



***Ganoderma Lucidum*: Extraction and Characterization of Polysaccharides, Yields and their Bioapplications**

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Abstract. The Reishi mushroom, *Ganoderma lucidum* is an edible herbal home remedy to boost the immune system, especially in the Asian countries. Its fruiting body can thrive well in a hot and humid climate and contain specific bioactive macromolecules like triterpenoids, phenolic compounds, steroids, nucleotides and their derivatives polysaccharides and glycoproteins which have strong therapeutic properties. In this mini-review, the focus is on medicinal *G. lucidum* polysaccharides, one of the effective constituents as a health-promoting agent and its methods of extraction and purification to reflect the current status of characterization techniques in clinical practices. An overview of conformational properties, different analytical techniques and other methods involved were briefly discussed. A detailed account of significant biological applications of *G. lucidum* polysaccharides like antitumor, anti-inflammatory, antiviral and anticancer activities was tabulated and discussed.

Keywords. *Ganoderma lucidum*, Polysaccharides, Extraction, Purification, Biological applications.

INTRODUCTION

Mushrooms have been known for their edible, medicinal resources and antitumor substances for many years. The fungi belonging to the genus *Ganoderma* are popular medicinal

mushrooms, widely used in China, Japan and Korea over the past two millennia (Silva, 2006). The most frequently cited *Ganoderma* species used in research publications on the cultivation, chemical analysis, pharmacology and medicinal effects are the *Ganoderma lucidum* (*G. lucidum*), an edible medicinal mushroom commonly known as *Reishi* or *Manentake* (Japanese) or *Lingzhi* (Chinese) (Stamets and Yao, 2002). The incredible curative properties have won it the titles of ‘supernatural mushroom’, ‘magic mushroom’ and ‘plant of longevity or immortality’, produced not only in its native East Asian countries such as China, India, Japan, Korea, Taiwan, and Malaysia but also in the USA. *G. lucidum* has been reported to have many pharmacological effects including immune-modulating, anti-atherosclerotic, anti-inflammatory, analgesic, chemopreventive, anti-tumour, radioprotective, sleep-promoting, antibacterial, antiviral (including anti-HIV), hypolipidemic, anti-fibrotic, hepatoprotective, diabetic, antioxidative and radical-scavenging, antiaging, hypoglycemic, and antiulcer properties (Jong and Birmingham, 1992).

Reishi mushroom has now become recognized as an alternative adjuvant in the treatment of leukaemia, carcinoma, hepatitis, and diabetes. Polysaccharides, triterpenes, sterols and peptidoglycans are the major chemical constituents of *G. lucidum*, along with oleic acid, soluble proteins, amino acids, ergosterol peroxide (5,8-epidioxy-ergosta-6,22E-dien-3-ol), and the cerebrosides (4E',8E)-N-D-2'-hydroxylstearoyl-1-O- β -D-glucopyranosyl-9-methyl-4-8-sphingadienine, and (4E',8E)-N-D-2'-hydroxypamitoyl-1-O- β -D-glucopyranosyl-9-methyl-4-8-sphingadienine and cyclo-octasulfur (Simmonds, 2003) along with inorganic ions like iron, manganese, germanium, magnesium, zinc, copper, and calcium. The fruiting body of *G. lucidum* is shown in figure 1. *G. lucidum* spore cell wall contains a high amount of polysaccharides, which are natural macromolecular compounds with complex and versatile biological activities. The spores also contain choline, betaine, tetracosanoic acid, stearic acid, palmitic acid, ergosta-7, 2, 2-dien-3-ol, nonadecanoic acid, behenic acid, tetracosane, hentriacontane, ergosterol, and β -sitosterol. One of the lipids isolated from *G. lucidum* is pyrophosphatidic acid (Liu, 1999).



Kingdom:	Fungi
Phylum:	Basidiomycota
Class:	Agaricomycetes
Order:	Polyporales
Family:	Ganodermaceae
Genus:	Ganoderma
Species:	lucidum

Fig.1. *Ganoderma lucidum* (Reishi or Lingzhi).

