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Review

Ganoderma – A therapeutic fungal biofactory

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Abstract

Ganoderma is a basidiomycete white rot fungus which has been used for medicinal purposes for centuries particularly in China, Japan and Korea. A great deal of work has been carried out on *Ganoderma lucidum*. The common names for preparations include Lingzhi, Munnertake, Sachitake, Reishi and Youngzhi. This review collates the publications detailing activities and compounds by representative species whilst considering the most valid claims of effectiveness. The biological activities reported of preparations from *Ganoderma* are remarkable and given most emphasis herein as distinct from structure/activity information. The metabolites consist of mainly polysaccharides and terpenoids. Many are activities against the major diseases of our time and so the present review is of great importance. The list of effects is huge ranging from anti-cancer to relieving blockages of the bladder. However, the reports have not all been tested scientifically with the convincing evidence is reserved for assays of pure compounds. It is a prime example of an ancient remedy being of great relevance to the modern era. There does appear to be an assumption that the therapeutic effects attributed to the fungus have been proven. The next step is to produce some effective medicines which may be hampered by problems of mass production.

Keywords: *Ganoderma*; Polyporaceae; Lingzhi; Terpenoids; Steroids; Polysaccharides; Cancer; HIV

Contents

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1. Introduction

Ganoderma is a prolific producer of novel "mycochemicals". The quantity of information on the fungus in this respect is quite remarkable; hence this is an extensive review. However, there is an uneasy combination of the mythical, pseudo-science and science and deciding one from the other requires experience. The organism is a white rot which is involved in the fundamental process of lignocellulose degradation in Nature, although this aspect will not be considered. An image of a *Ganoderma* specimen is provided in Fig. 1 and appears to be *G. applanatum* from the morphology alone. The Chinese, inter alia, have used preparations as medicines from a similar or identical fungus, for millennia (cited as early as 100 BC) and have given the organism the name "Lingzhi". In Japan the names include Munnertake, Sachitake and Reishi, whereas in Korea it has the name Youngzhi. However, whether this and any particular species of *Ganoderma* represent the same entity and throughout all periods in history is questionable. The taxonomic situation within *Ganoderma* is unclear as the species and genus concepts are confused. For example, similar fungi are found in *Fomes*, *Polyporus* and *Tomophagus*.

"Lingzhi" is the Chinese name given to the basiocarps (my preferred name) of *Ganoderma* as isolated in that vast and diverse country. However, there is a tendency for authors in the scientific literature to refer to such names making definitive conclusions more difficult (see Huie and Di, 2004). The preparation was and is considered to have healing properties to the body and mind: "Lingzhi" is still revered by some. The preparation does indeed contain certain bioactive ingredients (such as triterpenes and polysaccharides) that might be beneficial for the prevention and treatment of a variety of ailments. Remarkably, these include such very important diseases such as hypertension, diabetes, hepatitis, cancers, and AIDS. These types of biomedical investigations have been conducted predominately in China, Korea, Japan and the United States of America and so are extensive. It is unclear why other countries are not more involved.

Fig. 1. A typical *Ganoderma* basidiocarp.

Research, quality assurance and control of "Lingzhi" products depend on the isolation/purification of active ingredients and analytical/preparative separations (Huie and Di, 2004). A practical classification of the fungus is required to understand more clearly what the fungus represents. In addition, research is necessary on the physiology of production of the active metabolites to enable yields to be optimised in solid or liquid substrate bioreactors – assuming they can be so produced.

Preparations referred to as "Lingzhi" were/are used widely as a health supplement and "herbal" medicine worldwide. Of course, the fungus is not a herb (which are plants) and calling it such simply adds to the confusions surrounding names. A company in the USA has a web site with numerous *Ganoderma* products which makes extraordinary claims for medicinal properties (http://www.fungi.-com/). This raises issues of accreditation and validity. However, strapped-for-cash culture collections will look enviously at the commercial potential. Employing my computer search engine gave numerous "hits" from organisations selling such preparations which must surely be of concern to those in search of valid data.

