol do not have direct inhibition power on the tumors, but can delay tumorigenicity. Anti-inflammatory effects discussed earlier in the immunostimulating chapter have a direct result to have anti-tumor effects. Even anti-mutagenic effect of inotodiol and 3β-hydroxylanosta-8,24,dien-21-al from *Inonotus obliquus* is a way to inhibit tumors. Activation of the immune system has anti-tumor effects, but it is not a direct effect.

In almost all cases, triterpenes are the compounds that have the anti-cancer effects. The anti-cancer effect is usually taking place since triterpenes have the effect of inhibiting pathways needed for developing cancer. The exact answer why triterpenes can do this and polysaccharides cannot is not clear. Both mushrooms seem to be equally strong on this.

5.2.4 Cardiovascular activities

The cardiovascular system consists of the blood circulation and the lymphatic system. Medicinal mushrooms are said to have a cleaning effect on this system. In table 8, some cardiovascular activities of *Inonotus obliquus* and *Ganoderma lucidum* are listed depending on what compound cause them.

Table 8. Cardiovascular activities of *Inonotus obliquus* and *Ganoderma lucidum*.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Lower cholesterol level	•	•	•	•
Lower hypertension				•
Inhibit platelet aggregation				•
Reduce blood glucose level	•		•	

Both mushrooms have shown effects on lowering the total cholesterol level and both polysaccharides and triterpenes seem to have effect on it. It is not specified how β-glucans from *Inonotus obliquus* exactly work to lower the cholesterol level, it is only known that it has this effect. Polysaccharides from *Ganoderma lucidum* have hypolipidemic effects which not necessarily mean that they only lower cholesterol, they can also lower triglycerides in the blood. Both lanosterol and ganoderic acids Mf and T-O regulate and inhibit cholesterol synthesis. This indicates that triterpenes might be more effective in regulating the synthesis, while polysaccharides lower the levels that are already high. In general, it is known that β-glucans from both medicinal mushrooms and other foods have cholesterol lowering effects by isolating bile acid in the intestine, reducing their absorption

and return to the liver. The reduced bile acid leads to conversion of cholesterol to bile acid, which means the cholesterol level is decreasing. [91]

Triterpenes from *Ganoderma lucidum* have been shown to be effective in lowering hypertension, high blood pressure. Especially effective are the ganoderic acids, where ganoderic acid F has an atherosclerosis protective effect by inhibiting the angiotensin converting enzyme, which will result in lower blood pressure, and ganoderic acids B and C are very strong anti-hypertensive agents. Compounds from *Inonotus obliquus* may have some effect, but those from *Ganoderma lucidum* are much stronger.

Also inhibition of platelet aggregation has been reported by the ganodermic acid S that stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate. This phospholipid triggers platelet aggregation and by regulating the breakdown of the phospholipid it can reduce the risk of thrombi and heart attacks.

One diabetic symptom is high blood glucose. It is not directly related to cardiovascular activities, but here are glucose lowering properties counted in to this category, since it gives a more balanced blood concentration. Only polysaccharides from both mushrooms have shown effects of reducing blood glucose level, both in diabetic test animals, as well as in healthy animals.

These cardiovascular properties are not connected to each other in the same way as for instance the properties in the immunostimulating or anti-cancer effects are. This means it is not as easy to make direct conclusions about the connections between the properties and compounds. It can though be said that *Ganoderma lucidum* seems to be the mushroom that ‘cleans’ the blood more effectively, by reducing blood fats, glucose and lowering high blood pressure.

5.2.5 Anti-viral, -fungal and –allergic effects

Medicinal mushrooms are known for having anti-fungal effects, but also anti-viral effects have been shown. Some have inhibition action against HIV regulation as well as anti-allergic effects. These effects are listed in table 9 and together with

the compounds of *Inonotus obliquus* and *Ganoderma lucidum* that have these effect.

Table 9. Anti-viral, -fungal and -allergic effects of *Inonotus obliquus* and *Ganoderma lucidum*.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Inhibition of HIV replication		•		•
Anti-viral effect		•		•
Anti-fungal effect		•		
Anti-allergic effect				•

Triterpenes from *Inonotus obliquus*, betulinic acid and in some part betulin, have been reported to have inhibitive activities against HIV replication. During the HIV replication the virus is reproducing in the host cell. Several different triterpenes from *Ganoderma lucidum*, for instance ganoderiol F, ganodermanontriol, ganolucidinic acid A, and lucidumol B, have also inhibitive effects against HIV. In contrast to betulinic acid, they have anti-HIV protease activity. This is the last stage in the replication cycle where the virus matures with the help of the protease enzyme, and by inhibiting the protease activity the virus cannot spread further. It is supposed that carbon 3, 24, and 25 in the triterpenes are important factors for the anti-HIV protease activity.

