Graphical Abstract

Chemical features of *Ganoderma* polysaccharides with antioxidant, antitumor and antimicrobial activity

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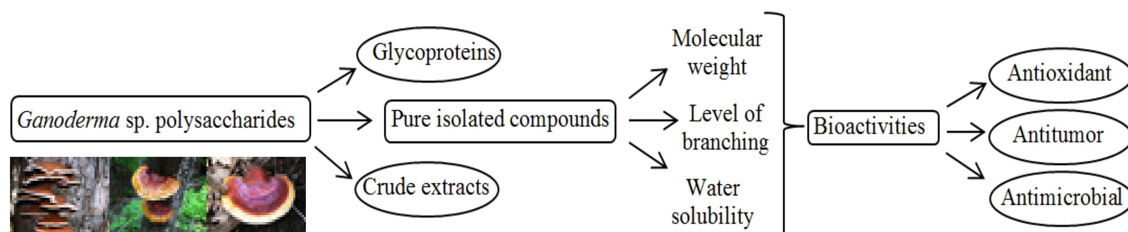

This review aims to contribute to the growing knowledge on bioactive (antioxidant, antitumor and antimicrobial) properties of polysaccharides, glycoproteins and polysaccharidic extracts obtained from *Ganoderma* species. The chemical features were analyzed and related to correspondent bioactivities.

Research highlights

Chemical features of *Ganoderma* bioactive polysaccharides were discussed.

Methods for extraction, isolation and identification were evaluated.

Bioactivity of polysaccharidic extracts and purified compounds were discussed.

Integration of data allowed deduction of structure-activity relationships.

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Chemical features of *Ganoderma* polysaccharides with antioxidant, antitumor and antimicrobial activities

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ABSTRACT

Ganoderma genus comprises one of the most commonly studied species worldwide, *G. lucidum*. However, other *Ganoderma* species have been also reported as important sources of bioactive compounds. Polysaccharides are important contributors to the medicinal properties reported for *Ganoderma* species, as demonstrated by the numerous publications, including reviews, on this matter. Yet, what are the chemical features of *Ganoderma* polysaccharides that have bioactivity? In the present manuscript, the chemical features of *Ganoderma* polysaccharides with reported antioxidant, antitumor and antimicrobial activities (the most studied worldwide) are analyzed in detail. The composition of sugars (homo- versus hetero-glucans and other polysaccharides), type of glycosidic linkages, branching patterns, and linkage to proteins are discussed. Methods for extraction, isolation and identification are evaluated and, finally, the bioactivity of polysaccharidic extracts and purified compounds are discussed. The integration of data allows deduction of structure-activity relationships and gives clues to the chemical aspects involved in *Ganoderma* bioactivity.

KEYWORDS: *Ganoderma*; Polysaccharides; Chemical features; Bioactivity; Structure-activity

1. Introduction

Ganoderma is a genus of polypore macrofungi growing in decaying logs or tree stumps (Kirk et al., 2011). Commonly known as Lingzhi, *Ganoderma* comprises the most studied species of medicinal mushrooms in the world. In ancient China, Lingzhi was believed to bring longevity, due to its mysterious power of healing the body and calming the mind (Huie and Di, 2004).

1.1. Bioactivity of Ganoderma

The above mentioned genus has been widely studied regarding its bioactive properties (Paterson, 2006; Nie et al., 2013), including antibacterial, antioxidant, antitumor and other effects (Wang et al., 1997; Wasser, 2002; Heleno et al., 2012; Li et al., 2012; Heleno et al., 2013; Popović et al., 2013; Zhonghui et al., 2013). The beneficial health properties of *Ganoderma* species are attributed to a wide variety of bioactive components, such as polysaccharides, triterpenes, sterols, lectins and other proteins (Wang et al., 2002; Ferreira et al., 2010).

Different kinds of bioactive polysaccharides have been extracted and isolated from the fruiting bodies of different *Ganoderma* species (Kozarsky et al., 2011; Liu et al., 2010; Kozarsky et al., 2012; Ma et al., 2013; Shi et al., 2013), and represent a structurally diverse class of biological macromolecules with a wide-range of physiological properties. The major bioactive *Ganoderma* polysaccharides are composed of (1→3), (1→6)-α/β-glucans, glycoproteins and water soluble heteropolysaccharides (Nie et al., 2013) with glucose, mannose, galactose, fucose, xylose and arabinose combined in different proportions and types of glycosidic linkages, as well as peptide bonds (Chen et al., 2008; Wang and Zhang, 2009). As polysaccharides are very complex molecules, their detailed characterization in specific glycosidic linkages, molecular weight and

sugars composition is mandatory in order to establish structure-biological activity relationships. Nevertheless most of the articles available in the literature, do not report these parameters, which is a drawback in the understanding of the most crucial chemical features for polysaccharides bioactive properties such as antioxidant, antitumor and antimicrobial activities.

1.2. Bioactivity of Ganoderma polysaccharides

Most of the studies on bioactivities of polysaccharides, glycopeptides or polysaccharidic crude extracts have been performed using *Ganoderma lucidum* (Nie et al., 2013). This species has been under special attention because of the potent antioxidant, antitumor and antibacterial activities of the polysaccharides, glycoproteins and polysaccharidic extracts obtained from the fruiting bodies (Jia et al., 2009; XiaoPing et al., 2009; Shi et al., 2013).

Antioxidant properties include free radicals scavenging abilities, reducing power and chelating effects on ferrous ions, among others (Liu et al., 2010; Kozarski et al., 2011). The radicals scavenging activity seems to be related to an increase in the activity of antioxidant enzymes: superoxide dismutase (SOD) which catalyzes dismutation of superoxide anion to hydrogen peroxide; catalase (CAT) which detoxifies hydrogen peroxide and converts lipid hydroperoxides to nontoxic substances; and glutathione peroxidase (GSH-Px) which maintains the levels of reduced glutathione (GSH) (YouGuo et al., 2009; XiaoPing et al., 2009).

Antitumor polysaccharides exert their bioactivity mostly *via* activation of the immune response of the host, enhancing the host's defense system (Mizuno et al., 1995b). The antitumor properties of water-soluble polysaccharide-enriched fractions from the fruiting bodies of *G. lucidum* seem to be related to the production stimulation of

interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6 from human monocyte-macrophages, and interferon (IFN)-γ from T lymphocytes (Wang et al., 1997).

There are only a few reports on antimicrobial activities of polysaccharides from *Ganoderma* species. This genus has been widely studied for its therapeutic properties, but less investigated as a source of new antibacterial agents (Gao et al., 2003a). Nevertheless, some polysaccharides from *Ganoderma* species exert antibacterial activity by inhibiting the growth of bacteria and, in some cases, by killing pathogenic bacteria (Skalicka-Woźniak et al., 2012).

Although being very active as antioxidants and antimicrobials, *Ganoderma* polysaccharides are mostly known as antitumor agents; however the mechanisms of action involved in their bioactivities are not well understood. Furthermore, most of the studies are performed under *in vitro* conditions, with very few experiments using *in vivo* assays.

This review aims to contribute to the knowledge of bioactivity (mainly antioxidant, antitumor and antimicrobial properties) of polysaccharides, glycoproteins and polysaccharidic extracts obtained from *Ganoderma* species. The most common extraction and isolation procedures are presented, including their chemical features. This includes discussion of monosaccharides' composition, type of glycosidic linkages, branching patterns and linkages to proteins, with these features being related to the corresponding bioactivities.

2. Extraction, isolation and identification of *Ganoderma* polysaccharides

2.1. Chemical features of the most common polysaccharides found in Ganoderma

According to different researchers, the polysaccharides isolated from *Ganoderma* are constituted by glucose, mannose, galactose, fucose, xylose and arabinose, with different

combinations and different types of glycosidic linkages, and which can be bound to protein or peptide residues (polysaccharide-protein or -peptide complexes) (Sone et al., 1985; Zhang et al., 2007; Chen et al., 2008; Wang and Zhang, 2009; Ferreira et al., 2010; Nie et al., 2013). These carbohydrates are characterized by their molecular weight, degree of branching, and higher (tertiary) structures (Ferreira et al., 2010), and have different compositions, comprising β-glucans, hetero-β-glucans, heteroglycans or α-manno-β-glucan complexes (Moradali et al., 2007).

Homo-glucans are linear or branched biopolymers having a backbone composed of α- or β-linked glucose units (such as (1→3), (1→6)-β-glucans and (1→3)-α-glucans), and might contain side-chains attached at different positions. Among the homo-glucans, β-glucans (primary components of the cell walls of higher fungi) are glucose polymers that can exist as a non-branched (1→3)-β-linked backbone or as a (1→3)-β-linked backbone with (1→6)-β-branches (Moradali et al., 2007; Ferreira et al., 2010).

These polysaccharides have either linear or branched molecules in a backbone composed of α- or β-linked glucose units, containing side-chains that are attached in different ways. Hetero-glucan side-chains contain glucuronic acid, xylose, galactose, mannose, arabinose or ribose moieties as a main component or in different combinations (Wasser, 2002; Ferreira et al., 2010).

Glycans are other polysaccharides that are found in *Ganoderma*. These polysaccharides, in general, contain units other than glucose in their backbone. They are classified as galactans, fucans, xylans, and mannans by the individual sugar components in the backbone (Moradali et al., 2007). Hetero-glycan side-chains contain arabinose, mannose, fucose, galactose, xylose, glucuronic acid, and glucose as a main component or in different combinations (Wasser, 2002; Moradali et al., 2007).

Polysaccharides can also be covalently bound to proteins or peptides as polysaccharide-protein or –peptide complexes, which possess antioxidant and antitumor potential (Jia et al., 2009; Ferreira et al., 2010). Glycoproteins are polysaccharide-protein complexes, and such compounds include β-glucan-protein, α-glucan-protein and heteroglycan-protein complexes. On the other hand, glycopeptides are a group structurally similar to glycoproteins, but with a smaller chain of amino acids (Ferreira et al., 2010). Finally, proteoglycans are another class of glycoproteins, which are heavily glycosylated. They consist of a core protein with one or more covalently attached glycosaminoglycan chain(s) (Moradali et al., 2007). An example of this is GLIS (*G. lucidum* immunomodulating substance), a bioactive proteoglycan isolated from the fruiting bodies of *G. lucidum*. GLIS contains carbohydrates and proteins in a ratio of 11.5:1, being the carbohydrate portion formed by seven different monosaccharides, predominantly D-glucose, D-galactose, and D-mannose in a molar ratio of 3:1:1 (Zhang et al., 2002). Thus, polysaccharides have been under special attention since they have an utmost capacity for carrying biological information because they have great potential for structural variability (Wasser, 2002).

Polysaccharides have a huge diversity in their chemical structure and composition, and several details can differently influence their specific bioactivities. Thus, the full characterization of these molecules is crucial in order to identify the main bioactive groups and study the respective mechanism of action.