As indicated previously, there are issues that require to be addressed in relation to the fungus: What is *Ganoderma*? How does it relate to *Fomes*, *Polyporus*, and *Tomophagus*? What constitute the preparations with non-Latinised names that are often referred to (e.g. Lingzhi, Songshan lingzhi, Reishi)? After all, these are the ancient medicines on which all the new research and interest are based. Have voucher specimens of *Ganoderma* been placed in culture collections with international accreditation, and are they available to others (see Paterson, 2005)? How useful is the information in obscure journals? Using only one sample for species recognition as has occurred (e.g. Di et al., 2003) and is invalid as assessments of variation cannot be obtained.

For example, a *G. lucidum* has been worked on extensively and yet was obtained from the family-owned company mentioned previously (see Ziegenbein et al., 2006). What degree of scientific accreditation applies to this private enterprise? The same questions can be asked of other culture collections, whether public or private, where high quality accessions, preservation and identifications may not apply: A culture collection in China will not necessarily have the equivalent standards to one in the USA.

"Lingzhi" has been examined predominantly for bioactive triterpenes and polysaccharides. It is perhaps surprising that other classes of intrinsically secondary metabolites have not been found. For example, polyketides and shikimic acid pathway metabolites are not well represented. A point to consider is that the fungus may transform compounds from the host on which it was isolated and not be able to produce the compounds per se. This may be true particularly of lignin-derived compounds as the white rot fungi degrade lignin to monomeric phenols and further to CO_2 and H_2O for energy (Leonowicz et al., 1999) which will have ramifications for mass production of treatments.

The use of the term ''Lingzhi'' needs to be avoided in scientific papers (cf. Huie and Di, 2004) as it is imprecise and the Latin names should prevail. I will tend to use inverted commas when referring to it as a compromise. ''Lingzhi'' has been used for the treatment of migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, diabetes, hypercholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis, and cardiovascular problems. One can only wonder how the treatments were assessed as being effective. It is claimed to possess (a) anti-cancer (including leukaemia), (b) anti-ageing and (c) anti-microbial/viral activities including anti-human immunodeficiency virus (HIV). Additionally, the presence of neuroactive compounds in the extracts of ''Lingzhi'' that mediated the neuronal differentiation and neuroprotection of rat PC12 cells has also been demonstrated recently (Huie and Di, 2004). This all seems like very good news indeed as even one of these effects would be remarkable if some new prescribed medicines were produced. Extraordinary claims such as these needs to have equivalent evidence to prove that they are factual.

Furthermore, *Ganoderma* is not a herb as mentioned previously: It is a complex fungus which has had very significant interactions with human beings over the centuries. The label ''herb'' needs to be avoided. Fungi form a separate kingdom from plants and are closer to animals in an evolutionary sense. These definitions are important. For example, *Ganoderma* is not likely to contain lignin but this is what is implied in Huie and Di (2004) in an interpretation of how an enzyme-based extraction procedure is effective. The enzyme was claimed to break bonds between cellulose and lignin hence permitting the extraction of the desired material. And so the rational for the extraction procedure with enzymes was wrong from the initial assumption. The following review will consider how the fungus became to be known as an ancient remedy for ailments and what the latest developments have been. Extensive references are provided because of the huge claims beings made of the biomedical activities of *Ganoderma*.

2. "Herbal" medicinal *Ganoderma*

Sliva (2006) and Stanley et al. (2005) mention that *G. lucidum* is a popular medicinal mushroom, and has been used in traditional Chinese medicine (TCM) in Asian countries over the past two millennia. The regular consumption was/is *believed* to preserve human vitality and to promote longevity. The fungus has been used for the prevention, or treatment, of numerous diseases including cancer although whether it was effective is presumably unknown. Western medicine began to study natural products from TCM and the popularity of herbal therapies for the treatment of cancer has increased in the USA. Of course, *Ganoderma* is a fungus and not a herb. Scientific elucidation of mechanisms responsible for the biological effects of these natural products would indicate if they were valid alternatives, or adjuvant cancer therapies; as would well-conducted therapeutic trials.