It is not only HIV that these two mushrooms have anti-viral effects against. The triterpene lanosterol has shown evidence of other anti-viral effects. Ganoderic acids have also anti-viral effects. They have especially shown inhibitory effects against hepatitis B virus. It is in overall known that triterpenes from medicinal mushrooms are having anti-viral as well as anti-microbial effects.

Most medicinal mushrooms have anti-fungal effects. The triterpene ergosterol is not found in human or animal bodies, which makes it an excellent anti-fungal compound. Even triterpenes from *Ganoderma lucidum* have anti-fungal activities, although the theoretical part did not report anything about it.

Triterpenes from *Ganoderma lucidum* have been reported to have anti-allergic potency. The effects behind ganoderic acid C are not specified while ganoderic acids A and D for instance have inhibiting actions against histamine release. Histamine is the neurotransmitter that triggers the inflammatory reaction. There is no information being reported if *Inonotus obliquus* has this kind of properties.

It is only triterpenes that are having inhibiting effects on viruses, fungi and allergic reactions. There does not seem to be any bigger differences between the two mushrooms, without the fact that *Inonotus obliquus* has not shown any signs of having anti-allergic effects. Why triterpenes are the compounds that have anti-viral, -microbial and –fungal effect is not clear. In virus and fungi they may have the effect of crossing lipid shells, which could be a reason.

5.2.6 Other effects

In addition to all these properties mentioned above, the adaptogenic effect that *Ganoderma lucidum* has must be pointed out specifically. It is the triterpenes that have this effect. Adaptogens help to adapt to the surroundings and situations around you. When you are stressed it gives an anti-stress effect, while it can boost the energy levels if you are suffering from fatigue. In the chapter of medicinal mushrooms, polysaccharides are said to have adaptogenic effect, though this is observed for neither *Inonotus obliquus* nor *Ganoderma lucidum*. Triterpenes are in general the compounds that have tonic effects, however, *Inonotus obliquus* has not shown any result of this.

The sleep quality and the time you sleep can be improved and prolonged, respectively, by polysaccharides from *Ganoderma lucidum*. It is not totally clear what kind of polysaccharides contribute to this, but since they most likely have something to do with the immunomodulating activity to do, especially cytokines,

it is likely that β-glucans are important factors for this reaction. It is though interesting why *Inonotus obliquus* does not show this kind of effects, even though it contains higher amount of β-glucans.

Triterpenes from *Ganoderma lucidum* have also been reported to have anti-hepatotoxic effects. Especially ganoderic acids R and S have shown significant results of inhibiting liver injury. Triterpenes usually work by inhibiting enzymes that affect a damaging process for the liver. There is no information that *Inonotus obliquus* would have this kind of effect.

6 Conclusions of the analysis

In this chapter the analytical part is being summarized and the final conclusions about the similarities and differences between *Inonotus obliquus* and *Ganoderma lucidum* are presented.

6.1 Interpretation of the analysis

For both *Inonotus obliquus* and *Ganoderma lucidum* polysaccharides and triterpenes are the main compounds. β-Glucans are the most important polysaccharides for both mushrooms. They are not exactly the same, since all medicinal mushrooms have their own specific glucans and it depends where they come from, mycelia or fruiting body or if they are cultivated or wild, but although they act in almost the same way with some variations. All triterpenes are different in the two mushrooms, but most of them have the same base, a lanostane base. On the whole they act in the same way, but triterpenes from *Ganoderma lucidum* are more active compared with *Inonotus obliquus*. *Inonotus obliquus* contains much more carbohydrates, while *Ganoderma lucidum* is richer in lipids. This can explain why triterpenes from *Ganoderma lucidum* are more active than triterpenes from *Inonotus obliquus*. It also gives a hint that polysaccharides from *Inonotus obliquus* are very powerful even though they have the same properties as polysaccharides from *Ganoderma lucidum*.

Based on the analysis of the properties of the two mushrooms a new table has been done that is almost similar to table 4 in chapter 5. Some details have been changed to give a clearer overall picture of the properties of the mushrooms and the compounds that induce them.

Table 10. Effects of polysaccharides and triterpenes from *Inonotus obliquus* and *Ganoderma lucidum* after the theoretical part have been analyzed.

	Inonotus obliquus		***Ganoderma lucidum***	
	Polysaccharides	**Triterpenes**	**Polysaccharides**	**Triterpenes**
Antioxidant activity	●	●	●	●
Immunostimulating effects	●		●	
Anti-cancer activity		●		●
Cholesterol lowering effects	●	●	●	●
Cardiovascular activities				●
Anti-viral effects		●		●
Anti-fungal effects		●		●
Glucose lowering effects	●		●	
Adaptogenic effects				●
Anti-allergic effects				●

Both mushrooms as well as both polysaccharides and triterpenes have antioxidant activity. *Inonotus obliquus* is said to be one of the most powerful antioxidants in the world and together with a high amount of carbohydrates and fiber it is very likely that it is β-glucans from *Inonotus obliquus* that are among the most powerful compounds in antioxidant activity. The analysis shows in general that polysaccharides act in a more specified way, when triterpenes only inhibit oxidation reactions or it is not specified how they work. This may indicate that polysaccharides are the more powerful ones as well.