2.2. Extraction procedures

A wide range of polysaccharides with different chemical structures has been extracted and isolated from *Ganoderma* species with demonstrated bioactive properties such as antioxidant (**Table 1**), antitumor (**Table 2**) and antimicrobial (**Table** 3) activities.

According to the polysaccharide characteristics, the selection of an extraction method is very important; it is based on the structure and water-solubility of the polysaccharide, and depends especially on the cell wall structure. The basic principle is to break the cell wall from the outer layer to the inner layer under mild-to-strong extraction conditions (pH and temperature) (Zhang et al., 2007). Based on this principle, most polysaccharides are extractable with hot water, or acidic, saline and dilute alkali solutions, or with dimethyl sulfoxide (Mizuno et al., 1995a; Wasser and Weis, 1999; Gao et al., 2004; Zhao et al., 2005; Ye et al., 2009; YouGuo et al., 2009; Liu et al., 2010; Dong et al., 2012; Nie et al., 2013; Zhonghui et al., 2013).

Hot water extraction is the most common methodology for extraction of polysaccharides from *Ganoderma*. High temperature is required to accelerate dissolution of polysaccharides from cell walls (Nie et al., 2013). Hence, traditional procedures for extraction begin with powders of raw materials being defatted by organic solvents or with 80% aqueous ethanol to eliminate low molecular weight compounds. After that, the material is successively extracted with water (*e.g.*, 100ºC for 3 h), or with saline and diluted alkali solutions at different temperatures (*e.g.*, 2% ammonium oxalate at 100ºC for 6 h, and 5% sodium hydroxide at 80ºC for 6 h). The hot water extraction yields water-soluble polysaccharides; on the other hand, extraction with alkali solution yields water-insoluble ones (Zhang et al., 2007; Nie et al, 2013).

Other techniques such as microwave, ultrasonic, ultrasonic/microwave, and enzymatic treatments are also used, which could promote the breakage of the cell wall and increase the yield of the extracted polysaccharides (Huang et al., 2007; Xu et al., 2007; Huang and Ning, 2010; Zhao et al., 2010; Huang et al., 2011; Ma et al., 2013; Shi et al., 2013). However, following the extraction procedure itself, it is necessary to remove free proteins. Sevag method is the typically used method for this in *Ganoderma* species (the

proteins are precipitated after repeated denaturation by shaking with a solution of octanol in chloroform) (Staub, 1999).

In order to obtain the crude polysaccharides after dialyzing against water, the de-proteinized solution is precipitated by alcohol, methanol or acetone. Finally, to obtain pure polysaccharides, purification is usually carried out through chromatographic techniques, such as ion-exchange, gel filtration and affinity chromatography (Zhang et al., 2007; Chen et al., 2008; Huang et al., 2011; Jiang et al., 2012). Essentially, ion-exchange chromatography through DEAE-cellulose columns separate neutral polysaccharides from acidic ones. Neutral polysaccharides are then separated into α-glucans (adsorbed fraction) and β-glucans (non-adsorbed fraction) using gel filtration and affinity chromatography. The same procedure with acidic polysaccharides (after elution with 1 M NaCl) yields purified polysaccharides (Mizuno, 1999). A combination of techniques may also be used, such as fractionation by ethanol, fractional precipitation, acidic precipitation with acetic acid or freeze-thawing (Liang et al., 1994; Zhang et al., 2007).

As mentioned above, the extraction procedures/conditions are applied according to the characteristics (*e.g.*, molecular weight, solubility) of the target polysaccharide(s). Most of the extraction procedures are well established and have been optimized in order to increase the extraction yield and efficiency by the use of new techniques such as ultrasonic/microwave and enzymatic treatments.

2.3. Isolation and identification procedures

Since glycan structures are diversified, it becomes difficult to define a universal protocol for their analysis. The primary structure of a polysaccharide is defined by the composition in monosaccharides, configuration and position of glycosidic linkages,

sequence of monosaccharides, as well as the nature, number and location of appended non-carbohydrate groups. The analytical methods used to determine the primary structures of polysaccharides include gas-liquid chromatography with flame ionization detection (GLC-FID), gas-liquid chromatography with mass spectrometry (GLC-MS) and high performance liquid chromatography (HPLC), techniques that allow evaluation of monosaccharides' composition; high performance anion-exe chromatography with pulsed amperometric detection (HPAEC-PAD), infrared (IR), exoglycosidase digestion with specific enzymes (limited to a few enzymes of high specificity) and mass spectrometry (MS) for the analysis of the configuration (α, β) of the anomeric carbon and position of the glycosidic linkages; and nuclear magnetic resonance (NMR) spectroscopic analysis that, besides the two previous features, allows inference of the sequence of the polysaccharide(s) (Varki et al., 1999; Zhao et al., 2005; Zhang et al., 2007; Ye et al., 2009; Dong et al., 2012; Shi et al., 2013). Monosaccharide's analysis provides precise molar ratios of individual sugars, and may suggest the presence of specific oligosaccharide classes, such as *N*- or *O*-glycans (Zhang et al., 2007).

These techniques also allow obtaining the molecular weight of the polysaccharides. Sone et al. (1985) used HPLC to obtain the molecular weight of the polysaccharides from fruiting bodies and cultured medium of *G. lucidum*. In addition, Chen et al. (2008) obtained the molecular weight of a water-soluble protein-bound polysaccharide through gel chromatography. Moreover, Zhao et al. (2010) determined the homogeneity and average molecular weight of polysaccharide fractions by high performance gel filtration chromatography (HPGFC). More recently, Ma et al. (2013) determined the molecular weight distribution of *G. lucidum* polysaccharides (GLP) using high-performance gel permeation chromatography (HP-GPC) with an HPLC apparatus.

Other features, like conformational properties (*e.g.*, polysaccharide dynamics), remain an area still under investigation. With development of high resolution instrumental processes, such as various light scattering techniques, x-ray diffraction analysis, small-angle neutron scattering (SANS), atomic force microscopy (AFM) and high resolution NMR spectroscopy, it has become possible to study the conformation and 3D structure of a polysaccharide at the molecular level. Indeed, through the use of molecular mechanics and computer assisted energy minimization methods, it is possible to simulate and visualize the 3D structure of polysaccharides (Zhang et al., 2007). Currently, there are numerous existing tools for the isolation and characterization of polysaccharides.

New isolation and identification techniques have been applied to polysaccharides analysis, facilitating the elucidation of their chemical structures. The use of efficient technologies such as NMR and MS, among others described above, allows the determination of specific chemical characteristics, such as the type of glycosidic linkages, sugars composition, and molecular weight. With this information it is possible to establish the main features on polysaccharides structure related to bioactivity, and give clues on this relationship.

3. Antioxidant activity

3.1. Ganoderma polysaccharides

Among the *Ganoderma* genus, there are several reports in the literature describing the antioxidant activity of polysaccharides isolated from *G. lucidum* (Li et al., 2007; YouGuo et al., 2009; XiaoPing et al., 2009; Liu et al., 2010; Kao et al., 2012; Ma et al., 2013; Shi et al., 2013; Zhonghui et al., 2013) (**Table 1**).

Homo-glucans and hetero-glucans isolated from this species have promising radical scavenging abilities, as evaluated by several *in vitro* antioxidant assays, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, reducing power, chelating ability, hydroxyl radical scavenging activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) scavenging activity, superoxide radical scavenging activity and hydrogen peroxide scavenging activity, respectively (Liu et al., 2010; Ma et al., 2013; Shi et al., 2013) (**Table 1**). A low molecular weight β-1,3-glucan (LMG) was able to significantly increase the viability (from 40% to 80%) of a mouse leukaemic monocyte macrophage cell line (RAW 264.7) with H_2O_2-induced oxidative stress, reduced reactive oxygen species (ROS) formation and also suppressed the activities of neutral and acidic sphingomyelinases (SMases) (Kao et al., 2012). A homo-polysaccharide composed by mannose also had very interesting antioxidant activity under *in vitro* and *in vivo* conditions. This polysaccharide displayed promising free radicals ($O_2^{\cdot-}$; $HO^{\cdot}$ and DPPH) scavenging ability and was able to increase the activity of the antioxidant enzymes, SOD (from 67.4 to 115.4 U/mL and 140 to 230 U/mL), CAT (from 7.82 to 13.91 U/mL and 13.0 to 22.0 U/mL) and GSH-Px (from 10.42 to 26.39 U/mL and 16.0 to 36.0 U/mL), as well as decrease malondialdehyde (MDA) levels (from 16.0 to 8.0 mmol/mL) in rats with cervical and ovarian carcinomas (YouGou et al., 2009; XiaoPing et al., 2009). Zhonghui et al. (2013) studied the antioxidant capacity of a *G. lucidum* polysaccharide (GL-PS) against exercise-induced oxidative stress, which was related with the dose; the activity of the antioxidant enzymes significantly increased: SOD (from 110 to 170 U/mg protein), CAT (from 1.58 to 1.95 U/mg protein) and GSH-Px (from 6.0 to 15.0 U/mg protein), while the levels of MDA decreased (from 8.2 to 4.8 nmol/mg protein). A hetero-glucan also isolated from *G. lucidum* showed antioxidant activity against mitochondria oxidative injury induced by γ-irradiation, causing a drastic

decrease in MDA (from 1.24 to 0.55 nmol/mg protein), lipid hydroperoxides (LOOH) (from 1.09 to 0.04 nmol/mg protein) and protein carbonyl formation (from 0.84 to 0.22 nmol/mg protein), while protein thiol formation increased (from 9.28 to 13.42 nmol/mg protein). This hetero-glucan also increased the activity of the antioxidant enzymes SOD (from 3.07 to 6.11 U/mg protein), CAT (from 3.25 to 7.08 U/mg protein) and GSH-Px (from 2.66 to 4.77 U/mg protein) (Li et al., 2007). The main linkages in the homo-glucans were β-(1-3), (1-4) and (1-6) glycosidic bonds, as also in hetero-glucans, composed of different sugars, such as mannose, glucose, rhamnose, galactose, galactose, xylose, arabinose and fucose in different proportions. Liu et al. (2010) isolated a homo-glucan and a hetero-glucan, both low molecular weight polysaccharides, and reported a higher antioxidant activity of the homo-glucan because of its lower molecular weight. Nevertheless, Ma et al. (2013) isolated hetero-glucans with different molecular weights, and the polysaccharide with the highest molecular weight gave the highest antioxidant activity.

3.2. Ganoderma glycopeptides

There are several reports on the *in vitro* and *in vivo* antioxidant activity of glycopeptides obtained from *Ganoderma* sp. (Yu-Hong et al., 2002; Zhang et al., 2003; Sun et al., 2004; Zhao et al., 2004; Chen et al., 2008; Jia et al., 2009; Li et al., 2009; Li et al., 2010; Li et al., 2011; Li et al., 2012a).