In recent years, polysaccharides extracted from *G. Lucidum* have been regarded as an important class of anticoagulants, immunomodulating and antitumour with antioxidant activities, antiproliferative activities, antiviral and antiprotozoal activities (Bao et al., 2002). *G. lucidum* polysaccharides such as β -D-glucans, heteropolysaccharides, and glycoprotein have been isolated and characterized; considered as the major contributors of bioactivity of the mushroom. β -D-glucans consist of a linear backbone of β -(1 \rightarrow 3)-linked D-glucopyranosyl groups with varying degrees of branching from the C6 position. In addition to water-soluble β -D-glucans, β -D-glucans co-exist with hetero-polysaccharide chains of xylose, mannose, galactose, uronic acid and β -D-glucans–protein complexes that are present at 10–50% in dry *G. lucidum* (Cheong et al., 1999). Presence of various reactive groups in their structure, polysaccharides can be easily modified chemically and biochemically. Moreover, the

presence of hydrophilic groups in their structure, such as hydroxyl, carboxyl and amino groups, enhance bio-adhesion with biological tissues, like epithelia and mucous membranes, forming non-covalent bonds, a useful strategy to improve the bioavailability of drugs included in drug delivery systems.

EXTRACTION OF POLYSACCHARIDES

Polysaccharides extraction is fairly time consuming and slow process, the literature suggests several published articles using different approaches for the extraction of polysaccharides from the spores of *G. lucidum*. The most common approaches were hot water extraction (HWE) for the extraction of water-soluble polysaccharides and alkaline extraction is used for the extraction of water-insoluble polysaccharides.

Traditional use of HWE was the cause for a lower yield, longer extraction times and high-temperature process. To get the better yields other techniques like Ultrasound Microwave-Assisted Extraction (UMAE) (Huang et al., 2010), Ultrasonic Assisted Extraction (UAE) Liyan et al., 2010), breaking the spores of the fungus *G. lucidum* by supercritical CO₂ t (Yu-Jie et al., 2009), breaking the spores of *G. lucidum* by fermentation with *Lactobacillus plantarum* (Chaiyavat et al., 2010) and alkaline extraction of polysaccharides (AEP) (Gao et al., 2011) were reported. The percentage yields of different techniques were given in table 1. The most effective procedure of AEP results showed optimized yields from the fruiting body of *G. lucidum* of 6.81% under alkaline extraction conditions.

Table 1. Polysaccharides extraction from *G. lucidum* in percentage yield using various methods.

Method of Extraction	% of Yield	Water-soluble/ in-soluble	Reference
Hot water extraction	0.4	Soluble	Chang and Lu, 2004
Hot water extraction	7.5	Soluble	Qian et al., 2010
Hot water extraction	3.7	Soluble	Pang et al., 2007
Hot water extraction Breaking spores by Supercritical CO ₂	2.98	Soluble	Yu-Jie et al., 2009
Breaking spores by fermentation using <i>Lactobacillus plantarum</i>	Not available	Not available	Chaiyavat et al., 2010
Ultrasound microwave-assisted extraction	3.9	Soluble	Huang and Zheng, 2010
Ultrasonic assisted extraction	2.07	Soluble	Liyan et al., 2010
Alkaline extraction of polysaccharides	8.21	In-soluble	Huang et al., 2010
Alkaline extraction of polysaccharides	1.41	In-soluble	Jinghua et al., 1998
Alkaline extraction of polysaccharides	6.81	In-soluble	Gao et al., 2011

Extraction Procedure

The dried sample was grounded into a fine powder and defatted with petroleum ether, ethyl acetate and methanol. Then mixed with 80% ethanol and shaken at 30°C for 24 h, to remove most of the polyphenols and monosaccharides. Water-soluble polysaccharides were extracted stepwise with a 0.2M phosphate buffer solution (PBS) of pH-7 at 25°C, 80°C and 120°C. In

each step, the PBS suspension was centrifuged and to the supernatant was added a large quantity of ethanol to precipitate the polysaccharide. The precipitates of *G. lucidum* polysaccharide (GLP) were designated GLP I, GLP II and GLP III in the order of increasing extraction temperature.