The immunostimulating activities are caused only by polysaccharides from both mushrooms. They work in almost the same way, but most likely the activation from *Inonotus obliquus* is stronger and it activates the humoral immunity more while *Ganoderma lucidum* has a better effect on the cell-meditated immunity.

Anti-cancer effect is of course a result of a boosted immunity, but also direct anti-cancer effects are caused by triterpenes from both mushrooms. Polysaccharides from sclerotia of *Inonotus obliquus* have shown direct anti-cancer effect, but this is very rare. *Inonotus obliquus* may be somewhat stronger, but it is also the mushroom that has triterpenes that only delay tumorigenicity instead of directly inhibiting tumors.

Both triterpenes and polysaccharides from both mushrooms have cholesterol lowering effects. It seems that triterpenes have the effect of inhibiting the cholesterol synthesis, while polysaccharides act in another way, probably by lowering already high levels. Most likely *Ganoderma lucidum* is the stronger one here.

Preventing effects on harmful cardiovascular activities is for instance lowering of high blood pressure and inhibition of platelet aggregation. Only triterpenes from *Ganoderma lucidum* have shown these actions.

Triterpenes from both *Inonotus obliquus* and *Ganoderma lucidum* have anti-viral effects against for instance HIV and hepatitis B. They seem to be equally strong.

Anti-fungal effects in the theoretical part were only reported from triterpenes of *Inonotus obliquus*, but even *Ganoderma lucidum* has strong effect against fungi. Most likely, triterpenes that have anti-fungal effect act in the same way as they do for anti-viral, exactly how is not clear from this analysis.

Blood glucose is being lowered by polysaccharides from both mushrooms. It may be that polysaccharides work a little bit like insulin that lowers blood glucose. Both mushrooms seem to have the same effect.

Ganoderma lucidum is classified as an adaptogen and this is because of the triterpenes. One the effect of an adaptogen is the anti-stress function. This can help to enhance the sleep quality. *Ganoderma lucidum* has also shown effects of enhancing the sleep quality, but this function was related to the immune system and thus caused by polysaccharides. It may explain the fact that polysaccharides from medicinal mushrooms can help the body to adapt to different situations and decrease stress. So triterpenes are not the only compounds that give adaptogenic effects, even though they clearly are the stronger ones.

Moreover, triterpenes from *Ganoderma lucidum* also have anti-allergical effects. This effect is taking part for instance due to the inhibition of histamine release that cause allergic reactions.

By comparing table 10 for *Ganoderma lucidum* with figure 11, the similarities are obvious. This gives a hint that the analysis is correct for *Ganoderma lucidum*.

6.2 Summary

The objective of the thesis was to analyze the similarities and differences between the medicinal mushrooms *Inonotus obliquus* and *Ganoderma lucidum*. They both contain polysaccharides and triterpenes as their main compounds. The most important polysaccharides for both of them are β-glucans. They are somewhat different from each other and it also depends on where they are from, though they act in the same way by for instance having antioxidant and immunostimulating activities, as well as lowering effects on blood cholesterol and glucose. Due to the fact that *Inonotus obliquus* contains much more carbohydrates and fibers than *Ganoderma lucidum*, it has to have much stronger effects on these properties, especially immunostimulating and antioxidant activities. *Ganoderma lucidum* has much more triterpenes that are also more active than in *Inonotus obliquus*. The triterpenes are different, but most of them have the same base. Triterpenes from both of the mushrooms have antioxidant and anti-cancer activities, and also anti-viral and anti-fungal effects, which means that they act mainly in the same way. *Ganoderma lucidum* contains more lipids than *Inonotus obliquus* and should thus have more or stronger effects caused by triterpenes, which seems to be true. These triterpenes have adaptogenic and anti-allergical effects, as well as inhibitory activities on cardiovascular activities that *Inonotus obliquus* does not have. The differences in their contents make differences in the properties, even though they otherwise are very similar.

6.3 Error analysis

The error analysis points out things that could have been done differently in the thesis or factors that may have affected the result.