The most abundant component isolated from *G. atrum* (PSG-1) is a glycoprotein with a molecular weight of 1.013 KDa, composed of 10.1% of protein with 17 general amino acids, and different sugars namely, mannose, galactose and glucose linked by *O*-glycosidic linkages (Chen et al., 2008). PSG-1 was studied for its antioxidant activity against anoxia/re-oxygenation injury in neonatal rat cardiomyocytes, anoxia/re-

oxygenation-induced oxidative stress in mitochondrial pathway, oxidative stress induced by D-galactose in mouse brain, and age-related oxidative stress in mice. The authors reported very potent antioxidant activity by protecting cardiomyocytes from anoxia/re-oxygenation. It significantly increased the activity of antioxidant enzymes, decreased the levels of MDA, and attenuated ROS formation, thereby having the potential to promote health and improve aging-associated pathologies by modifying the redox system and improving the immune function (Li et al., 2009; Li et al., 2010; Li et al., 2011; Li et al., 2012a).

Yu-Hong et al. (2002) studied the antioxidant activity of a glycopeptide (GLP) isolated from *G. lucidum* against the injury of macrophages induced by ROS. It was composed of 14 amino acids, D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose, and D-glucose as sugars, linked by β-glycosidic linkages, and with a molecular weight of 0.585 KDa. GLP showed *in vitro* and *in vivo* antioxidant activity by increasing the survival rate of macrophages, and protecting the mitochondria against injury by membrane-permeant oxidant (*t*BOOH). GLP was also studied for its antioxidant activity on streptozotocin (STZ)-diabetic rats, being able to increase non-enzymatic and enzymatic antioxidants, serum insulin level and to reduce lipid peroxidation (Jia et al., 2009).

Sun et al. (2004) studied GLP antioxidant activity in different oxidation systems (soybean and lard oils as oxidation substrates), and described an excellent activity comparable to the synthetic antioxidant butylated hydroxytoluene (BHT) in soybean oil. This glycopeptide was able to block soybean lipoxygenase activity, showed scavenging activity toward hydroxyl radicals produced in a deoxyribose system, quenched superoxide radical ion produced by pyrogallol autoxidation, displayed antioxidant

activity in rat liver tissue homogenates and mitochondrial membrane peroxidation systems, and also blocked the auto-hemolysis of rat red blood cells.

A glycopeptide isolated from *G. lucidum*, with a molecular weight of 0.5849 KDa, composed of 17 amino acids and rhamnose, xylose, fructose, galactose, mannose and glucose as sugars, linked by β-glycosidic linkages, had antioxidant activity by reducing ROS formation, MDA levels and increasing the activity of manganese superoxide dismutase in rat cerebral cortical neuronal cultures exposed to hypoxia/re-oxygenation (Zhao et al., 2004). This glycopeptide also showed antioxidant activity (free radicals scavenging ability) by protecting against alloxan-induced pancreatic islets damage under *in vitro* and *in vivo* conditions (Zhang et al., 2003).

3.3. Crude polysaccharidic Ganoderma *extracts*

The antioxidant activity of crude polysaccharidic extracts obtained from *Ganoderma* sp., have been recently described (Shi et al., 2010; Yang et al., 2010; Heleno et al., 2012; Kozarski et al., 2012; Zhao et al., 2012; Pan et al., 2013).

A polysaccharidic extract from *G. lucidum* showed antioxidant activity in rats with gastric cancer by increasing the activity of antioxidant enzymes (SOD, CAT and GSH-Px) (Pan et al., 2013). Other polysaccharidic extracts, also obtained from *G. lucidum*, displayed radicals scavenging ability, reducing power and lipid peroxidation inhibition, with the extract obtained from spores as the most effective (Heleno et al., 2012). Kozarski et al. (2012) reported the antioxidant activity of polysaccharidic extracts from *G. applanatum* and *G. lucidum* namely, radicals scavenging activity, reducing power, lipid peroxidation inhibition and chelating abilities.

Zhao et al. (2012) reported the radio-protective effects of a *G. lucidum* polysaccharidic extract on mouse deoxyribonucleic acid (DNA) damage induced by cobalt-60 gamma-irradiation, and described that DNA strand-break and micronuclei frequency were

significantly reduced, while GSH-Px activity and nucleated cell count in bone marrow significantly increased. This polysaccharidic extract also increased SOD activity and decreased MDA levels.

Polysaccharidic extracts prepared from *G. lucidum* also lowered serum levels of MDA and intercellular adhesion molecule-1 in heart and liver of mice with ischemic reperfusion, and increased antioxidant enzymes activity (Shi et al., 2010). In diabetic rats, the polysaccharidic extract was able to reduce oxidative injury and inhibit apoptosis by increasing antioxidant enzymes activity, and modifying B-cell lymphoma 2 (bcl-2) expression and bcl-2-associated X protein (bax)/bcl-2 ratio (Yang et al., 2010). The studies performed over the last decades concerning antioxidant properties of polysaccharides, glycoproteins and crude extracts described that the radicals scavenging activity seems to be mostly related with the increase in the activity of antioxidant enzymes such as SOD, CAT and GSH-Px (Yu-Hong et al., 2002; XiaoPing et al., 2009; YouGuo et al., 2009; Pan et al., 2013).

There are not many studies on the antioxidant activity of *Ganoderma* polysaccharides, and the existing ones only report polysaccharides from *G. lucidum*. Most of these studies were carried out under *in vitro* conditions; and reports using *in vivo* assays are scarce and do not describe the mechanism of action involved. Instead, they only describe an increase in antioxidant enzymes activity after exposure to a specific injury. Additionally, those polysaccharides were isolated but not completely chemically characterized. The available data generally include molecular weights and, in some cases, sugars composition; glycosidic linkages are rarely characterized. Therefore, it is not possible to highlight a key chemical feature directly related with the antioxidant activity of *Ganoderma* polysaccharides, since there is a lack of information on their chemical characteristics. Based on the existing reports with available information about

structural features, it can only be speculated that homo-glucans and hetero-glucans with β (1→3) glycosidic linkages have strong antioxidant properties (Liu et al., 2010; Kao et al., 2012).

4. Antitumor *Ganoderma* polysaccharides

4.1. Ganoderma *polysaccharides*

The crude water-soluble extract of *G. lucidum* has been used in traditional Chinese medicine as antitumor and immunomodulating agent (Zong et al., 2012). Most reports concerning the antitumor activity of polysaccharides from *Ganoderma* demonstrate that it is mainly related to the host-mediated immune function (Gao et al., 2005a; Paterson, 2006). *Ganoderma* polysaccharides have received special attention from the scientific community, especially those from the species *G. lucidum*, and their antitumor activity has been studied both *in vitro* and *in vivo*.

Hence, bioactive polysaccharides have been isolated from the fruiting bodies of *G. lucidum* (Bao et al., 2002; Zhao et al., 2010) and from the mycelia cultivated in liquid culture medium (Kim et al., 1993; Peng et al., 2005; Liu et al., 2012). Some polysaccharides have also been isolated from the culture medium of growing mycelium (extracellular polysaccharides) (Sone et al., 1985).

Antitumor effects of polysaccharides isolated from *G. lucidum*, such as the branched heteroglucan, arabinoxyloglucan (GL-1), were initially observed in subcutaneously transplanted sarcoma-180 ascites growing in mice (Miyazaki and Nishijima, 1981; **Table 2**). This polysaccharide contains a backbone and side-chains involving D-glucopyranosyl, α-(1→4), β-(1→6) and β-(1→3) linkages; arabinose is present as a part of the non-reducing terminal residues, and xylose is present as a part of the side-chain. This hetero-glucan strongly inhibited the growth of sarcoma-180 solid-type tumor (inhibition ratio, 95.6 - 98.5%) after intra-peritoneal injection (20 mg/Kg) for 10 days in

imprinting control regions (ICR) of mice (Miyazaki and Nishijima, 1981; **Table 2**). Sone et al. (1985) also described the antitumor activity of *G. lucidum* polysaccharides either from the fruiting bodies or the mycelium against sarcoma-180 solid tumor. Once again, the studied polysaccharides had (1→3)-β-D-glucan bonds and some (1→4)-linked glucosyl units (**Table 2**).

The antitumor potential of *Ganoderma* polysaccharides is usually related to their immunomodulatory activity. Since polysaccharides have a large molecular weight, these compounds cannot penetrate cells, but they bind to immune cell receptors. It has been proven that there are fungal pattern-recognition molecules for the innate immune system. However, the mechanism by which the innate immune system recognizes and responds to fungal cell wall carbohydrates is a very complex and multifactorial process (Lowe et al., 2001).

Yan and collaborators suggested that the activity of polysaccharides from *G. lucidum* was mediated through the complement receptor type 3 (CR3 receptor), which binds β-glucan polysaccharides (Yan et al., 1999). Indeed, *G. lucidum* polysaccharide (GLP), known as a homo-glucan from *G. lucidum,* isolated by hot aqueous extraction and ethanol precipitation from the fruiting bodies of this medicinal mushroom, exerted its antitumor activity in sarcoma-180 solid tumor by inducing a cascade of immuno-modulatory cytokines. It could induce a marked increase in the gene expression levels of IL-lα (2-fold), IL-lβ (3-fold), TNF-α (2-fold), IL-12 p35 (up to 6-fold), and IL-12 p40 in the splenocytes. In the macrophages, GLP promoted a remarkable increase in the gene expression levels of IL-lβ (2.5- to 3-fold), TNF-α (up to 6-fold), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (up to 2-fold) (Ooi et al., 2002; **Table 2**). GLP also exhibited antitumor effects on solid tumor induced by Ehrlich's ascites carcinoma cells. Indeed, 100 mg/kg of this polysaccharide showed 80.8% and 77.6%

reduction in tumor volume and tumor mass, respectively, when administered 24h after tumor cell implantation. Moreover, GLP with the same dose but administered prior to tumor inoculation, showed 79.5% and 81.2% inhibition of tumor volume and tumor mass, respectively (Soniamol et al., 2011). GLP not only has (1→3)-β-D-glucan bonds, but also has (1→6)-β-D branches. Furthermore, structural features such as (1→3)-β-linkages in the main chain of the glucan, and additional (1→6)-β-branch points, seem to be important factors for the observed antitumor activity.

The same features were verified for the heteroglucans from *G. tsugae* described by Peng et al., (2005), which were composed by (1→3)-β-D-glucans and (1→4)-α-D-glucans and also possess antitumor activity against sarcoma-180 solid tumor (**Table 2**). Actually, the fruiting body of *G. tsugae* is used to promote health and longevity in Oriental countries (Haghi, 2011), which can be, in part, justified for these findings.

More recently, other heteropolysaccharides from *Ganoderma* have been studied both *in vivo* and *in vitro*, establishing inhibitory activity in tumor cell lines, apoptosis induction and inhibition of tumors transplanted in mice (Liu et al., 2012; Zhang et al., 2012; Ma et al., 2013;**Table 2**).