Furthermore, Chen et al. (2006) in an extensive paper mention that many ''herbal'' medicines are used widely as immuno-modulators in Asian countries. *G. lucidum* is one of the most used ''herbs'' in Asia and preclinical studies have established that the polysaccharide fractions have potent effects. Also, one wonders what ''established'' means here – is it proven? As mentioned, clinical evidence for this is scarce. Pero et al. (2005) state that, the combination of nutritional supplements to achieve synergistic benefit is a common practice in the nutraceutical industry. However, establishing added health benefit from a combination of natural ingredients is often assumed, untested and without regard to the principle of metabolic competition between the active components (see Aydemir, 2002).

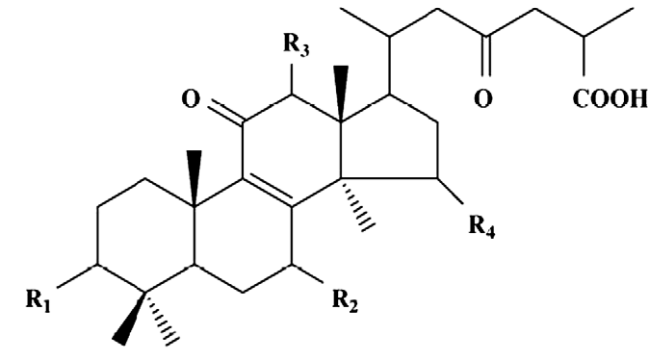
The dried powder is currently used worldwide in the form of dietary supplements. Stanley et al. (2005) have demonstrated that *G. lucidum* induces apoptosis, inhibits cell proliferation, and suppresses cell migration of highly invasive human prostate cancer cells PC-3. However, the molecular mechanism(s) responsible for the inhibitory effects have not been fully elucidated. Hajjaj et al. (2005) state that *G. lucidum* is a medicinal fungus belonging to the Polyporaceae family and is know in Japan as Reishi. Furthermore, Müller et al. (2006) emphasise that over many centuries, ''herbal'' remedies have treated a variety of ailments and the empirical/observational approach has produced a number of leads for formulated medicines. Lin et al. (2006) refer to *G. tsugae* as the Chinese mushroom Songshan lingzhi, which is cultivated in Taiwan and used extensively to treat diseases. Johnston (2005) alludes to the fact that *G. lucidum* is used in TCM for the treatment of cancers. In truth, the fungus must be one of the most convincing examples of ancient folk remedies being translated into new drug leads, and may represent an excellent paradigm for the general principal. Screening methods are very useful for assessing the constituents of preparations before the isolation of compounds and this is next presented.

3. Metabolite profiling

Differentiation was possible of ''Lingzhi'' from several other fungal crude drugs, such as hoelen, omphalia and polyporus using TLC (Huie and Di, 2004). Ganoderic acids B and C (see Fig. 1 for ganoderic acids) are unique constituents of ''Lingzhi''. Incidentally, profiles in Ofodile et al. (2005) indicated interesting differences between species, in addition to the activities reported. TLC was applied for the differentiation of species and unique triterpene patterns were reported (Huie and Di, 2004). Samples could be grouped into 18, which were similar clusters to those by HPLC. Similarly, distinctive TLC patterns of triterpenes were obtained from basidiocarps of *G. lucidum* and *G. tsugae*. A rapid agar

plug method of cultures and solvent extraction of basidiocarps of *Ganoderma* from oil palm were described in Miller et al. (1995). Some of the metabolites were analysed by mass spectrometry and did not appear to be lanostanoid compounds. Di et al. (2003) used high-performance TLC for the profiling of carbohydrates. This was the first time this approach had been used. Unique patterns were observed between *G. applanatum* and *G. lucidum*. The analyses of more strains and taxa are required generally (see Figs. 2 and 3).