The residue obtained from the last PBS suspension was then treated with 1% ammonium oxalate and acetic acid was added to the supernatant to precipitate the water-insoluble polysaccharides and was designated as GLP IV the residue obtained was treated with 5% NaOH at 25°C, acetic acid was added to precipitate the polymers and designated as GLP V. Ethanol was added to the supernatant to get the final polysaccharides GLP VI. Crude polysaccharides of *G. lucidum* at different stages of extraction were obtained. These extraction procedures were similar to those reported for *Reishi* mushroom (Sone et al., 1985) and *G. tsuaga* mushroom (Wang et al., 1993). The complete extraction process of polysaccharides from *G. lucidum* reported elsewhere (Mizuno, 1999).

PURIFICATION OF POLYSACCHARIDES

The extracted polysaccharides were purified by a combination of techniques, such as ethanol precipitation, fractional precipitation, and acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration, and affinity chromatography. The ethanol precipitation excludes the impurities from the polysaccharides. The separation of acidic and neutral polysaccharides can be achieved by anion-exchange chromatography on diethyl-amino-ethanol cellulose (DEAE-C) column. The neutral polysaccharide in the mixture is first eluted by an appropriate running buffer; the acidic polysaccharide is then eluted at a higher salt concentration. Neutral polysaccharides later separated into α -glucans (adsorbed fraction) and β -glucans (non-adsorbed fraction) with the help of gel filtration and affinity chromatography. This process now allows for the highly specific and efficient purification of some carbohydrates. The complete purification process of *G. lucidum* polysaccharides is given in figure 2.

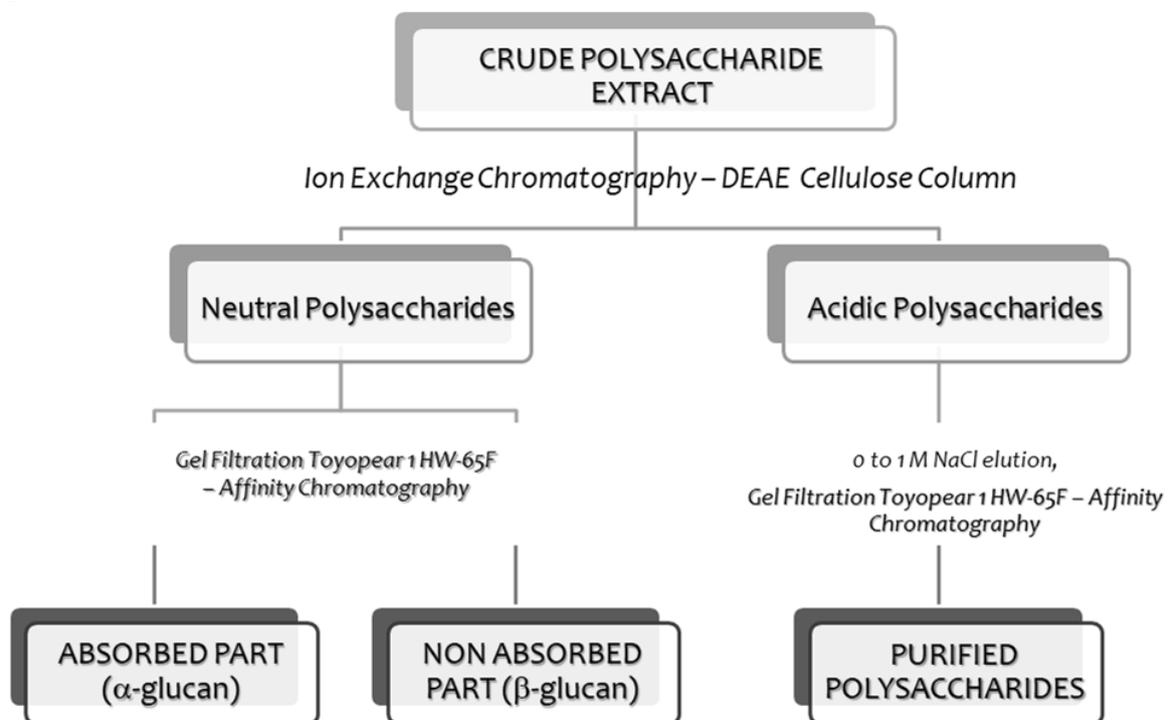


Fig. 2. Purification of polysaccharides by chromatography
CONFORMATIONAL PROPERTIES AND ANALYTICAL TECHNIQUES