Other polysaccharides from *G. lucidum* with immunomodulatory properties have been described, namely PG-1 and PG-2, which increased the proliferation and pinocytic activity of macrophages and played an inhibitory effect on the growth of a human breast cancer cell line (MDA-MB-231) (Zhao et al., 2010; **Table 2**).

There are also reports on the antitumor potential of other polysaccharides from *Ganoderma* species but without their chemical characterization. For example, other authors reported the antitumor properties of mannogalactoglucans and (1→3)-β-glucuronoglucans from *G. lucidum* tested *in vitro* (in cell lines) and of glucogalactans from *G. tsugae* tested *in vivo* (pre-clinical animal models), through their

immunomodulatory activity (Zhuang et al., 1994; Cho et al., 1999; Wasser, 2002; Moradali et al., 2007; Zhang et al., 2007; Ferreira et al., 2010).

4.2. Ganoderma *polysaccharide-protein or -peptide complexes*

As mentioned above, polysaccharides isolated from *Ganoderma* may be also bound to protein or peptide residues. These polysaccharide-protein or -peptide complexes have also been described as having antitumor properties. *G. lucidum* polysaccharide peptide (GLPP), potently inhibited human lung carcinoma cell line (PG), proliferation *in vitro* and reduced the xenograft (of the PG cell line) in albino laboratory-bred strain of the house mouse (BALB/c) nude mice *in vivo*. This compound proved to have anti-angiogenic activity, which can be the basis of its antitumor effects. This polysaccharide-peptide with relative molecular weight (MW) of 512500, is composed by D-rhamnose, D-xylose, D-fructose, D-galactose, and D-glucose linked together by β-glycosidic linkages (Cao and Lin, 2004).

A fucose-containing glycoprotein fraction from the water-soluble extract of *G. lucidum* seems also to be responsible for its immunomodulating and antitumor activities through the stimulation of the expression of cytokines, especially IL-1, IL-2 and IFN-γ (Wang et al., 2002). Although the active fraction contained the majority of D-glucose, D-mannose and D-galactose, the only active component identified in the glycopeptide fraction contained fucose residues. In addition, the crude extract of *G. lucidum* did not stimulate expression of cytokines, whereas the glycoprotein fraction significantly induced expression of IL-1, IL-2, and IFN-γ (Wang et al., 2002).

A well-known proteoglycan from *G. lucidum* is the previously mentioned GLIS (Section 2.1). This proteoglycan with a molecular weight of about 2000 kDa, and carbohydrate portion consisting of hetero-polysaccharides composed predominantly of

D-glucose, D-galactose and D-mannose, exhibits an effective antitumor effect by increasing both humoral and cellular immune activities (Zhang et al., 2010).

A water-soluble protein-bound polysaccharide from the fruiting bodies of *G. atrum* (PSG-1), besides the antioxidant properties previous reported, displayed potent antitumor activity in sarcoma180 transplanted mice by induction of tumor apoptosis through mitochondrial pathways, and its antitumor effect was related to immuno-enhancement (Li et al., 2011a). This compound, proved to improve immunity by inhibiting proliferation of a mouse colon carcinoma cell line (CT26) via activation of peritoneal macrophages. *In vivo*, PSG-1 considerably suppressed the tumor growth in CT26 tumor-bearing mice (Zhang et al., 2013).

A *G. lucidum* polysaccharide-peptide conjugate with a molecular weight of 0.5125 KDa and polysaccharide chain assembled in β-glycosidic linkages, also exhibited antitumor potential in different studies. For example, it significantly inhibited tumor growth in a murine sarcoma180 model, and inhibited proliferation of Human Umbilical Vein Endothelial Cells (HUVECs) by inducing cell apoptosis and decreasing the expression of secreted vascular endothelium growth factor (VEGF) in human lung cancer cells (Li et al., 2008; Cao and Lin, 2006).

4.3. Ganoderma *polysaccharidic extracts/fractions*

Polysaccharidic fractions from *Ganoderma* have also been described as having potential antitumor activity. Ganopoly is one of the most well-known aqueous polysaccharidic fractions from *G. lucidum* with antitumor potential. Treatment of mice with Ganopoly for 10 days could significantly reduce tumor weight in a dose-dependent manner in S-180-bearing mice. Furthermore, the polysaccharide caused significant cytotoxicity in the human tumor cell lines: Human Caucasian Cervical Epidermoid Carcinoma (CaSki),

Human Cervical Cancer (SiHa), Human Hepatoma (Hep3B), Human Hepatocellular Liver Carcinoma (HepG2), Human Colon Carcinoma (HCT116) and Human Colon Adenocarcinoma Grade II (HT29) Cells *in vitro*, with marked apoptotic effects observed in CaSki, HepG2 and HCT116 cells (Gao et al., 2005a). Other studies showed that Ganopoly could enhance immune responses in patients with advanced-stage cancer, which could be an approach for overcoming immunosuppressive effects of chemotherapy/radiotherapy (Gao et al., 2003b; Gao et al., 2005b).

Some studies also suggest that antitumor activity of polysaccharides from fresh fruiting bodies of *G. lucidum* (PS-G), is achieved through stimulation of the production of IL-1β, TNF-α, and IL-6 from human monocyte-macrophages and IFN-γ from T lymphocytes. These studies were carried out in the human promyelocytic leukaemia (HL-60), and human lymphoma cell lines (U937) (Wang et al., 1997). *G. lucidum* polysaccharide (GL-B), consisting of seven fractions of polysaccharides isolated from this species, was tested both *in vitro* (HL-60, and sarcoma-180 cells), and *in vivo* (sarcoma-180 cells injected sub-dermally into the axillary fossa of the right foreleg of BALB/c mice), and this established that its antitumor potential is also related to TNF-α and IFN-γ (Zhang and Lin, 1999). Co-administration of *G. lucidum* polysaccharides and cyclophosphamide potentiated the antitumor activity of this drug (used to treat cancer and immune diseases) in mice. These results indicate that either *G. lucidum* or its active components have antitumor activity in mice, and that *Ganoderma* polysaccharides have a synergic effect on the antitumor activity of cyclophosphamide (Lin and Zhang, 1999).

G. tsugae mycelium and fruiting body polysaccharidic fractions have also been investigated. Seven glycans with strong antitumor activities were obtained from 14 water-soluble and 15 water-insoluble fractions extracted from *G. tsugae* fruiting bodies. The bioactivity against sarcoma-180/mice was tested, and tumor inhibition ratios from

26.1 to 100% were observed (Wang et al., 1993). Water-soluble fractions were protein-containing glucogalactans associated mainly with mannose and fucose, but also containing arabinose and rhamnose; water-insoluble fractions represented protein-containing β-(1→3)-glucans with different protein content and some of them with (1→6)-β-D-glucosyl branched chains. The molecular weight averages ranged from 8×10^3 to 700×10^3 (Wang et al., 1993). Sixteen water-soluble polysaccharides were extracted from *G. tsugae* mycelium and examined for their antitumor effects on sarcoma-180 in mice (Zhang et al. 1994). The active polysaccharides obtained were: i) a glycan-protein complex containing 9.3% protein, with a hetero-glyco-chain of mannose and xylose; ii) a glucan-protein complex containing 25.8% protein and iii) a glycan-protein with glucose as the main component, and associated with arabinose, mannose, xylose, and galactose. The molecular weight ranged from 10×10^{-3} to 16×10^{-3} (Zhang et al. 1994). Comparison of active water-soluble polysaccharides obtained from the fruiting body and mycelium showed that the first were gluco-galactan-protein complexes, but those of the mycelium were homo-glucan-protein complexes or a hetero-glycan composed of mannose and xylose (Wasser, 2002). However, and once again, the structure with β-(1→3)-glucans and, in some cases, with (1→6)-β-D-glucosyl branched chains was present in these bioactive polysaccharidic fractions.

Other polysaccharidic fractions were also obtained from the water soluble extracts of *G. applanatum*. These preparations had antitumor properties against transplanted sarcoma-180 in mice, and, for one of the obtained fractions, a complete regression of tumors was observed in more than half of animals; inhibition ratios were over 95%, with no sign of toxicity (Sazaki et al., 1971). These fractions were considered to be a glucan consisting partially of a mixture of β-(1→3) and (1→4) linked D-glucose residues.

Polysaccharidic extracts from the mycelium of *G. lucidum* also exhibited antitumor effects against fibrosarcoma in male and female mice and inhibited the metastasis of a lung tumor.

Different studies showed that bioactive polysaccharides and extracts could stimulate blood mononuclear cells to increase cytokines, tumor necrosis factor, interferon and interleukins production, induce apoptosis and meaningfully increased the lifespan of the tumor-implanted mice (Paterson, 2006; Ramberg et al., 2010; Roupas et al., 2010; Liao et al., 2013).

4.4. Structure-bioactivity relationship

Polysaccharides are one of the biologically active groups of compounds found in mushrooms, namely in *Ganoderma* genus, which have antitumor properties (Wasser, 2002; Lindequist et al., 2005; Paterson, 2006; Ferreira et al., 2010; Patel and Goyal, 2012; Nie et al., 2013). Thus, *Ganoderma* has been considered a bioactive therapeutic fungus (Paterson, 2006) and its antitumor potential has been explored (Wang et al., 1997; Yuen and Gohel, 2005).

The study and description of the chemical features of *Ganoderma* polysaccharides are very important as they allow us to infer or deduce structure-bioactivity relationships. Different polysaccharides from the *Ganoderma* genus have been isolated and characterized especially in the past three decades.

The first reports of *Ganoderma* polysaccharides structure date back to 1981, when Miyazaki and Nishijima characterized a water-soluble branched arabinoxyloglucan from *G. lucidum,* which contained β-(1→4)-, β-(1→6)-and β-(1→3)-D-glucopyranosyl residues in the backbone and side-chains. These authors inferred that the essential

structure for the antitumor activity of polysaccharides from *Ganoderma* might be a branched glucan core involving (1→3)-β-, (1→4)-β- and (1→6)–β- linkages.

More recently, Bao et al. (2002) isolated three polysaccharides, two heteroglucans (PL-1 and PL-4) and one glucan (PL-3) from the fruiting bodies of the same species. This study showed that PL-1 had a backbone consisting of 1,4-linked α-D-glucopyranosyl residues and 1,6-linked β-D-galactopyranosyl residues with branches at *O*-6 of glucose residues and *O*-2 of galactose residues, composed of terminal glucose, 1,6-linked glucosyl residues and terminal rhamnose, respectively. PL-3 was a highly branched glucan composed of 1,3-linked β-D-glucopyranosyl residues substituted at *O*-6 with 1,6-linked glucosyl residues. PL-4 was comprised of 1,3-, 1,4-, 1,6-linked β-D-glucopyranosyl residues and 1,6-linked β-D-mannopyranosyl residues. More recently, Wang et al. (2011) isolated five water-soluble heteropolysaccharides from the cultured fruiting body of *G. lucidum,* designated as GL-I to GL-V. These compounds proved to be heteropolysaccharides, mainly composed of glucose, galactose, mannose and arabinose. GL-I was the most branched of the heteropolysaccharides (27.0% degree of branching), while GL-V was mostly a linear glucan.