Triterpenoid profiling for the chemotaxonomy of *G. lucidum* and related species has been demonstrated by HPLC. Also, the determination and comparison of production yields was obtained (Huie and Di, 2004). Samples could be divided into 18 groups based on characteristics of the HPLC pattern of triterpenoids, in good agreement with morphological examination (and TLC). Rapid and efficient characterization of ''Lingzhi'' was obtained. In addition,



Ganoderic acid C_2 (1): R_1=R_2=β-OH, R_3=H, R_4=α-OH
Ganoderic acid B (2): R_1=R_2=β-OH, R_3=H, R_4=O
Ganoderic acid AM_1 (3): R_1=β-OH, R_2=R_4=O, R_3=H

Fig. 2. Chemical structure of some ganoderic acids.

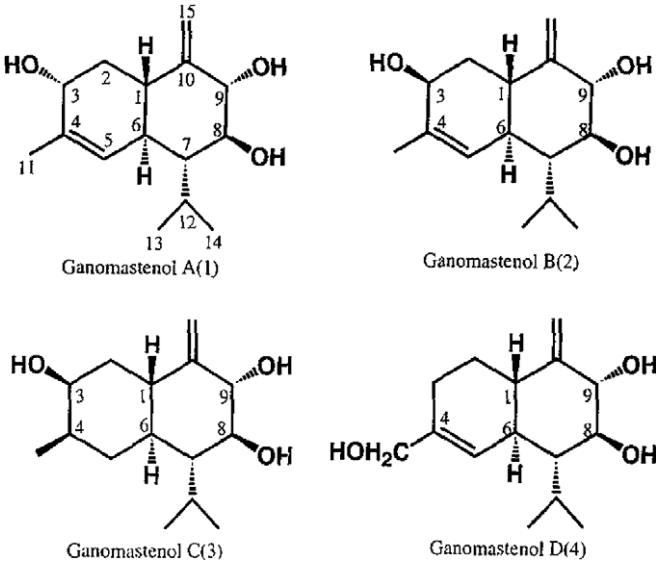


Fig. 3. Chemical structure of some ganomastenols.

strains of *G. tsugae* and *G. lucidum* were analysed for triterpenoid of their basidiocarps. An HPLC procedure was employed for the isolation of oxygenated triterpenoids from crude extracts which has chemotaxonomic applications (Lin and Shiao, 1987). Strain specific profiles were obtained for *G. lucidum* (Nishitoba et al., 1986), and similar patterns were observed by Hirotani et al. (1993a). Chen et al. (1999) deduced some strains were similar and yet others different. The profiles provide useful chemotaxonomic data and activity/quality information.

Fourier transform infrared absorption and HPLC for the classification/identification of commercial *G. lucidum* and *G. tsugae* products into three different types (cultured mycelia, fruiting bodies and mixed mycelia/fruiting bodies) are also reported. Other workers have isolated nine triterpenoids from the basidiocarps of *G. tsugae*: ganoderic acids E, C5, C6, G and ganoderenic acid D (see Table 1) were analysed for the first time by HPLC. A method for determining ganoderic acid B in different parts of *G. lucidum* has been validated. The pilei (caps) were found to be the optimal source of ganoderic acid B, followed by the stipes (stems), and the spores were the poorest source. Similarly, the determination of ganoderic acid A, ganoderic acid C, ganoderic acid D and ganoderic acid E in cultured and wild *G. lucidum* was achieved, and this method was claimed to be highly suitable for the quality control of ''herbal'' formulations containing ''Lingzhi'' (Huie and Di, 2004). A validated method was reported for the simultaneous analysis of ganosporeric acid A, lucidenic acid A, ganoderic acids B and C from *G. lucidum*. Interestingly, it was found that the contents of four triterpenes in the fruiting bodies were higher than that in the spores, and the triterpene contents in the spores with a sporoderm-breaking rate of 85% were found to be higher than those obtained from spores having complete sporoderms. Another method was described for the quantitative analysis of the ganoderic alcohols and acids in the spores and five basidiocarps of *G. lucidum* collected from different countries. Six ganoderic alcohols (lucidumols A and B, ganoderiols A and F, ganodermanontriol A, ganodermanondiol) were well separated. Seven ganoderic acids also were well separated except for ganoderic acids C1 and H on the same column using 2% ethanol–acetonitrile (7:3) as the mobile phase. The results indicated that the total triterpene content of the spores was 5–20 times higher than that of the fruiting bodies (Huie and Di, 2004). In contrast, other researchers have shown that the spores were the poorest source of triterpenes. Miller et al. (1995) describe an HPLC method using gradient solvent elution and UV detection which was used for *Ganoderma* from oil palm, and it is desirable that the bioactivities of oil palm isolates are determined.