Since the analysis is based on literature, there could have been more references than 91. The most optimal would have been to go through all scientific material that is possible to find to get as good and wide result as possible. It is though a question of about thousands of papers published per mushroom, which all cannot be analyzed for a thesis. With a larger amount of references it could also be possible to classify the different properties to see which compounds have most effects. The references here were not enough to be able to make any deeper conclusions based on the amount of properties. For instance it would be very interesting to count reports on the immunostimulating activities caused by β-glucans of *Inonotus obliquus* and *Ganoderma lucidum* to see if *Inonotus obliquus* gives a clearly higher result. It has to be pointed out that most of the scientific studies that are made about medicinal mushrooms are focused on themes like cancer studies, which are subjects having monetary effects or a huge interest to find pharmaceutical solutions for people in the industrialized world. Subjects that cannot give the same pharmaceutical benefits or do not have the same interest, for instance adaptogenic effects, do not get the same attention in scientific papers, which is sad since it does not give as objective picture as possible. In the other way adaptogenic effects can be difficult to measure compared with cancer studies and a longer timeline is needed.

There is much information we do not know about the compounds that have been studied in the scientific material. As it already has been mentioned several times, there is a big difference where the compounds come from; fruiting body or mycelium, if the compounds are cultivated or processed et cetera. In several materials, it is not specified where the compounds are from or if there could be other compounds in an extract. This gives an uncertain result, but here again could a larger reference material enhance the result. The whole study could also have gotten a larger expanse if more compounds or compound-complexes had been taken into account here. For instance properties caused by compounds like

flavonoids and phenols or complexes together with them, could have been interesting to analyze.

Much of the result in the study is based on the contents of carbohydrates and lipids in *Inonotus obliquus* and *Ganoderma lucidum*. There is no guarantee that these numbers are the same for all mushrooms. As we could see in table 2, the relationship between polysaccharides and triterpenes in *Ganoderma lucidum* can vary a lot. It is the same with the amount of carbohydrates and lipids in the mushrooms. They may vary depending on where they are from or when the test has been done.

This study is meant to be objective and is based only on scientific material. After working very intensively with medicinal mushrooms for more than one year and continuously reading about them almost daily, I have learned so much that it sometimes has been difficult to separate facts directly from references and my own knowledge in the study.

6.4 Further studies

There are several furthers studies that could be done in this subject, since it is a quite new subject in the Western world. *Inonotus obliquus* and *Ganoderma lucidum* are said to be the king and the queen of medicinal mushrooms, so it is very interesting to focus on comparisons between them and also see how they support each other, which can enhance the effects they have.

As mentioned in the error analysis, the study could be made so much wider by increasing the number of references and focusing on more properties and more compounds. Especially to get better and more data to conclude which compounds are more effective based on both how many studies that show the same result and studies where compounds are from different parts of the mushroom but still show the same result.

A very interesting subject to go deeper into is the immunostimulating activities. First more basic information about different cytokines, leukocytes and antibodies is needed and how they work, as well as how they are connected. With more data about them it is easier to understand better how polysaccharides from medicinal mushrooms act. Especially rewarding would be to find the link between anti-inflammatory activities and immune enhancing effect where NF-κB, TNF-α, and NO are figuring as important cytokines that both can increase and inhibit inflammations.

The absolutely best study to be done would be to perform own research on the medicinal mushrooms. The variations here could be multiple. The effects of mushrooms from different places could be investigated and compared, as well as compounds from different parts of the mushrooms, not only from fruiting body or mycelia, but there is also difference if the compounds are taken from a younger or older part of the same mushroom. To get the best result, the trials should be repeated. Overall to get a clearer picture how the amounts of compounds vary depending on where the samples are from would probably help everyone in further studies.

References

[1] Zong, A., Cao, H., Wang, F., Anticancer polysaccharides from natural resources: A review of recent research, *Carbohydr Polym,* **90** (2012), p. 1395-410.

[2] Sullivan, R., Smith, J., Rowan, N., *Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments,* University of Strathclyde, London 2002.

[3] Halpern, G. M., *Healing mushrooms: Ancient wisdom for better health,* 1st, Square One Publishers, Garden City Road, NY 2007.

[4] Clayden, J., Greeves, N., Warren, S., Wothers, P., *Organic chemistry,* Oxford University Press, New York 2001.

[5] Muffler, K., Leipold, D., Scheller, M., Haas, C., Steingroewer, J., Bley, T., et al., Biotransformation of triterpenes, *Process Biochemistry,* **46** (2011), p. 1-15.

[6] Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Miroslav Kvasnica, M., Biedermann, D., et al., Pharmacological activities of natural triterpenoids and their therapeutic implications, *Natural Product Reports,* (2006),

[7] Parmar, S. K., Sharma, T. P., Airao, V. B., Bhatt, R., Aghara, R., Chavda, S., et al., Neuropharmacological effects of triterpenoids, *Phytopharmacology,* **4** (2013), p. 354-72.

[8] Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., et al., A higher-level phylogenetic classification of the fungi, *Mycol Res,* **111** (2007), p. 509-47.

[9] Wasser, S., Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides, *Appl Microbiol Biotechnol,* **60** (2003), p. 258-74.

[10] Halmetoja, J., *Pakurikääpä,* J. Halmetoja Studios, Tampere 2012.