The biological activity/antitumor potential of polysaccharides seems to be highly correlated with their chemical composition and configuration, as well as their physical properties, being exhibited by a wide range of glycans extending from homopolymers to highly complex heteropolymers (Ooi and Liu, 1999). As stated initially by Miyazaki and Nishijima (1981), more recent studies continue to point to the importance of structural features such as (1→3)-β-linkages in the main chain of the glucan and additional (1→6)-β- branch points as essential factors for the antitumor activity of polysaccharides (Wasser, 2002). Therefore, β-glucans containing mainly 1→6 linkages exhibit less activity, possibly due to their inherent flexibility of having too many

possible conformations (Zhang et al., 2007; Ferreira et al., 2010). However, antitumor polysaccharides may have other chemical structures, such as hetero-β-glucans (Mizuno et al., 1995b), heteroglycan (Gao et al., 1996), β-glucan-protein (Kawagishi et al., 1990), α-manno-β-glucan (Mizuno et al., 1995b), α-glucan-protein (Mizuno et al., 1995b) and heteroglycan-protein complexes (Zhuang et al., 1993; Mizuno et al., 1996). It has been described that the antitumor activity of mushroom polysaccharides containing glucose and mannose may be due to their immunomodulating activity, since a polysaccharide receptor, which has been demonstrated to have high specificity for glucose and mannose, has been found on human macrophages (Lombard, 1994). Triple helical conformation of (1→3)-β-glucans is considered an important structural feature for their immuno-stimulating activity, but how the triple helical conformation of (1→3)-β-glucan precisely affects their antitumor action still remains unclear. Indeed, (1→3)-β-glucans exhibit antitumor activity related to their triple helical conformation (Zhang et al., 2007).

Higher antitumor potential seems to be also correlated with higher molecular weight (Mizuno et al., 1996; Wasser, 2002), lower level of branching and greater water solubility of β-glucans (Ferreira et al., 2010). Thus, although other features such as molecular weight or level of branching are very important for the antitumor potential of the polysaccharides, the molecules described above have the main glycosidic bonds required for this activity, which seems to be highly related with the results obtained.

The antitumor potential is the most explored bioactivity of *Ganoderma* polysaccharides, being extensively studied; the chemical structures are completely characterized, and even some mechanisms of action are proposed by some authors (Yan et al., 1999; Ooi et al., 2002). Analysing the available data, it can be highlighted that the essential structure for the antitumor potential of polysaccharides is a branched glucan core involving

(1→3)-β-, (1→4)-β- and (1→6)-β-linkages (Miyazaki and Nishijima, 1981). Nevertheless, clinical human trails are needed to better understand the bioactivity of these interesting and extremely potent molecules, so that the investigation can progress in order to use these molecules in the development of new nutraceuticals or drugs.

4.5. Antioxidant and antitumor potential

The antitumor activity of *Ganoderma*, namely *G. lucidum*, seems to be also strongly related with its antioxidant properties, since water soluble polysaccharides extracted from *G. lucidum* were effective in preventing DNA strand breaks (Paterson, 2006).

An aminopolysaccharide fraction from *G. lucidum* (G009) was found to have the ability to protect against ROS, which is implicated in the pathophysiology of cancer (Pincemail, 1995). G009 inhibited iron-induced lipid peroxidation and inactivated hydroxyl radicals and superoxide anions. Furthermore, G009 also reduced oxidative DNA damage, suggesting that the aminopolysaccharide fraction of *G. lucidum* possesses chemopreventive potential (Lee et al., 2001).

Two cerebrosides (glycosphingolipids consisting of D-glucose, sphingosine and 2-hydroxypalmitoyl or 2-hydroxystearoyl fatty acid moiety, respectively), were also isolated from the fruiting body of *G. lucidum* (Mizushina et al., 1998). Both molecules inhibited DNA polymerases, suggesting their possible use for cancer therapy by inhibiting DNA replication (Sliva, 2003).

With all the studies conducted so far, polysaccharides have been suggested to have an ability to enhance the host's defense system in both antioxidant and antitumor abilities (Mizuno et al., 1995a; Pan et al., 2013). The work performed so far, especially in *G. lucidum*, indicates that fractions of polysaccharides were not as effective as their equivalent dose in the crude extract of the whole mushroom, suggesting that the

bioactivity of this medicinal mushroom may be due to the synergistic effects of multiple compounds, such as triterpenes (Liu et al., 2002). This idea is supported by some studies, such as the study in which a polysaccharidic mixture containing isoflavone aglycones produced from the cultured mycelia of *G. lucidum* inhibited angiogenesis in BALB/c mice with implanted chambers containing a suspension of colon-26 cells (Miura et al., 2002).

5. Antimicrobial *Ganoderma* polysaccharides

5.1. Medicinal mushrooms as antimicrobial agents

Fungi are well known for the production of important antibiotic compounds, such as penicillin. However, the occurrence of antibiotics in the class of fungi known as mushrooms is less well documented (Miles and Chang, 1997). Mushrooms belong to the kingdom of Fungi, they were thought to have weak antifungal activities (Mizuno, 1995) and therefore have rarely been investigated for their bioactivity as antifungal agents. It is only recently that they have become of interest due to their secondary metabolites exhibiting a wide range of antimicrobial activities.

Ganoderma species have been widely investigated for their therapeutic properties as antitumor and antiviral agents but have been far less investigated as a source of new antibacterial agents. A review by Gao et al. (2003a) on the antibacterial and antiviral value of *Ganoderma* species supported this observation, as there were few citations on research in this area. It is interesting to note that the majority of antibacterial investigations on *Ganoderma* species have been performed on the fruiting body and there are relatively few on extracts from the liquid cultivated mycelium.

5.2. Current antimicrobial research on Ganoderma *species*

Western and Eastern medicine have adopted different regulatory systems for herbal and mushroom preparations (Wasser, 2011). Western medicine has made little use of medicinal mushroom products partly due to their complex structure and lack of acceptable pharmacological purity (Sullivan et al., 2006). In the search for microbiologically active compounds from *Ganoderma* species, the majority of research has been performed on extracts from the fruiting body and mycelium, and there are a few studies on antimicrobial activity of isolated fractions or pure polysaccharides. It appears that there are a number of biologically active compounds to be found in the mycelium and fruiting body, but antimicrobial activity evaluation of chemically characterized polysaccharides is very limited. It could be only noted that (1→3)-β-D-glucan with (1→6)-β-D branches could act as antimicrobial agent *in vivo*.

5.3. Antibacterial activity of Ganoderma *polysaccharides*

The antibacterial activity of polysaccharides from *G. lucidum* fruiting bodies was reported (**Table 3**) (Skalicka-Woźniak et al., 2012). Thirty six samples were analyzed. Four strains of *G. lucidum* (GL01, GL02, GL03 and GL04) were cultivated on the growth substrates of three different sawdust types: birch (Bo), maple (Kl) or alder (Ol) amended with wheat bran in three different concentrations: 10, 20 and 30% (w/w). Even though the richest in polysaccharides was the GL01 strain, the highest yields of the polysaccharides were observed in the GL04Kl3 sample (112.82 mg/g of dry weight). The antibacterial activity of the polysaccharides was determined *in vitro* using the micro-dilution broth method. A panel of eight reference bacterial strains was used and all the tested polysaccharides showed moderate antibacterial activity. The *Micrococcus luteus* American Type Culture Collection (ATCC) 10240 strain was the most sensitive with minimal inhibitory concentrations (MICs) 0.63-1.25 mg/mL. Nevertheless, the

analyzed polysaccharides exhibited inhibitory effects against all the bacterial strains tested, with MICs ranging from 0.62 to 5.0 mg/mL. The minimal bactericidal concentrations (MBCs) of the samples were comparable (2.5 or 5.0 mg/mL). Only slight differences were observed between MICs and MBCs of the polysaccharide samples obtained from the strains of the *G. lucidum* fruiting bodies, and the ones obtained from the sawdust cultivation substrates. The low MBC/MIC ratios suggest that polysaccharides acted as bactericidal agents. The screening of antibacterial activity indicates that there were no significant differences in the antimicrobial activity between the polysaccharides obtained from the four strains of *G. lucidum* fruiting bodies and the ones obtained from different sawdust cultivation substrates. The polysaccharides tested exerted the strongest inhibitory effect towards *M. luteus* (MIC 0.62 or 1.25 mg/mL) (Skalicka-Woźniak et al., 2012).

In another study, *G. lucidum* polysaccharides were extracted with boiling water, and further tested for antimicrobial activity against three plant pathogens (*Erwinia carotovora*, *Penicillium digitatum*, *Botrytis cinerea*) and five food harmful microorganisms (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Rhizopus nigricans*) by the agar diffusion method. The results showed that the polysaccharide liquid had a powerful inhibitory effect on *E. carotovora*, a weak inhibitory effect on *P. digitatum* and a nearly non-inhibitory effect on *B. cinerea*, for the plant pathogens. Regarding food harmful microorganisms, the polysaccharide liquid had a strong inhibitory effect on *B. subtilis* and *B. cereus*, a weak inhibitory effect on *E. coli* and *A. niger*, and a nearly non-inhibitory effect on *R. nigricans* (Bai et al., 2008).

Polysaccharides from the mycelia and basidiocarp of *Ganoderma applanatum* were found to possess activity against *Acitenobacter aerogenes*, *Acrobacter aerogenes*, *Arthrobacter citreus*, *Bacillus brevis*, *B. subtilis*, *Corynebacterium insidiosum*, *E. coli*,

Proteus vulgaris, Clostridium pasteurianum, Micrococcus roseus, Mycobacterium phlei, Sarcina lutea and *Staphylococcus aureus* (Bhattacharyya et al., 2006).

The extracellular polysaccharides obtained from *Ganoderma formosanum* culture medium were separated into three major fractions, PS-F1, PS-F2, and PS-F3, based on their molecular size (Wang et al., 2011a). Although the different monosaccharide's composition in each fraction, D-mannose was the major constituent among all fractions, and in the two major fractions PS-F2 and PS-F3, the second most abundant sugar was D-galactose, followed by D-glucose. *G. formosanum* thus synthesizes a different form of polysaccharide as compared with other *Ganoderma* species (*e.g.*, *G. lucidum*) in which D-glucose is usually the major component (Wang et al., 2002). These results show that D-mannose and D-galactose are the major constituents of *G. formosanum* polysaccharides. The differences in carbohydrate composition among fungal polysaccharides could be due to strain variations or caused by different ways of cultivation (solid-state culture *versus* liquid-state culture). The polysaccharides were produced in a submerged mycelial culture. The fungal cell wall polysaccharides synthesized under different growth conditions may exhibit different biological effects. Methods of extraction may also affect the polysaccharides obtained from *G. lucidum* fruiting bodies, which could contain either β-1,3-glucans or α-1,4-linked polymannose (Miyazaki and Nishijima, 1981). It appears that both the sugar components and structures of the hetero-polysaccharides in the fungal cell wall are diverse and complicated. It is suggested that the extracellular polysaccharides of *G. formosanum* (PS-F2, and perhaps PS-F1 and PS-F3) have the potential to be used as immuno-stimulatory and antibacterial agents against *Listeria monocytogenes* injected in mice.