Polysaccharides having hyper-branched structures, to characterize such structures for their chemical structure and chain conformations are not an easy task. The chemical structures were analysed by FTIR spectroscopy, Raman spectroscopy, NMR spectroscopy- Liquid-state NMR (1D and 2D) and Solid-state NMR, several chromatographic techniques like Gas Chromatography (GC), GC–Mass (GC–MS) and High-Performance Liquid Chromatography (HPLC) were employed for fractionation of polysaccharides. Chain conformations of polysaccharides in solutions were investigated using static and dynamic light scattering, viscosity analysis based on the theory of dilute polymer solutions, and Atomic Force Microscopy (AFM) including single molecular AFM and AFM-based single-molecule Force Spectroscopy, fluorescence correlation spectroscopy and NMR spectroscopy.

Characterization of polysaccharides

The chemical structures of polysaccharides, such as the sugar composition, type of glycosyl linkage and the branched structures, were characterized by spectral analysis, chemical analysis and chromatography.

FTIR spectroscopy

FTIR spectroscopy technique was used in investigating the vibrations of molecules and polar bonds between the different atoms. Fourier transform infrared (FT-IR) spectroscopy is a physicochemical method based on measurement of vibration of a molecule excited by IR radiation at a specific wavelength range. Functional groups present in a molecule tend to absorb IR radiation in the same wavenumber range regardless of other structures in the molecule, and spectral peaks are derived from the absorption of bond vibrational energy changes in the IR region. Structures of polysaccharides, such as monosaccharide types, glucosidic bonds and functional groups, can be analysed using FTIR spectroscopy (Mathlouthi and Koenig, 1986). In the range of 1100–1010 cm^{-1} , three strong absorption peaks appear for pyranoside, and two peaks for furanoside.

Raman spectroscopy

Compared with FTIR spectroscopy, Raman spectroscopy is highly sensitive to detect the vibrations of molecules and non-polar bonds of the same atom. Raman spectroscopy is the best suitable technique to characterize the helical conformation and the plane fold of bio-macromolecules (Zhu et al., 2006). The Raman spectra of saccharides segregated into four regions: the bands in the range of 350–600 cm^{-1} are assigned to skeletal modes of pyranose rings; the anomeric region is from 600 to 950 cm^{-1} , the glycosidase stretching modes appear in the region 950–1200 cm^{-1} ; and the CH_2 and C-OH deformations region is from 1200 to 1500 cm^{-1} .

NMR spectroscopy

NMR spectroscopy has become the most powerful and non-invasive physicochemical technique for determining polysaccharide structures providing detailed structural information of polysaccharides, including identification of monosaccharide composition, elucidation of α - or β -anomeric configurations, the establishment of linkage patterns, and sequences of the sugar units in polysaccharides.

Liquid-state NMR - The liquid-state NMR has become recognized as an important developing tool for chemical structural analysis of polysaccharides (Bubb 2003). Most polysaccharides can be dissolved in water and dimethyl sulfoxide (DMSO), thus denatured water and DMSO (D_2O and DMSO-d_6) are common solvents for polysaccharides in the liquid-state NMR experiments. The proton signals of polysaccharides overlap in the range of 3.5–5.5 ppm in the ^1H NMR spectrum, it is difficult to assign them (Leeuwen et al., 2008)

investigated the ^1H NMR spectroscopy of the primary structural characterization of α -D-glucans in detail, in which chemical shift patterns for (α 1 \rightarrow 2)-, (α 1 \rightarrow 3)-, (α 1 \rightarrow 4)- and (α 1 \rightarrow 6)-linked D-glucose residues were analysed. In contrast, the range of ^{13}C chemical shifts of polysaccharides is much wider than that of ^1H chemical shift, which comes from 60 to 110 ppm.