[11] Cheung, P.C.K., *Mushrooms as Functional Food,* 1st, John Wiley & Sons, United Kingdom 2008, 260 p.

[12] Zhang, M., Cui, S. W., Cheung, P. C. K., Wang, Q., Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity, *Trends Food Sci Technol,* **18** (2007), p. 4-19.

[13] Moradali, M., Mostafavi, H., Ghods, S., Hedjaroude, G., Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi), *Int Immunopharmacol,* **7** (2007), p. 701-24.

[14] Kim, Y. O., Han, S. B., Lee, H. W., Ahn, H. J., Yoon, Y. D., Jung, J. K., et al., Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of inonotus obliquus, *Life Sci*, **77** (2005), p. 2438-56.

[15] Kim, Y. O., Park, H. W., Kim, J. H., Lee, J. Y., Moon, S. H., Shin, C. S., Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of inonotus obliquus, *Life Sci*, **79** (2006), p. 72-80.

[16] Ham, S., Kim, S., Moon, S., Chung, M. J., Cui, C., Han, E., et al., Antimutagenic effects of subfractions of chaga mushroom (inonotus obliquus) extract, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, **672** (2009), p. 55-9.

[17] Lee, I., Kim, Y., Jang, Y., Jung, J., Yun, B., New antioxidant polyphenols from the medicinal mushroom inonotus obliquus, *Bioorg Med Chem Lett*, **17** (2007), p. 6678-81.

[18] Cui, Y., Kim, D., Park, K., Antioxidant effect of inonotus obliquus, *J Ethnopharmacol*, **96** (2005), p. 79-85.

[19] Boh, B., Berovic, M., Zhang, J., Zhi-Bin, L., Ganoderma lucidum and its pharmaceutically active compounds In: Biotechnology Annual Review, Elsevier, 2007, 265-301.

[20] Jia, J., Zhang, X., Hu, Y., Wu, Y., Wang, Q., Li, N., et al., Evaluation of in vivo antioxidant activities of ganoderma lucidum polysaccharides in STZ-diabetic rats, *Food Chem*, **115** (2009), p. 32-6.

[21] Berger A, Rein D, Kratky E, Monnard I, Hajjaj H, Meirim I, Piguet-Welsch C, Hauser J, Mace K, Niederberger P, Cholesterol-lowering properties of Ganoderma lucidum in vitro, ex vivo, and in hamsters and minipigs, *Lipids in Health and Disease*, (2004) 2.

[22] Kuo, M., Weng, C., Ha, C., Wu, M., Ganoderma lucidum mycelia enhance innate immunity by activating NF-κB, *J Ethnopharmacol*, **103** (2006), p. 217-22.

[23] Winkler, D., CORDYCEPS SINENSIS: A precious parasitic fungus infecting tibet, *Field Mycology*, **11** (2010), p. 60-7.

[24] Koh, J.H., Kim, K.M., Kim, J.M., Song, J.C., Suh, H.J., Antifatigue and antistress effect of the hot-water fraction from mycelia of Cordyceps sinensis, - *Biological and Pharmaceutical Bulletin*, **26** (2003) 691-694.

[25] Hsu, C., Huang, Y., Tsai, S., Sheu, C., Huang, B., In vivo and in vitro stimulatory effects of cordyceps sinensis on testosterone production in mouse leydig cells, *Life Sci*, **73** (2003), p. 2127-36.

[26] Shashidhar, M. G., Giridhar, P., Udaya Sankar, K., Manohar, B., Bioactive principles from cordyceps sinensis: A potent food supplement – A review, *Journal of Functional Foods,*

[27] Mori, K., Obara, Y., Hirota, M., Azumi, Y., Kinugasa, S., Inatomi, S., et al., Nerve Growth Factor-Inducing Activity of Hericium erinaceus in 1321N1 Human Astrocytoma Cells, *Biological and Pharmaceutical Bulletin,* **31** (2008) 1727-1732.

[28] Mori, K., Inatomi, S., Ouchi, K., Azumi, Y., Tuchida, T., Improving effects of the mushroom yamabushitake (hericium erinaceus) on mild cognitive impairment: A double-blind placebo-controlled clinical trial, *Phytotherapy Research,* **23** (2009), p. 367-72.

[29] Mori, K., Kikuchi, H., Obara, Y., Iwashita, M., Azumi, Y., Kinugasa, S., et al., Inhibitory effect of hericenone B from hericium erinaceus on collagen-induced platelet aggregation, *Phytomedicine,* **17** (2010), p. 1082-5.

[30] Lee, J. S., Cho, J. Y., Hong, E. K., Study on macrophage activation and structural characteristics of purified polysaccharides from the liquid culture broth of hericium erinaceus, *Carbohydr Polym,* **78** (2009), p. 162-8.