In the study of antibacterial activity of exopolysaccharide (EPS) from basal medium and malt medium obtained from different mushrooms, *G. lucidum* EPS showed the highest

activity against the growth of *B. cereus* among other bacterial species (23 ± 0.61 mm and 18 ± 0.38 mm, respectively) (Mahendran et al., 2013).

Ganoderma polysaccharides have not been much studied regarding antimicrobial properties. Nevertheless, the available studies report mainly their activity against several pathogenic bacteria. Several authors reported antimicrobial activity of *G. lucidum* different extracts but not isolated polysaccharides (Sheena et al., 2003; Quereshi et al., 2010). Heleno et al. (2013) reported strong antibacterial, antifungal and also demelanizing properties of *G. lucidum* extract, even better than the standards ampicillin and streptomycin in some cases. Thus, polysaccharides isolated from these species should also be analyzed since they can have a strong participation in the antimicrobial properties exhibited by *G. lucidum*.

6. *Ganoderma* polysaccharides with non-reported bioactivity

Polysaccharides from *Ganoderma* species with previously non-reported bioactivity are briefly discussed here. The reported polysaccharides with non-tested bioactivity belong to alkali-soluble polysaccharides and/or water insoluble polysaccharides and water soluble polysaccharides (**Table 4**).

The methods for isolation, purification and identification are given in **Table 4**, as well as sugars composition and molecular weight. A water insoluble, but alkali-soluble glucan G-A was previously isolated from *G. japonicum*. (Ukai et al., 1982). Chen et al., (1998) have isolated water-insoluble glucans, namely GL4-1 and GL4-2 from the fruiting bodies of *G. lucidum*. A water soluble and low branched polysaccharide (SGL-III) was isolated from the spores of *G. lucidum* (Zhao et al., 2005). A neutral, water soluble, heteropolysaccharide (GLPS3) was isolated from germinating spores of *G. lucidum* (Zhang et al., 2006). A water soluble β-glucan (DESSK5) was reported in the

basidiocarp of *G. resinaceum* (Amaral et al., 2008). A water soluble polysaccharide, heteropolysaccharide LZ-C-1 was isolated from *G. lucidum* (Ye et al., 2009). Neutral polysaccharide, soluble in water was isolated by Huang et al., (2011) from *G. lucidum* fruiting body. Novel heteropolysaccharides (GL-1 to GL-5) were also isolated from the fruiting bodies of *G. lucidum* (Wang et al., 2011a). Finally, a novel water soluble and neutral β-D-glucan (GLSA50-1B) was isolated from the spores of *G. lucidum* (Dong et al., 2012).

The chemical features of the described polysaccharides with non-reported bioactivity are similar to the ones described for the polysaccharides with some reported bioactivities. These substances might be further used for evaluation of its biological potential, as their chemical properties are promising.

7. Concluding remarks

The beneficial health properties of *Ganoderma* species have been attributed to a wide variety of bioactive components present in this genus, such as polysaccharides. Most of the studies to date have focused on this class of compounds, since they have a considerable capacity for carrying biological information due to their high structure variability.

Over the last three decades, many polysaccharides from *Ganoderma* species have been extracted by different methodologies according to their structure, water-solubility and mainly according to their cell wall structure. Therefore, polysaccharides have been extracted mostly by hot water and have been isolated and identified by different chromatographic techniques, such as mass spectroscopy or nuclear magnetic resonance. The α- or β-(1→3)-, (1→6)-glucans and hetero-polysaccharides with different

combinations of sugars have been extracted from different species, having molecular weights ranging from thousands to millions of Daltons.

Polysaccharide structural features and bioactivities have been widely explored. These molecules have antioxidant, antitumor and antibacterial potential, which has been shown both *in vitro* and *in vivo*. These properties seem to be particularly related to polysaccharide molecular weight, level of branching, and water solubility. In all the studies reported, when a structure-activity relationship is considered, some characteristics of the polysaccharides may vary (*e.g.*, molecular weight). For example, if the low molecular weight polysaccharides appeared to have higher antioxidant potential, more recent studies established that polysaccharides with a higher molecular weight also have this activity, as well as antitumor potential. Concerning the sugars composition, homo and heteropolysaccharides composed by different sugars such as mannose, glucose, rhamnose, galactose, xylose, arabinose, fructose, or fucose and linked by β-glycosidic linkages revealed high bioactivity. So far, there is no uniformity in the structural features and characteristics of the bioactive polysaccharides. Moreover, besides the natural isolated polysaccharides, the polysaccharide-protein or –peptide complexes also present bioactivity, as well as polysaccharidic extracts or fractions, in which synergistic processes with other molecules improve the potential.

Nevertheless, one feature seems to be common, and appears to be in the basis of the polysaccharide bioactivity: the glycosidic linkages. Indeed, features such as the (1→3)-β-linkages in the main chain of the glucan and additional (1→6)-β- branch points, display higher bioactivity potential.

Since these molecules were isolated and submitted to biological assays without complete chemical characterization, especially in the evaluation of antioxidant and antimicrobial activities, it is difficult to conclude what are the key chemical

characteristics for the bioactivities discussed. Accordingly, the best molecular weights cannot be specified, as also the sugars' composition or level of branching that confer higher antioxidant, antitumor or antimicrobial potential to polysaccharides. Perhaps it is this variability that makes them such interesting compounds. With the reported studies, it can only be stated that the antioxidant properties of *Ganoderma* polysaccharides, particularly the radicals scavenging activity, seem to be more related with the increase of antioxidant enzymes (SOD, CAT and GSH-Px) activity, while the antitumor potential seems to be particularly related to the host's immunity function, by exerting a series of immuno-enhancement properties, such as cytokine production. In some cases, the antitumor activity has been related to the antioxidant properties, since some polysaccharides extracted from *G. lucidum* have been shown to be effective in preventing DNA damage, suggesting a possible chemopreventive potential.

There is not much data regarding the antimicrobial activity of polysaccharides from *Ganoderma*. However, the interest in this field has recently increased due to the discovery of secondary metabolites isolated from fungi, which have antimicrobial potential. Indeed, some polysaccharides from *Ganoderma* species have antibacterial activity, inhibiting bacterial growth or inducing death of pathogenic bacteria.

Based on all the results herein reviewed, and although further testing is necessary (*e.g.*, human clinical trials), the scientific evidence available to date suggests that *Ganoderma* may become a good health food supplement, namely for cancer patients. Nevertheless, the complete chemical characterization of the polysaccharides is of extremely importance so that it could be possible to better understand the main features responsible for their powerful abilities. Moreover, with that knowledge the investigation could be better conducted in order to develop new nutraceuticals and pharmacological

formulations such as the existing ones with *G. lucidum* extracts, glycoproteins or polysaccharides (Wang et al., 2006; Wong et al., 2006; Tu et al., 2011).

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Table 1: Analytical procedures, chemical characterization and antioxidant activity of polysaccharides isolated from *Ganoderma lucidum*.

Origin	Extraction/isolation procedure	Identification technique	Polysaccharide type	Main glycosidic bonds	Sugars composition	Molecular weight	Antioxidant activity assays	Antioxidant activity values	References
China (Fruiting body) (cultivated)	Ultrasonic extraction; Sevag method; ethanol precipitation; ultrafiltration membranes	HP-GPC; HPLC-FID; GC	Heteroglucans (GLP, GLP1, GLP2, GLP3, GLP4)	na	Mannose; rhamnose; glucose; Galactose	GLP- n.a.; GLP1- >10KDa; GLP2- 8-10 KDa; GLP3- 2.5-8 KDa; GLP4- < 2.5 KDa	*In vitro* DPPH scavenging activity; Reducing power (RP); Fe^{2+}chelating activity ORAC	DPPH EC_{50} : GLP 0.28 mg/mL; GLP1 0.27 mg/mL; GLP2 0.34 mg/mL; GLP3 0.42 mg/mL; GLP4 > 0.8 mg/mL RP EC_{50}: GLP 0.42 mg/mL; GLP1 0.36 mg/mL; GLP2 0.27 mg/mL; GLP3 0.36 mg/mL; GLP4 > 0.5 mg/mL Fe^{2+} EC_{50}: GLP 0.10 mg/mL; GLP1 0.07 mg/mL; GLP2 0.07 mg/mL; GLP3 0.06 mg/mL; GLP4 0.058 mg/mL ORAC: GLP 1200 µmol trolox/g; GLP1 1500 µmol trolox/g; GLP2 1780 µmol trolox/g; GLP3 1400 µmol trolox/g; GLP4 1300 µmol trolox/g	Ma et al., 2013
China (Fruiting body) (cultivated)	Hot water extraction; ethanolprecipitation; Sevag method; dialysis	na	na	na	na	na	*In vivo* SOD activity; GSH-Px activity; CAT activity; MDA levels	200 mg/kg body weight increased the antioxidant activity of the enzymes and decreased the MDA levels in mice with exercise-induced oxidative stress	Zhonghui et al., 2013
na (Mycelium) (cultivated)	Ultrasonic assisted extraction; hydrolysis; Sevag method; ethanol precipitation; anion-exchange DEAE Sephadex A-50 column; regenerated cellulose bag filter; dialysis	GC-MS- FID; FT/IR	Heteroglucans (GLPI, GLPII, GLPIII, GLPIV)	na	GLPI- arabinose; rhamnose; xylose; mannose; glucose GLPII- arabinose; xylose; glucose GLPIII- arabinose; rhamnose; xylose; galactose; mannose; glucose	na	*In vitro* $HO^{\cdot}$ scavenging activity; DPPH scavenging activity; Reducing power (RP); Fe^{2+}chelating activity; ABTS scavenging activity; SOD-like activity	$HO^{\cdot}$ EC_{50}: GLPI 1.25 mg/mL; GLPII 0.156 mg/mL; GLPIII 0.156 mg/mL; GLPIV 0.156 mg/mL DPPH EC_{50}: GLPI 2.2 mg/mL; GLPII 1.25 mg/mL; GLPIII 0.156 mg/mL; GLPIV 0.156 mg/mL RP EC_{50}: GLPI 9.00 mg/mL; GLPII 7.5 mg/mL; GLPIII 5.00 mg/mL; GLPIV 3.00 mg/mL	Shi et al., 2013