Solid-state NMR - Solid-state NMR in contrast with liquid-state NMR the line widths become broader mainly due to the anisotropic character and dipolar interaction (Xue, 1993). The anisotropic parts of the interactions from the molecules can be removed when the solid sample rotates at 54.7° . Magic-angle-spinning (MAS) is essential to achieve high-resolution ^{13}C solid-state NMR spectra (Mcbrierty and Packer, 1993). The intensity of the solid ^{13}C signals can be enhanced using cross-polarization (CP) technology, in which the polarization transfers from ^1H to ^{13}C . In recent years, solid-state NMR is used to analyze the chemical structures of polysaccharide to overcome the solubility problem, since the samples can be measured in a solid and dehydrated form have reported the ratio between proteins and polysaccharides was directly determined through solid ^{13}C CP/MAS spectroscopy (Spevacek and Brus, 2008; Pizzoferrato et al., 2000).

Chromatography

The monosaccharide compositions, types of glycosidic linkages and branching of polysaccharides may be also analysed by chromatography. GC, GC-MS and HPLC methods are employed after polysaccharides are hydrolysed by trifluoroacetic acid (TFA) (Urai, 2007) or derived by the methylation (Ciucanu and Kerek, 1984), periodic acid oxidation (Abdel-Akher et al., 1952) and Smith degradation (Datta et al., 1999).

Chain conformational analysis of polysaccharides in solution

Conformation of polysaccharides in solutions; especially in aqueous solutions, can be investigated according to the theory of dilute polymer solutions. The intrinsic viscosity η is a characteristic property of polysaccharide solution. Huggins and Kraemer's equations are used to estimate the η value by extrapolating to infinite dilution (Goh et al., 2006a).

$$\eta_{\text{sp}}/C = \eta + K'\eta^2C$$

$$(\ln \eta_r)/C = \eta + K''\eta^2C$$

Where K' is the Huggins constant and K'' is the Kraemer constant, η_{sp}/C is the reduced specific viscosity, and $(\ln \eta_r)/C$ is the inherent viscosity.

Other methods

- a The AFM-based single-molecule force spectroscopy (AFM-SMFS) technology is a powerful tool to characterize the force-induced conformational transitions, the dynamics, and supramolecular structures of polysaccharides at the molecular level (Abu-lail and Camesano, 2003).
- b Fluorescence correlation spectroscopy (FCS) is interesting to determine the conformations and sizes of polysaccharides at a lower concentration of 10^{-8}mol/l (Meunier and Wilkinson, 2002).

BIOLOGICAL APPLICATIONS

G. lucidum has been used to treat various human diseases such as allergy, arthritis, bronchitis, gastric-ulcer, hyperglycemia, hypertension, chronic hepatitis, hepatopathy, insomnia, nephritis, neurasthenia, scleroderma, inflammation, and cancer. The fruiting bodies or spores of *G. lucidum* were linked to possible therapeutic effects (Table 2).

The mechanisms of action involve gut microbiota, meaning the polysaccharides act as prebiotics in the digestive system (Friedman, 2016). Different compounds with various

biological activities were extracted from mycelia. Current biological/biomedical applications of *G. lucidum* (Wasser and Weis, 1997) were given in figure 3.