[31] Bisen, P. S., Baghel, R. K., Sanodiya, B. S., Thakur, G. S., Prasad, G. B. K. S., Lentinus edodes: A macrofungus with pharmacological activities, *Current Medicinal Chemistry,* **17** (2010), p. 2419-30.

[32] Mayell, M., Maitake extracts and their therapeutic potential - A review, *Alternative Medicine Review,* **6** (2001), p. 48-60.

[33] Matsui, K., Kodama, N., Nanba, H., Effects of maitake (grifola frondosa) D-fraction on the carcinoma angiogenesis, *Cancer Lett,* **172** (2001), p. 193-8.

[34] Chen, G., Ma, X., Liu, S., Liao, Y., Zhao, G., Isolation, purification and antioxidant activities of polysaccharides from grifola frondosa, *Carbohydr Polym,* **89** (2012), p. 61-6.

[35] Cui, J. and Chisti, Y., Polysaccharopeptides of coriolus versicolor: Physiological activity, uses, and production, *Biotechnol Adv,* **21** (2003), p. 109-22.

[36] Harhaji, L., Mijatović, S., Maksimović-Ivanić, D., Stojanović, I., Momčilović, M., Maksimović, V., et al., Anti-tumor effect of coriolus versicolor methanol extract against mouse B16 melanoma cells: In vitro and in vivo study, *Food and Chemical Toxicology,* **46** (2008), p. 1825-33.

[37] Wisitrassameewong, K., Karunarathna, S. C., Thongklang, N., Zhao, R., Callac, P., Moukha, S., et al., Agaricus subrufescens: A review, *Saudi Journal of Biological Sciences,* **19** (2012), p. 131-46.

[38] Aida, F. M. N. A., Shuhaimi, M., Yazid, M., Maaruf, A. G., Mushroom as a potential source of prebiotics: A review, *Trends Food Sci Technol,* **20** (2009), p. 567-75.

[39] World Health Organization, Guidelines for the assessment of herbal medicines, http://whqlibdoc.who.int/hq/1991/WHO_TRM_91.4.pdf, 26.8.2013.

[40] European Commission, Herbal medicinal products, http://ec.europa.eu/health/human-use/herbal-medicines/, 26.8.2013.

[41] European parlament, DIRECTIVE 2004/24/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 31 march 2004 amending, as regards traditional herbal medicinal products, directive 2001/83/EC on the community code relating to medicinal products for human use, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0085:0090:en:PDF, 26.8.2013.

[42] Europeans Medicines Agency, Committee on herbal medicinal products (HMPC), http://www.emea.europa.eu/ema/index.jsp?curl=pages/about_us/general/general_content_000264.jsp&mid=WC0b01ac0580028e7c, 26.8.2013.

[43] Wong, K., Lai, C. K. M., Cheung, P. C. K., Immunomodulatory activities of mushroom sclerotial polysaccharides, *Food Hydrocolloids,* **25** (2011), p. 150-8.

[44] Pérez, S., Kouwijzer, M., Mazeau, K., Engelsen, S. B., Modeling polysaccharides: Present status and challenges, *Journal of Molecular Graphics,* **14** (1996), p. 307-21.

[45] Koyama, T., Gu, Y., Taka, A., Fungal medicine, fuscoporia obliqua, as a traditional herbal medicine: Its bioactivities, in vivo testing and medicinal effects, *Asian Biomedicine,* **2** (2008), p. 459-69.

[46] Wachtel-Galor, S., Yuen, J., Buswell, J. A., Benzie, I. F. F., Ganoderma lucidum (lingzhi or reishi): A medicinal mushroom In: Herbal Medicine: Biomolecular and Clinical Aspects, Benzie, I. F. F. and Wachtel-Galor, S., editors. 2nd, Boca Raton (FL): CRC Press, 2011, Chapter 9.

[47] Ríos, J., Effects of triterpenes on the immune system, *J Ethnopharmacol,* **128** (2010), p. 1-14.

[48] Shashkina, M. Y., Shashkin, P. N., Sergeev, A. V., Chemical and medicobiological properties of chaga (review), *Pharmaceutical Chemistry Journal,* **40** (2006), p. 560-8.

[49] Zheng, W., Miao, K., Liu, Y., Zhao, Y., Zhang, M., Pan, S., et al., Chemical diversity of biologically active metabolites in the sclerotia of *inonotus obliquus* and submerged culture strategies for up-regulating their production, *Appl Microbiol Biotechnol,* **87** (2010), p. 1237-54.

[50] Nakajima, Y., Sato, Y., Konishi, T., Antioxidant small phenolic ingredients in *inonotus obliquus* (persoon) pilat (chaga), *Chem Pharm Bull (Tokyo),* **55** (2007), p. 1222-6.

[51] Lee, M., Hyeon-Hur, Chang, K., Lee, T., Ka, K., Jankovsk, L., Introduction to distribution and ecology of sterile conks of *inonotus obliquus*, *Mycobiology,* **36** (2008), p. 199-202.