Wang et al. (2006) analysed various *Ganoderma* species for triterpenoids and the qualitative profiles of the species indicate utility for separating the taxa. However, both *G. lucidum* and *G. sinense* are the official species of Lingzhi recorded in the 2005 Chinese Pharmacopoeia and they were considered to have the same therapeutic effects. The

Table 1
A composite table comparing some polysaccharides and terpenoids/steroids from *Ganoderma* spp. reported in the journal *Phytochemistry* from the 1970s to present

Compounds	*Ganoderma* species									
Polysaccharides	*lucidum*	*applanatum*	*lipsiense*	*carnosum*	*sugae*	*mastoporum*	*neo-japonicum*	*amboinense*	Indonesian sp.	*australe*
Heteroglucan										
PL-1	+									
PL-2	+									
Homoglucan										
PL-3	+									
Terpenoids and steroids										
(24*S*)-24-methylcholesta-7,16-dien-3β-ol		+								
(24ξ)-24-methyl-5α-cholest-7-en-3β-ol		+								
(24*S*)-24-methyl-5α-cholest-7,16-dene-3β-ol		+								
24-Methylcholesta-7,22-dien-3β-ol	+	+								
24-Methylcholesta-5,7,22-trien-3β-ol ergosterol	+	+								
24-Methylcholest-7-en-3β-ol	+	+								
(24*S*)-24-methyl-5α-cholest-7-ene-3β ol (Ia)		+								
Sesquiterpene (−)-7-hydroxycalamenene		+								
Friedelin (terpene)		+								
Alnusenone		+								
Friedoolean-5-en-3one		+								
Ergosta-5,7,22-triene-3β-ol		+								
Ergosterol	+	+	+							+
Ergosta-7,22-diene-3-one		+								
Ergosterol peroxide[1]		+								
Ergosterol peroxide	+			+						
Ergosta-7,22-diene-3β-yl palmitate	+									
Ergosta-7,22-diene-3β-yl linoleate	+									
Ergosta-7,22-diene-3β-yl pentadecanoate	+									
Ergosta-7-diene-3β-yl linoleate	+									
Ergosta-7,22-dien-3β-ol			+	+	+					
Ergosta-7,22-dien-3-one			+							
Steroids: ergosta-7,22-dien-3β-ol, ergosta-7,22-dien-3β-yl linoleate								+		
Steroid: ergosta-7,22-dien-3β-yl palmitate								+		
Steroid: 2β,3α,9α-trihydroxyergosta-7,22-diene								+		
Steroid: 5α,8α-epidioxyergosta-6,9(11),22-trien-3β-ol								+		
Triterpenoid esters										
Ganoderic acid A, B, C1, H	+									
Ganoderic acid N?			+							
Ganoderic acid methyl ester	+		+							
Ganoderic derivatives	Sekishi/Shishi strains +, Kokushi −									
Ganoderic acid V_1: (24*E*)-3β,20ξ-dihydroxy-7,11,15-trioxo-5α-lanosta-8,24-dien-26-oic acid	+ Shishi strain									
Ganoderic acid V_1: (24*E*)-3β,20ξ-dihydroxy-7,11,15-trioxo-5α-lanosta-8,24-dien-26-oic acid	+ Shishi strain									
Triterpenoids Ten ganoderic acid, ganoderenic acid and lucidenic acid derivatives	+									

(*continued on next page*)

Table 1 (*continued*)