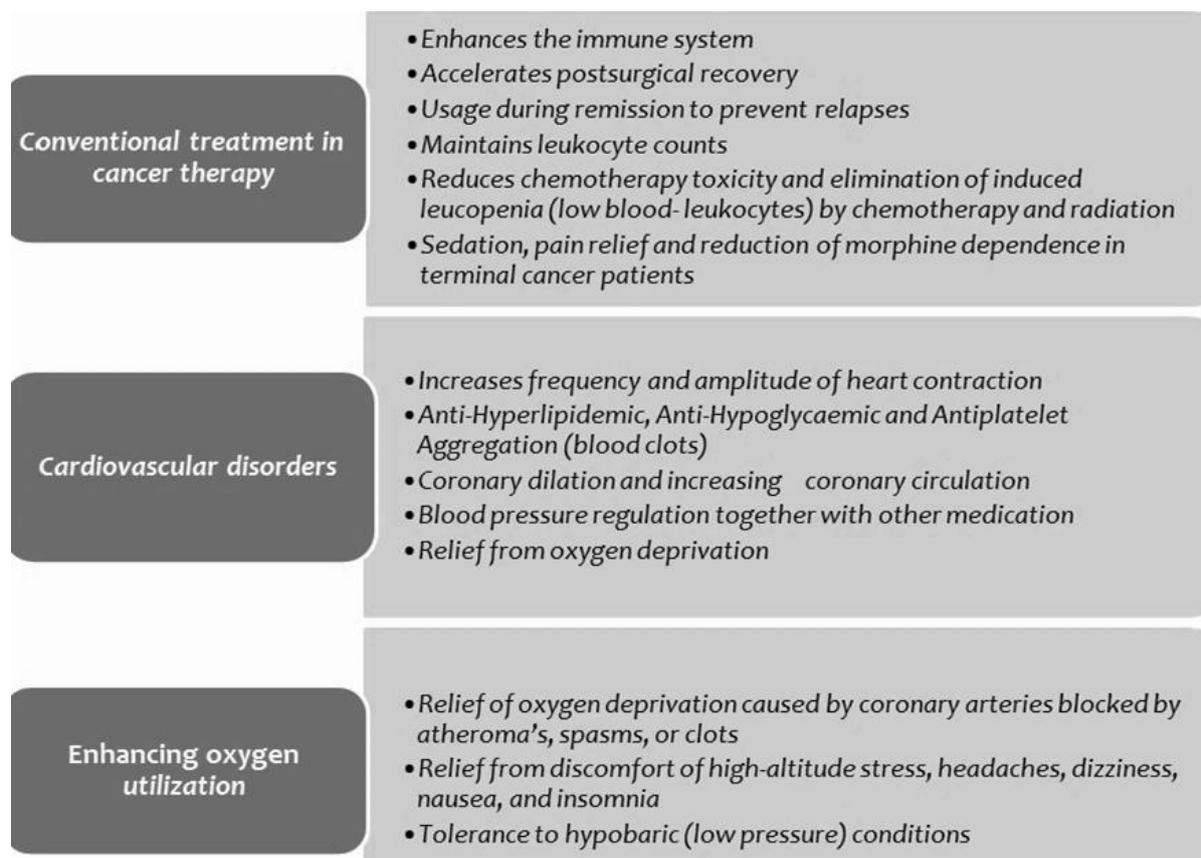


Fig. 3. Current biological/biomedical applications of *G. lucidum*

Polysaccharides of *G. lucidum* have been used for a broad spectrum of health benefits from preventative measures and maintenance of health to the regulation or treatment of chronic as well as acute life-threatening illness. Nowadays more research is focussed on bioactive molecules from *G. lucidum* including polysaccharide as a chemotherapeutic agent to treat cancer (Lawal et al., 2019). In China, clinical trials on the approved drug are undergoing on *G. lucidum* polysaccharides to treat myopathy and other diseases (Zeng et al., 2017). Some of the significant biological/ biomedical applications of this mushroom were given in table 3.

Table 2. Biologically active components in *Ganoderma lucidum*.