[52] Finnish Food Safety Authority Evira, Novel foods in finland, http://www.evira.fi/portal/en/food/manufacture+and+sales/novel+foods/frequently+asked+questions+regarding+novel+foods/, 26.8.2013.

[53] Finnish Food Safety Authority Evira, Novel foods, http://www.evira.fi/portal/en/food/manufacture+and+sales/novel+foods/, 26.8.2013

[54] European Commission, Health and consumers - food safety - novel foods, http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/index.cfm?action=mod_search.details&seqfce=421, 26.8.2013

[55] Finland's environmental administration, Jokamiehenoikeudet, http://www.ymparisto.fi/default.asp?contentid=423339&lan=FI, 26.8.2013.

[56] Suomen ympäristö, Jokamiehenoikeudet ja toimiminen toisen alueella, http://www.ymparisto.fi/download.asp?contentid=138438&lan=fi, 26.8.2013.

[57] Hu, H., Zhang, Z., Lei, Z., Yang, Y., Sugiura, N., Comparative study of antioxidant activity and antiproliferative effect of hot water and ethanol extracts from the mushroom inonotus obliquus, *Journal of Bioscience and Bioengineering,* **107** (2009), p. 42-8.

[58] Mazurkiewicz, W., Analysis of aqueous extract of inonotus obliquus, *Acta Poloniae Pharmaceutica - Drug Research,* **63** (2006), p. 497-501.

[59] Hyun, K. W., Jeong, S. C., Lee, D. H., Park, J. S., Lee, J. S., Isolation and characterization of a novel platelet aggregation inhibitory peptide from the medicinal mushroom, inonotus obliquus, *Peptides,* **27** (2006), p. 1173-8.

[60] Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., Teixeira, J. A., Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review, *Biotechnol Adv,* **29** (2011), p. 365-73.

[61] Ma, L., Chen, H., Zhu, W., Wang, Z., Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom inonotus obliquus, *Food Res Int,* **50** (2013), p. 633-40.

[62] Fan, L., Ding, S., Ai, L., Deng, K., Antitumor and immunomodulatory activity of water-soluble polysaccharide from inonotus obliquus, *Carbohydr Polym,* **90** (2012), p. 870-4.

[63] Zhang, N., Chen, H., Ma, L., Zhang, Y., Physical modifications of polysaccharide from inonotus obliquus and the antioxidant properties, *Int J Biol Macromol,* **54** (2013), p. 209-15.

[64] Choi, S. Y., Hur, S. J., An, C. S., Jeon, Y. H., Jeoung, Y. J., Bak, J. P., et al., Anti-inflammatory effects of inonotus obliquus in colitis induced by dextran sodium sulfate, *Journal of Biomedicine and Biotechnology,* (2010), p. 1-5.

[65] Kim, Y. O., Park, H. W., Kim, J. H., Lee, J. Y., Moon, S. H., Shin, C. S., Anti-cancer effect and structural characterization of endo-polysaccharide from cultivated mycelia of inonotus obliquus, *Life Sci,* **79** (2006), p. 72-80.

[66] Rhee, S. J., Cho, S. Y., Kim, K. M., Cha, D., Park, H., A comparative study of analytical methods for alkali-soluble b-glucan in medicinal mushroom, chaga (inonotus obliquus), *LWT - Food Science and Technology,* **41** (2008), p. 545-9.

[67] Fulda, S., Betulinic acid for cancer treatment and prevention, *International journal of molecular sciences,* **9** (2008), p. 1096-107.

[68] Yin, M., Anti-glycative potential of triterpenes: A mini-review, *Biomedicine,* **2** (2012), p. 2-9.

[69] Nomura, M., Takahashi, T., Uesugi, A., Tanaka, R., Kobayashi, S., Inotodiol, a lanostane triterpenoid, from inonotus obliquus inhibits cell proliferation through caspase-3-dependent apoptosis , *Anticancer research,* **28** (2008), p. 2691-6.

[70] Handa, N., Yamada, T., Tanaka, R., An unusual lanostane-type triterpenoid, spiroinonotsuoxodiol, and other triterpenoids from inonotus obliquus, *Phytochemistry,* **71** (2010), p. 1774-9.

[71] Leskosek-Cukalovic, I., Despotovic, S., Lakic, N., Niksic, M., Nedovic, V., Tesevic, V., Ganoderma lucidum — medical mushroom as a raw material for beer with enhanced functional properties, *Food Res Int,* **43** (2010), p. 2262-9.

[72] Gao, P., Hirano, T., Chen, Z., Yasuhara, T., Nakata, Y., Sugimoto, A., Isolation and identification of C-19 fatty acids with anti-tumor activity from the spores of ganoderma lucidum (reishi mushroom), *Fitoterapia,* **83** (2012), p. 490-9.