Compounds	*Ganoderma* species									
Polysaccharides	*lucidum*	*applanatum*	*lipsiense*	*carnosum*	*sugae*	*mastoporum*	*neo-japonicum*	*amboinense*	Indonesian sp.	*australe*
Triterpenoids, ganoderic acid T, ganoderic acid S, ganoderic acid R, ganoderic acid P, ganoderic acid Q, ganoderic acid 0	+ mycelium									
7-*O*-methyl-ganoderic acid 0	+ mycelium									
Ganoderenic acid A			+							
Ganoderenic acid D	+		+							
Three linoleic acid steryl esters **3**–**5**			+							
Ganoderiols A, B, F	+									
Ganodermanontriol	+									
Cerevisterol	+									
3β-5α-dihydroxy-6β-methoxyergosta-7,22-diene	+									
Lanostane-type triterpene										
26,27-Dihydroxylanosta-7,9(11),24-trien-3,16-dione				+						
Oleic acid methyl ester				+						
Glycerol trioleate				+						
Known lanostanoids										
3β-hydroxy-5α-lanosta-8,24-dien-21-oic acid					+					
3-Oxo-5α-lanosta-8,24-dien-21-oic acid					+					
New lanostanoids										
3α-acetoxy-5α-lanosta-8,24-dien-21-oic acid (tsugaric acid A)					+					
3α-acetoxy-16α-hydroxy-24ξ-methyl-5α-lanosta-8,25-dien-21-oic acid (tsugaric acid B)					+					
Cadinene sesquiterpenes										
Ganomastenols A–D cultured mycelia and broth						+				
Polyoxygenated lanostanoid triterpenes										
Applanoxidic acids E–H		+ (trop. Indonesia)								
Drimane-type sesquiterpenoids: cryptoporic acid H, I							+			
5′-deoxy-5′-methylsulphinyladenosine (both epimers)	+									
Oxygenated triterpenes: lanosta-7,9(11),24-trien-3α,15α-dihydroxy-26-oic acid	+ mycelum									
Polyhydroxyl steroid: 2β-methoxyl-3α,9α-dihydroxyergosta-7,22-diene								+		
Lanostanoid: ganoderic acid AM_1, 3β-hydroxy-7,11,15,23-tetraoxo-lanosta-8-en-26-oic acid								+		
Lucidone A								+		
Two homolanostanoid triterpene hydroxyacids									+	
Carboxyacetylquercinic acid									+	
Lanosta-7,9(11),24-trien-3β,15α-dihydroxy-26-oic-acid	+ mycelium									
Lanosta-7,9(11),24-trien-3β,22β-diacetoxy-15α-hydroxy-26-oic acid	+ mycelium									
Lanosta-7,9(11),24-trien-15α,22β-diacetoxy-3β-hydroxy-26-oic acid	+ mycelium									
Five new lanostanoid triterpenes	+									
Ergosterol derivative: ergosta-4,7,22-triene-3,6-dione	+ mycelium									
3β,7β,15α-trihydroxy-11,23-dioxo-5α-lanost-8-en-26-oic acid (ganoderic acid E)	+									

3,7,11,15,23-Pentaoxo-5α-lanost-8-en-26-oic acid (ganoderic acid F)	+		
Peptidoglycans: ganoderans B and C	+		
Lanostane-type triterpenoid ganoderic acid C	+		
Ergosterol palmitate			+
Ergosta-7,22-dien-3-one			+
Lanosta-7,9(11),24-trien-3β,21-diol			+
Free sterols	+	+	
Ergosta-7,22-dien-3β-ol		+	
Ergosta-7,22-dien-3-on		+	

+ = detected (Strigina et al., 1971; Domínguez et al., 1972; Ripperger and Budzikiewicz, 1975; Jain and Gupta, 1984; Kac et al., 1984; Tomoda et al., 1986; Nishitoba et al., 1987; Shiao et al., 1988; Chairul et al., 1991; Hirotani et al., 1991, 1995; Lin et al., 1993; Kawagishi et al., 1993; Sofni et al., 1994; Kawagishi et al., 1997; Keller et al., 1997; Lin et al., 1997; El-Mekkawy et al., 1998; Rösecke and König, 2000; Bao et al., 2002; Ziegenbein et al., 2006).

average content of total triterpenoids in *G. lucidum* was 10 times higher than that in *G. sinense*, which indicated the therapeutic effects of these two species might be quite different. Therefore, they are recommended to be used as two different "herbal" medicines. Other obvious conclusions would be that the compounds are not responsible for the effects, or that the effects have not been scientifically proven. This represents a common problem with some of the data and interpretations reviewed herein. In general, there appears to be an assumption that the therapeutic effects attributed to the fungus have been proven.