(fruiting body) (na)	hydrolysis; size-exclusion chromatography	MS; NMR; MALDI-TOF MS	Homoglucan				MTT assay (RAW264.7 cells); ROS formation; nSMase and aSMase activities	with H_2O_2-induced oxidative stress from 40% to 80% and significantly reduced ROS formation nSMase inhibition: IC_{50} 120 µg/mL; aSMase inhibition: IC_{50} 100 µg/mL	
China (fruiting body) (na)	Hot water extraction; D301R macroporous adsorption/ion exchange resin column; DEAE-Cellulose-32 column; gel filtration chromatography	Size exclusion HPLC; methylation analysis; GC, GC-MS; EI-MS; IR spectra	GLP_L1: Homoglucan GLP_L2: Heteroglucan	β (1-3), (1-4) and (1-6)	GLP_L1: Glucose. GLP_L2: Glucose; galactose, mannose	GLP_L1: 5.2 KDa; GLP_L2: 15.4 KDa	*In vitro* $HO^·$scavenging activity; $O_2^{·-}$scavenging activity; Fe^{2+}chelating activity; Reducing power (RP); H_2O_2 scavenging activity	$HO^·$ EC_{50}: GLP_L1 0.63 mg/mL; GLP_L2 2.50 mg/mL $O_2^{·-}$ EC_{50}: GLP_L1 2.12 mg/mL; GLP_L2 10.0 mg/mL Fe^{2+} EC_{50}: GLP_L1 6.0 mg/mL; GLP_L2 > 10.0 mg/mL RP EC_{50}: GLP_L1- GLP_L2 > 10.0 mg/mL H_2O_2 EC_{50}: GLP_L1 6.0 mg/mL; GLP_L2 > 10.0 mg/mL	Liu et al., 2010
China (fruiting body) (cultivated)	Hot water extraction; ethanolprecipitation; Sevag method; dialysis; precipitation with cetyl trimethyl ammonium hydroxide; DEAE cellulose column; anion exchange column of DEAE-Sepharose Fast Flow	TLC	Homopolysaccharide	na	Mannose	na	*In vivo* SOD activity; CAT activity; GSH-Px activity; TAOC level; TBARS (MDA levels)	300mg/kg body weight decreased MDA levels and increased SOD, CAT, GSH-Px activities and TAOC levels in rats with ovarian cancer	YouGuo et al., 2009
China (fruiting body) (cultivated)	Hot water extraction; ethanolprecipitation; Sevag method; dialysis; precipitation with cetyl trimethyl ammonium hydroxide; DEAE cellulose column; anion exchange column of DEAE-Sepharose Fast Flow.	TLC	Homopolysaccharide	na	Mannose	na	*In vitro* $O_2^{·-}$scavenging activity; $HO^·$scavenging activity; DPPH scavenging activity. *In vivo* SOD activity; CAT activity; GSH-Px activity	$O_2^{·-}$ EC_{50}: 1.5 mg/mL $HO^·$ EC_{50}: 2.2 mg/mL DPPH EC_{50}: 1.0 mg/mL 300 mg/kg body weight increased SOD, CAT and GSH-Px activities in rats with cervical carcinoma	XiaoPing et al., 2009
China (fruiting body) (cultivated)	Hot water extraction; ethanolprecipitation; Sevag method; dialysis	na	Heteroglucan	β-	D- Rhamnose; D-xylose; D-fructose; D-galactose; D-mannose; D-	0.5848 KDa	*In vitro* TBARS; LOOH; Protein carbonyls formation;	60 mg/mL decreased levels of TBARS, LOOH and protein carbonyls formation, and increased protein thiols formation and SOD, GSH-Px, CAT activities in mitochondria with	Li et al., 2007

		glucose	Protein thiols formation; SOD activity; CAT activity; GSH-Px activity	γ-irradiation induced oxidative stress

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); aSMase – acidic SMase; CAT – Catalase; DEAE – Diethylaminoethanol; DPPH – 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, IC_{50} – Concentration of polysaccharide providing 50% of antioxidant activity; EI – Electron ionization; FID – Flame ionization detector; FT-IR – Fourier transform infrared spectrophotometer; GC – Gas chromatography; GSH-Px – Glutathione peroxidase; H_2O_2 – Hydrogen peroxide; $HO^{\cdot}$ – Hydroxyl radical; HPAEC – High-performance anion-exchange chromatography; HP-GPC – High-performance gel permeation chromatography; HPLC – High performance liquid chromatography; IR – Infrared; LOOH – Lipid hydroperoxides; MALDI-TOF – Matrix assisted laser desorption ionization-time of flight; MDA – Malondialdehyde; MS – Mass spectrometry; MTT – 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; NMR – Nuclear magnetic resonance; nSMase – Neutral SMase; $O_2^{\cdot -}$ – Superoxide radical; ORAC – Oxygen radical absorbance capacity; RAW 264.7 – Mouse leukaemic monocyte macrophage cell line; ROS – Reactive oxygen species; RP – Reducing power; SMase – Sphingomyelinase; SOD – Superoxide dismutase; TAOC – Total antioxidant capacity; TBARS – Thiobarbituric acid reactive substances; TLC – Thin layer chromatography.na –data not available.

Table 2: Analytical procedures, chemical characterization and antitumor activity of polysaccharides isolated from *Ganoderma* species.

***Ganoderma* species**	**Origin**	**Extraction/isolation procedure**	**Identification technique**	**Polysaccharide type**	**Main glycosidic bonds**	**Sugars composition**	**Molecular weight**	**Antitumor activity assays/ Dose**	**Antitumor model used in the study**	**Antitumor activity values**	**References**
G. lucidum (fruiting body)	Japan (cultivated)	Hot water extraction; ethanol precipitation; Sevag method; DEAE-cellulose column chromatography with sodium hydrogen carbonate	PC; GLC; IR; ^{13}C NMR	Branched heteroglucan (arabinoxyloglucan)	β-D-(1→3), β-D-(1→6), and (1→4)-α- and -β-	Glucose; xylose; arabinose	40000	*In vivo* Tumorigenicity assay: calculation of the tumor inhibition ratio (%) in ICR mice 5-20 × 10 (mg/Kg × day)	Sarcoma 180 solid tumor	42.0 – 98.5 % of inhibition ratio	Miyazaki and Nishijima, 1981
G. lucidum (fruiting body)	Japan (cultivated)	First extraction with cold PBS (separation of the soluble and insoluble fractions). Hot water, and cold and hot 1M sodium hydroxide; treatment with cetyl pyridinium chloride (CPC) and glucoamylase; hydrolysis with acid	GLC; PC; HPLC	Water-soluble heteropolysaccharides		Glucose; galactose; mannose; xylose; arabinose; fucose	Na	*In vivo* Tumorigenicity assay: calculation of the tumor inhibition ratio (%) in IRC-JCL mice 10 × 10 (mg/Kg × day)	Sarcoma 180 solid tumor	na	Sone et al., 1985
				Water-insoluble glucans	(1→3)-β-D-glucan with a few short (1→4)-linked glucosyl units	Glucose	Na			10.9 – 97.9 % of inhibition ratio	
G. lucidum (growing culture of mycelium)	Japan (cultivated)	Ethanol precipitations/ Toyopearl HW-65S column chromatography	GLC; PC; HPLC	Branched homoglucan	(1→3)-β-D-glucan	Glucose	Na	*In vivo* Tumorigenicity assay: calculation of the tumor inhibition ratio (%) in IRC-JCL mice 10 × 10 (mg/Kg × day)	Sarcoma 180 solid tumor	91.6 % of inhibition ratio	Sone et al., 1985
G. lucidum (fruiting body)	na	Hot water followed by ethanol precipitation	Na	Branched homoglucan	(1→3)-β-D-glucan with (1→6)-β-D branches	Glucose	GLPO < 12.000 GLP I > 12.000	*In vivo* Tumorigenicity assay (BALB/c mice; Details not available)	Sarcoma 180 solid tumor	Induced a cascade of immunomodulatory cytokines	Ooi et al., 2002
G. tsugae (mycelium)	China (cultivated)	Immersion in 0.2 M sodium phosphate buffer (pH 7.0); Sevag method; H_2O_2; dialysis; isolation with phosphate buffer,	IR; GC; ^{13}C NMR	Heteroglucans	(1→3)-β-D-glucans and (1→4)-α-D-glucans; and (1→6)-branched (1→3)-β-D-	Rhamnose; fucose; xylose; mannose; galactose; glucose; *N*-	GTM3-465×10^{-4} GTM4-468×10^{-4} GTM5-176×10^{-4}	*In vivo* Tumorigenicity assay: calculation of the tumor inhibition ratio (%) and enhancement of	Sarcoma 180 solid tumor	12.9– 55.06 % of inhibition ratio 11.5 – 39.1 % enhancement of body weight ratio	Peng et al., 2005

		distilled water and 0.5 M sodium hydroxide			glucan	acetylglucosamine	GTM6- 161×10^{-4}	body weight ratio (%) in BALB/c mice 5-37.5 × 10 (mg/Kg × day)			
G. lucidum (fruiting body)	China (cultivated)	Extraction with 95% ethanol; ultrasonic-aid extraction (UAE); DEAE cellulose-52 chromatography and Sephadex G-100 size-exclusion chromatography	Spectrophotometry (UV); HPGFC; IR	Heteropolysaccharide	na	Glucose; galactose; mannose; rhamnose; fucose	GP-1- 1.926 KDa GP-2- 1086 KDa	*In vitro* MTT assay 5, 25, 50 µg/mL	Human breast cancer cell line (MDA-MB-231)	0.347–0.352 (OD values at 490 nm) corresponding to inhibition ratios of 0.50 – 6.72 %; Immunomodulatory activity (increasing macrophage cells proliferation)	Zhao et al., 2010
G. lucidum (mycelium)	China (cultivated)	Hot water; ethanol precipitation; Sevag method; dialysis	IR; UV; NMR	Heteropolysaccharide	α-D-Glc(1→6), α-D-Glc, α-D-Man (rhamnose and arabinose residues in the side chain)	Rhamnose; arabinose; mannose; glucose; galactose	3.500 KDa	*In vitro* and *in vivo* MTT assay; Cell cycle assay by Flow cytometry; Tumorigenicity assay: calculation of the tumor inhibition ratio (%) in ICR mice 500, 1000, 2000 µg/mL (for the in vitro assays) 0.5 and 2.0 × 8 (mg/Kg × day) (for the *in vivo* assays)	Human hepatocarcinoma cell line (HepG2) Tumour xenografts in ICR mice	0 – ≈ 35 % of inhibition rate (*in vitro*) 31.39–55.02 % of inhibition rate (*in vivo*); Apoptosis enhanced by supplemental dose of the intracellular polysaccharides	Liu et al., 2012; Zhang et al., 2012
G. lucidum (fruiting body)	China (cultivated)	Pretreatment with ethanol; Sevag method; Ultrasonic cell disruption; ultrafiltration	HP-GPC; HPLC-FID; GC	Heteroglucans	na	Mannose; Rhamnose; glucose; galactose	GLP- na; GLP1- >10KDa; GLP2- 8-10 KDa; GLP3- 2.5-8 KDa; GLP4- < 2.5 KDa	*In vitro* MTT assay 0.05, 0.25 and 1 mg/mL	Adrenal gland from rat -PC12 cell line	≈12.5 - ≈52.5 % of inhibition activity	Ma et al., 2013

BALB/c mice – albino laboratory-bred strain of the house mouse from which a number of common sub-strains are derived; DEAE – Diethylaminoethyl; GC – Gas chromatography; GLC – Gas liquid chromatography; HPGFC – High performance gel filtration chromatography; HP-GPC – High performance gel permeation chromatography; HPLC – High performance liquid chromatography; HPLC-FID – High performance liquid chromatography coupled to a flame ionization detector; ICR mice – Imprinting control regions, strain of albino mice

originating in Switzerland; IRC-JCL – Another strain of albino mice originating in Switzerland; IR – Infrared spectroscopy; MTT – 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; NMR – Nuclear magnetic resonance spectroscopy; OD – Optical Density; PBS – Phosphate buffered saline; PC – Paper chromatography; UV – Ultraviolet. na –data not available.