[73] Metsähallitus, Saaristomeren kansallispuiston suursienet, http://julkaisut.metsa.fi/assets/pdf/lp/Asarja/a112.pdf, 26.8.2013.

[74] Ympäristöhallinnon yhteinen verkkopalvelu, Suojelualueet missä lakkakääpä kasvaa, http://www.ymparisto.fi/fi-FI/haku?n=25246&s=lakkak%C3%A4%C3%A4p%C3%A4&d=1&o=Rank&page=2, 26.8.2013.

[75] Wang, X., Xi, R., Li, Y., Wang, D., Yao, Y., The species identity of the widely cultivated ganoderma, 'G. lucidum' (ling-zhi), in china , *PLoS ONE,* **7** (2012), p. 1-12.

[76] Smith, J. E., Rowan, N. J., Sullivan, R., Medicinal mushrooms: A rapidly developing area of biotechnology for cancer therapy and other bioactivities , *Biotechnology Letters,* **24** (2002), p. 1839-45.

[77] Paterson, R. R. M., Ganoderma – A therapeutic fungal biofactory, *Phytochemistry,* **67** (2006), p. 1985-2001.

[78] Calviño, E., Manjón, J. L., Sancho, P., Tejedor, M. C., Herráez, A., Diez, J. C., Ganoderma lucidum induced apoptosis in NB4 human leukemia cells: Involvement of akt and erk, *J Ethnopharmacol,* **128** (2010), p. 71-8.

[79] Smina, T. P., Mathew, J., Janardhanan, K. K., Devasagayam, T. P. A., Antioxidant activity and toxicity profile of total triterpenes isolated from ganoderma lucidum (fr.) P. karst occurring in south india, *Environ Toxicol Pharmacol,* **32** (2011), p. 438-46.

[80] Ulbricht, C., Abrams, T. R., Bent, S., Boon, H., et al., Reishi mushroom (ganoderma lucidum): Systematic review by the natural standard research collaboration, *Journal of the Society of Integrative Oncology,* **8** (2010), p. 148-59.

[81] Guo, L., Xie, J., Ruan, Y., Zhou, L., Zhu, H., Yun, X., et al., Characterization and immunostimulatory activity of a polysaccharide from the spores of ganoderma lucidum, *Int Immunopharmacol,* **9** (2009), p. 1175-82.

[82] Wasser SP, Didukh MY. Culinary-medicinal higher basidiomycete mushrooms as a prominent source of dietary supplements and drugs from the 21st century. Proceedings of the 5th International Conference of mushroom biology and Mushroom products; China. 2005.

[83] Cheung, P. C. K., The nutritional and health benefits of mushrooms, *Nutrition Bulletin,* **35** (2010), p. 292-9.

[84] Gao, Y., Zhou, S., Jiang, W., Huang, M., Xihu Dai, X., Effects of Ganopoly® (A *ganoderma lucidum* polysaccharide extract) on the immune functions in Advanced-Stage cancer patients, *Immunological Investigations,* **32** (2003), p. 201-15.

[85] Nie, S., Zhang, H., Li, W., Xie, M., Current development of polysaccharides from ganoderma: Isolation, structure and bioactivities, *Bioactive Carbohydrates and Dietary Fibre,* **1** (2013), p. 10-20.

[86] Cui, X., Cui, S., Zhang, J., Wang, Z., Yu, B., Sheng, Z., et al., Extract of ganoderma lucidum prolongs sleep time in rats, *J Ethnopharmacol,* **139** (2012), p. 796-800.

[87] Chu, Q., Wang, L., Cui, X., Fu, H., Lin, Z., Lin, S., et al., Extract of ganoderma lucidum potentiates pentobarbital-induced sleep via a GABAergic mechanism, *Pharmacology Biochemistry and Behavior,* **86** (2007), p. 693-8.

[88] Shaoqiong, X., Wanqing, L., Zhirong, Y., Zhidong, W., Related gene expressions in anti-keratinocyte aging induced by ganoderma lucidum polysaccharides, *J Med Coll PLA*, **23** (2008), p. 167-75.

[89] Sanodiya, B. S., Thakur, G. S., Baghel, R. K., Prasad, G. B. K. S., Bisen, P. S., Ganoderma lucidum: A potent pharmacological macrofungus, *Current Pharmaceutival Biotechnology*, **10** (2009), p. 717-42.

[90] Ruan, W. and Popovich, D. G., Ganoderma lucidum triterpenoid extract induces apoptosis in human colon carcinoma cells (caco-2), *Biomedicine & Preventive Nutrition*, **2** (2012), p. 203-9.

[91] Chen, J. and Huang, X., The effects of diets enriched in beta-glucans on blood lipoprotein concentrations, *Journal of Clinical Lipidology*, **3** (2009), p. 154-8.