Compound	Function/Outcome	Reference
(1→3)-β-D-glucans	Inhibition of growth of sarcoma S 180 tumour in mice	Sone et al., 1985
PS-G, protein-bound polysaccharides (95% polysaccharides and 5% peptides)	Activation of the immune response, stimulation of the IL-1β, IL-6, TNF-α, and IFN-γ production by macrophages & T lymphocytes, inhibition of neutrophil apoptosis, induction of neutrophil phagocytosis	Wang et al., 1997 Hsu et al., 2002 Hsu et al., 2003 Kim et al., 1999
G009, amino polysaccharides	Antioxidants	Lee et al., 2001
Glycoproteins (with fucose)	Stimulation of IL-1, IL-2 and IFN-γ expression in spleen cells	Wang et al., 2002
GLIS, proteoglycans	Activation of b-lymphocytes	Zhang et al., 2002
Cerebrosides	Inhibition of DNA-polymerase	Mizushina et al., 1998
Ganoderic acid (U, V, W, X, Y)	Cytotoxic for hepatoma cells	Shiao et al., 1994
Ganoderic acid (A, C)	Inhibition of farnesyl protein transferase	Toth et al., 1983
Lucidimol (A, B), Ganodermanondiol, Ganoderiol F, Ganodermanontriol	Cytotoxic for sarcoma and lung carcinoma cells	Min et al., 1998 El-Mekkawy et al., 1998 Min et al., 2000
Ganoderic acid F	Inhibition of angiogenesis	Kimura et al., 2002
Phenols	Antioxidant	Mau et al., 2002
Lipids	Growth inhibition of hepatoma, sarcoma S-180 and reticulocyte sarcoma L-II in vivo	Liu et al., 2002

Table 3. Glucidum polysaccharides: significant biological applications.

Significant Biological Applications of Polysaccharides from <i>G. lucidum</i>	Reference
Immunomodulation and potential anti-tumour activities	Zeng et al., 2011
A novel polysaccharide from Se-enriched <i>G. lucidum</i> induces apoptosis of human breast cancer cells	Shang et al., 2011
Effect of <i>Reishi</i> polysaccharides on human stem cells/progenitor cells	Chen et al., 2010
<i>Ganoderma lucidum</i> polysaccharides attenuate endotoxin-induced intercellular cell adhesion molecule-1 expression in cultured smooth muscle cells and the neointima in mice	Lin et al., 2010
Ling-Zhi polysaccharides potentiate cytotoxic effects of anticancer drugs against drug-resistant urothelial carcinoma	Huang et al., 2010
Immunomodulatory and adjuvant activities of a polysaccharide extract of <i>G. lucidum</i> in vivo and in vitro	Lai et al., 2010
The in vitro and in vivo experimental evidence disclose the chemopreventive effects of <i>Ganoderma lucidum</i> on cancer invasion and metastasis	Chia and Gow, 2010
<i>Ganoderma lucidum</i> induced apoptosis in NB4 human leukaemia cells	Eva et al., 2010
The effects of <i>Ganoderma</i> alcohols isolated from <i>Ganoderma lucidum</i> on the androgen receptor binding and the growth of LNCaP cells	Jie et al., 2010
Ganoderic acid T inhibits tumour invasion in vitro and in vivo through inhibition of MMP expression	Nian et al., 2010
<i>Ganoderma lucidum</i> (Fr.) P. Karst enhances activities of heart mitochondrial enzymes and respiratory chain complexes in the aged rat	Sudheesh et al., 2009
An immunomodulatory protein, Ling Zhi-8, induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappa B and MAPK pathways	Lin et al., 2009
Inhibitory effects of <i>G. lucidum</i> on tumorigenesis and metastasis of human hepatoma cells in animal models	Weng et al., 2009
<i>G. lucidum</i> induces the expression of CD40/CD86 on peripheral blood monocytes	Kazem and Majid, 2009
Effect of <i>G. lucidum</i> on the activities of mitochondrial dehydrogenases and complex I and II of electron transport chain in the brain of aged rats	Ajith et al., 2009
The signalling cascades of <i>G. lucidum</i> extract in stimulating non-amyloidogenic protein secretion in human neuroblastoma SH-SY5Y cell lines	Pinweha et al., 2008
Possible involvement of long-chain fatty acids in the spores of <i>G. lucidum</i> to its antitumor activity	Fukuzawa et al., 2008
Effect of <i>G. lucidum</i> capsules on T-lymphocyte subsets in football players on "living high-training low"	Zhang et al., 2008