Polysaccharides of "Lingzhi" and "related species" have been reported to be composed of a variety of monosaccharides, including glucose, galactose, mannose, arabinose, xylose, fucose, rhamnose, glucuronic acid and galacuronic acid and could be separated by paper chromatography (Huie and Di, 2004). The use of HPLC (or TLC) for carbohydrates is reported rarely. High performance size exclusion chromatography has been used for the quality control of "Siwei Lingzhi". However, the chemicals from *Ganoderma* have been described and this is considered in the following section.

4. Chemical constituents

Polysaccharides and triterpenes have been most thoroughly investigated from *G. lucidum* and related species. However, sterols, lectins and proteins have also been described. A list of the components which have been discussed in the journal *Phytochemistry* is provided in Table 1. This does not represent a chemotaxonomic treatment as certain taxa have been analysed much more than others. Nevertheless, some species produce the same compounds and strain variation is revealed.

4.1. Triterpenes/triterpenoids

Cole et al. (2003) list four sesqueterpene compounds from *G. mastoporum*: ganomastenol, ganomastenol B, ganomastenol C, and ganomastenol D (see Table 1). Cole and Schweikert (2003) include ergosterol; (24*S*)24-methyl-5α-cholesta-7,16-dien-3β-ol; (24*S*)24-methylcholesta-7,22-dien-3-one (Ergosta-7,22-dien-3-one) as C_{28} sterols from *G. applanatum*. However, the names are also "equal to" four other *Fomes* species indicating a general systematic problem. An equivalent situation exists for the C_{30} sterols friedelin and alnusenone (friedoolean-5-en-3-one): These are listed as being from *G. applanatum*. Officinalic acid and senexdiolic acid (also C_{30}s) are listed from *F. officinalis* and *F. senex*, respectively. From *G. lucidum* only are presented (a) 40 ganoderic acids, (b) 14 ganoderiols, (c) 5 ganolucidic acids, and (d) 15 lucidenic acids. It would be interesting to determine which other species (or genera) produce these compounds. Understandably, the authors put a disclaimer about the taxonomic veracity of the names they use in the volumes.

Furthermore, Akihisa et al. (2005) describe (a) two triterpenoids: 20(21)-dehydrolucidenic acid A and methyl 20(21)-dehydrolucidenate A, and (b) five new 20-hydroxylucidenic acids: 20-hydroxylucidenic acid D_2, 20-hydroxylucidenic acid F, 20-hydroxylucidenic acid E_2, 20-hydroxylucidenic acid N, and 20-hydroxylucidenic acid P isolated from the basidiocarps of *G. ludicum*. Shim et al. (2004) isolated four new lanostane-type triterpenes from the methanol extract of the fruiting bodies of *G. applanatum*. These are: (a) 3β,7β,20,23ξ-tetrahydroxy-11,15-dioxolanosta-8-en-26-oic acid, (b) 7β,20,23ξ-trihydroxy-3,11,15-trioxolanosta-8-en-26-oic acid, (c) 7β,23ξ-dihydroxy-3,11,15-trioxolanosta-8,20*E*(22)-dien-26-oic acid, and (d) 7β-hydroxy-3,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester. The authors suggest that more of these classes of compounds will be detected in future studies. The free sterols of *G. applanatum* and *G. lucidum* has been determined to contain mainly (ergosterol) and 24-methylcholesta-7,22-trien-3-ol. Also, the first isolation of 8,9-epoxyergosta-5,22-dien-3,15-diol from *G. lucidum* was reported (Huie and Di, 2004). So which biomedical activities have been described?