Table 3: Analytical procedures, chemical characterization and antimicrobial activity of polysaccharides isolated from *Ganoderma lucidum*.

***Ganoderma* species**	**Origin**	**Extraction/isolation procedure**	**Antimicrobial activity assay**	**Microorganisms used**	**Type of assay**	**Reference**
G. lucidum	Poland (cultivated)	Hot water followed by ethanol precipitation/ DEAE-cellulose column chromatography	Microdilution method	*Staphylococcus epidermidis*, *S.aureus*, *Bacillus subtilis*, *Micrococcus luteus Escherichia coli Klebsiella pneumonia, Pseudomonas aeruginosa Proteus mirabilis*	*In vitro*	Skalicka-Woźniak et al., 2012
G.lucidum	China (wild)	Hot water	Agar diffusion method	*Erwinia carotovora,Penicillium digitatum, Botrytis cinerea, Bacillus cereus Bacillus subtilis, Escherichia coli Aspergillus niger, Rhizopus nigricans*	*In vitro*	Bai et al., 2008
G. applanatum	India (wild)	na	Cup diffusion method	*Acitenobacter aerogenes Acrobacter aerogenes Arthrobacter citreus, Bacillus brevis*, *B.subtilis Corynebacterium insidiosum Escherichia coli, Proteus vulgaris Clostridium pasteurianum, Micrococcus roseus Mycobacterium phlei, Sarcina lutea Staphylococcus aureus*	*In vitro*	Bhattacharyya et al., 2006
G.formosanum (mycelia)*	Taiwan (cultivated)	Ethanol extraction to allow the precipitation dissolution in boiled Tris/HCl buffer, the sample was fractionated on a Sepharose CL-6B gel filtration column	na	*Listeria monocytogenes*	*In vivo* on mice; enhanced microbial killing, which is mostly mediated by monocytes/macrophages and neutrophils	Wang et al., 2011a
G. lucidum (mycelia)	India (cultivated)	na	Well diffusion method	*Escherichiacoli, Staphylococcus aureus Proteus* sp, *Bacillus subtilis, Pseudomonas aeroginosa Klebsiella* sp ,*Bacillus cereus*	*In vitro*	Mahendran et al., 2013

DEAE – Diethylaminoethanol. na –data not available.

*Branched homoglucan; (1→3)-β-D-glucan with (1→6)-β-D branches; composed by D-mannose, D-galactose, D-glucose, L-arabinose, L-fucose, D-fructose and L-rhamnose.

Table 4: Polysaccharides isolated from *Ganoderma* species without reported bioactivity.

***Ganoderma* species**	**Origin**	**Extraction/isolation procedure**	**Identification technique**	**Polysaccharide type**	**Main glycosidic bonds**	**Sugars composition**	**Molecular weight**	**References**
G. japonicum (fruiting body)	Japan (wild)	Hot dichloromethane and hot methanol; Hot water; Dialysis; gel filtration on Sepharose CL-4B	GLC-MS; ^{1}H-NMR; IR; PC	Alkali-soluble glucan	β -(1→3)-linked D-glucopyranosyl residues with side-chains of single, β - (1→6)-linked D-glucopyranosyl groups	Glucose; laminarabiose	82000	Ukai et al., 1982
G. lucidum (fruiting body)	China (cultivated)	PBS; Ethanol precipitations; 1N NaOH	^{13}C-NMR;^{1}H-NMR; IR	Water insoluble glucans	(1→3)- α -D-glucans	Glucose	GL4-1 - 1.95x10^{5}; GL4-2 – 1.33x10^{-4}	Chen et al., 1998
G. lucidum (spores)	na (cultivated)	Hot water followed by ethanol precipitation	^{13}C-NMR; GC; GC-MS; IR	Water soluble polysaccharide	(1→3)-linked-Glc; (1→6)-linked-Gal; (1→4)-linked-Gal; (1→6)-linked-Glc	Glucose; galactose	1.41x10^{4}	Zhao et al., 2005
G. tlucidum (germinating spores)	na (cultivated)	Deproteinization by Sevag method and frozen-thaw method, fractionated by ultrafiltration and gel chromatography on CL-6B column.	GC; HPLC; IR; NMR	Heteropolysaccharide	Na	Glucose; galactose	1.41x10^{5}	Zhang et al., 2006
G. resinaceum (fruiting body)	Brazil (wild)	Chloroform-methanol; Hot water; dialysis; Freeze-thawing; ultrafiltration	GC-MS; GPC; NMR	Water soluble glucan	(1→3)-linked β -glucan	Glucose; mannose; galactose; xylose	2.6x10^{4}	Amaral et al., 2008
G. lucidum (fruiting body)	China (wild)	Hot water followed by ethanol precipitation; ultrafiltration	FT-IR;HPAPC; NMR	Water soluble polysaccharide	1,6-disubstituted-α-galactopyranosyl, 1,2,6-trisubstituted-α-galactopyranosyl, 1,3-disubstituted-β -glucopyranosyl and 1,4,6-trisubstituted-β -glucopyranosyl residues	Fucose; glucose; galactose	7000Da	Ye et al., 2009
G. lucidum (fruiting body)	China (cultivated)	Utrasonic/microwave assisted extraction	FT-IR; GC-MS; HPSEC; NMR	Water soluble neutral polysaccharide	Backbone: 1,4-disubstituted- β -glucoseopyranose and	Glucose; galactose	2.5x10^{6}kDa	Huang et al., 2011

					1,4,6-trisubstituted-β –glucoseopyranosyl; Branched chains: 1,6-disubstituted- β -glucopyranosyl and 1,4-disubstituted- β–galactoseopyranosyl			
G. lucidum (fruiting body)	China (cultivated)	Ethyl-acetate; Sevag method; Dialysis	FT-IR; GC-MS; NMR	Water soluble Heteropolysaccharides	(1→4)-galactan heteropolysaccharide; (1→3)-glucan; 1,4,6-glucan, (1→3)-galactan, (1→6)-galactan, (1→4)-grabinan, (1→3)-mannan, and/or (1→4)-xylan linkages	glucose, galactose, mannose, arabinose	GL-I – 6.1x10^4; GL-V – 10.3x10^4	Wang et al., 2011
G. lucidum (spores)	China (cultivated)	Hot-water extraction, graded ethanol precipitation, anion-exchange chromatography	GPL; HPGPC; NMR	Water soluble glucan	Backbone: 1,6-linked β -D-Glcp; Side chain: 1,4-linked Glcp residues	Glucose	103 kDa	Dong et al., 2012

FT-IR – Fourier transform infrared spectrophotometer; GC- Gas chromatography; GC-MS – Gas chromatography coupled to mass spectrometry; GLC-MS – Gas liquid chromatography coupled to mass spectrometry; GPC – Gel permeation chromatography; HPGPC – High performance gel permeation chromatography; HPLC – High performance liquid chromatography; HPSEC – High pressure size exclusion chromatography; IR – Infrared spectroscopy; NMR – Nuclear magnetic resonance spectroscopy; PC – Paper chromatography; na –data not available.

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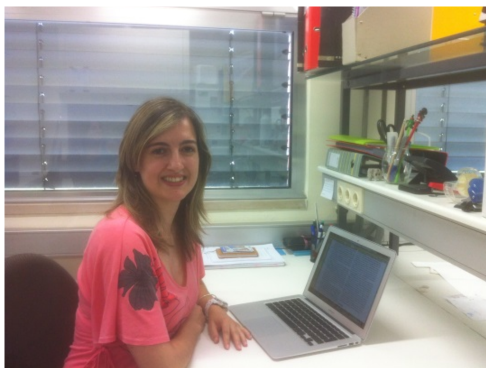

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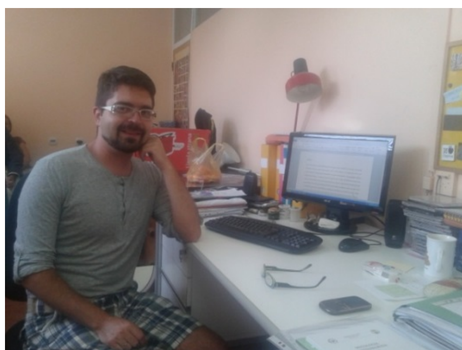

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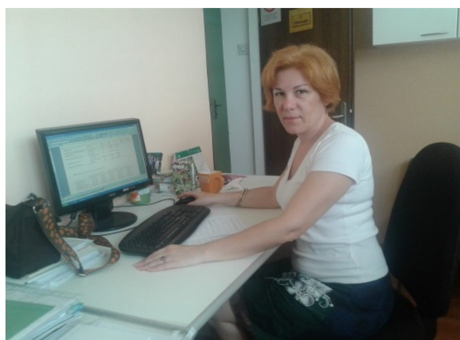

Marina Soković has graduated at Faculty of Biology, University of Belgrade and got PhD degree in the same University. She was a Teaching assistant at Faculty of Biology, University of Belgrade. She did her postdoc research in the Plant Research International, Wageningen University (The Netherlands). She is the leader of international Projects and scientific consultant at Innovation Serbian Projects. She is the leader of the research group in laboratory of Mycology. The main research topics are in the field of phytochemistry and chemotaxonomy of flowering plants, biological activity of secondary metabolites of medicinal plants and fungi (microfungi and mushrooms), investigation of pathogenic fungi (plant, human and animal pathogens and food poisoning microorganisms). She published the results of her research work so far in 170 international SCI journals. She supervised master's theses and doctoral dissertations. Dr Sokovic is a reviewer in a number of prominent international journals and was a guest editor in respected international Journals being also a member of several international associations relevant to the field.