Effects of <i>G. lucidum</i> spores on HepG2 cells proliferation and growth cycle	Li et al., 2008
A randomized clinical trial of an ethanol extract of <i>G. lucidum</i> in men with lower urinary tract symptoms	Noguchi et al., 2008b
Serum amyloid A mediates the inhibitory effect of <i>G. lucidum</i> polysaccharides on tumour cell adhesion to endothelial cells	Li et al., 2008
The dual roles of Ganoderma antioxidants on urothelial cell DNA under carcinogenic attack	Yuen and Gohel, 2008
Polysaccharides can induce human monocytic leukaemia cells into dendritic cells with immuno-stimulatory function	Chan et al., 2008
Effect of an extract of <i>G. lucidum</i> in men with lower urinary tract symptoms: a double-blind, placebo-controlled randomized and dose-ranging study	Noguchi et al., 2008a
Telomerase-associated apoptotic events by mushroom <i>G. lucidum</i> on premalignant human urothelial cells	Yuen et al., 2008
<i>G. lucidum</i> polysaccharides in human monocytic leukaemia cells: from gene expression to network	Kun et al., 2007
Herbal mixtures of <i>G. lucidum</i> improve recovery time in patients with herpes genitalis and labialis	Hijikata et al., 2007
Androgen receptor-dependent and -independent mechanisms mediate <i>G. lucidum</i> activities in LNCaP prostate cancer	Zaidman et al., 2007
<i>G. lucidum</i> polysaccharides enhance CD14 endocytosis of LPS and promote TLR4 signal transduction of cytokine expression	Hua et al., 2007
Novel polysaccharide preparation (GLPP) from Anhui-grown <i>G. lucidum</i> in tumour treatment and immune-stimulation	Pang et al., 2007
<i>G. lucidum</i> polysaccharide peptide reduced the production of pro-inflammatory cytokines in activated rheumatoid synovial fibroblast	Ho et al., 2007
Inhibition of oxidative stress-induced invasiveness of cancer cells by <i>G. lucidum</i> is mediated through the suppression of interleukin-8 secretion	Thyagarajan et al., 2006
Antitumor activity of extracts of <i>G. lucidum</i> and their protective effects on damaged HL-7702 cells induced by radiotherapy and chemotherapy	Wang and Weng, 2006
<i>Reishi</i> polysaccharides induce immunoglobulin production through the TLR4/TLR2-mediated induction of transcription factor blimp-1	Lin et al., 2006
Polysaccharide purified from <i>G. lucidum</i> induces gene expression changes in human dendritic cells and promotes T helper 1 immune response in BALB/c mice	Li et al., 2006
<i>G. lucidum</i> extract inhibits proliferation of SW 480 human colorectal cancer cells.	Xie et al., 2006
<i>G. lucidum</i> extract stimulates glucose uptake in L6 rat skeletal muscle cells	Jung et al., 2006
Effects of water-soluble <i>G. lucidum</i> polysaccharides on the immune functions of patients with lung cancer	Gao et al., 2005

CONCLUSION

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G. lucidum mushroom is consumed commercially all over the world, because of its unique taste and curative properties. Because of the presence of numerous bioactive compounds, this mushroom is a popular herb as it contains a good amount of polysaccharides, which can be extracted by the ethanol-water solution. These polysaccharide molecules when absorbed into the human blood circulatory system, stimulates the immune modulators by activating the cellular and humoral components and increased production of macrophages. Based on the particle size and extraction time, the most common approach of hot water extraction and ultrasound microwave-assisted extraction, of water-soluble polysaccharides, is feasible economically. In addition, the alkaline extraction of polysaccharides of water-insoluble polysaccharides resulted in good extraction yield, as the alkaline treatment easily breaks down the dietary fibre of *Reishi* mushroom and speed up the release of polysaccharide extraction. The obtained polysaccharide extract is further purified by gel filtration and affinity chromatography technique that can be useful in scientific studies. The fractionation of polysaccharides using new methodologies utilizing conformational properties was discussed that will open avenues for functional foods and herbal drugs. The biological preclinical studies displaying the multiple health potentials of *G. lucidum* polysaccharides as antitumor, anti-inflammatory, antiviral, anticancer activities etc. were reviewed for future directions.

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