5. Whole fungus/crude extracts bioactivities

Ganoderma have been investigated as anti-tumour and antiviral agents and less so as anti-bacterial agents (Gao et al., 2003). Anti-bacterial activity has been observed against Gram-positive bacteria from the basidiocarp extracts of *G. lucidum* (Kim et al., 1993) and *G. orgonense* (Brian, 1951). Furthermore, Sudirman and Mujiyati (1997) observed that seven Indonesian *Ganoderma* species inhibited the growth of *Bacillus subtilis*. Yoon et al. (1994) investigated the additive effect on the activity of an aqueous extract of *G. lucidum* with four known antibiotics and observed that the anti-bacterial activity increased. There are relatively few studies on extracts from the liquid cultivated mycelium. Coletto and Mondino (1991) noted that methanolic extracts of the mycelia and culture extracts of *G. recinaceum* and *G. lucidum* inhibited *B. subtilis*. *G. recinaceum* also inhibited *Staphylococcus aureus*. Ethanolic extracts from *G. lucidum* mycelium demonstrated significant anti-inflammatory effects (Kendrick, 1985). Shieh et al. (2001) concluded that the hepatic and renal protective mechanism of *G. lucidum* might be because of its superoxide scavenging effect. In addition, Lin et al. (1995) observed that the greatest anti-hepatotoxic activity also exhibited the highest free radical scavenging properties. Low molecular weight aqueous fractions strongly inhibited multiplication of HIV-1 (Kim et al., 1997). The basidiocarps of *G. lucidum* lowered plasma cholesterol levels in rats (Kabir et al., 1988).

In terms of the very recent literature, it is noted from the company web site mentioned previously, that preparation from five *Ganoderma* "species" are, "*known* to fortify the immune system while exerting anti-inflammatory and anti-allergenic effects". These carry a footnote saying that the statements have not been evaluated by the US Food and Drug Administration. This is mentioned here as a blurring of scientific rigour which appears occasionally to be an issue when dealing with *Ganoderma*. We need to get away from this virtual-air-conditioned science and back to a situation where scientist actually collect and identify their own specimens (Korf, 2005).

To continue, the effects of *G. lucidum* on cancer cells was investigated (Sliva, 2006) and inhibition of proliferation and apoptosis was observed in leukemia, lymphoma, and myeloma cells. Moreover, the inhibition of acute myoloblastic leukemia cells was associated with cell cycle arrest and apoptosis, whereas the inhibition of lymphoma was mediated by the upregulation of expression. *G. lucidum* inhibits distinct signalling pathways in different cancer cells. Finally, standardized *G. lucidum* extracts containing 0.15% ganoderic acid C2 were used. However, it is uncertain if this amount of ganoderic acid could be responsible for the effect on haematopoietic cells. Finally, the composition and amount of biologically active triterpenes depend on the places of production, cultivation conditions, extraction procedures and the strains of *G. lucidum*.

Extracts of *G. lucidum* inhibited markedly intracellular signalling and invasive behaviour of cancer cells, whereas others did not. This complexity can also bring significant advantages. For example, certain components in the natural products can reduce the cytotoxicity of the whole product (and vice versa – my addition). Also, the interaction between different biologically active components can be responsible for their effects in vivo. Different compounds can modulate unrelated signalling and therefore, can possess synergistic effects (see Aydemir, 2002). Thus, triterpenes in *G. lucidum* suppress growth and invasive behaviour of cancer cells, whereas the polysaccharides stimulate the immune system resulting in the production of cytokines and activation of anti-cancer activities of immune cells (Sliva, 2006).

In summary, the organism inhibits (a) proliferation and invasive behaviour of breast and prostate cancer cells; (b) growth and induces apoptosis of breast and prostate cancer cells; (c) growth of hepatoma cells; and (d) secretion of vascular endothelial growth factor suppressing angiogenesis and transforming growth factor from prostate cancer cells. In addition, it induces apoptosis of colon cancer cells (Sliva, 2006). Stanley et al. (2005) demonstrated that *G. lucidum* induces apoptosis, inhibits cell proliferation, and suppresses cell migration of human prostate cancer cells. However, the molecular mechanism(s) has not been fully elucidated.

G. lucidum may have an immuno-modulating effect in patients with advanced colorectal cancer (Chen et al., 2006). Further studies are needed to explore the benefits and safety to cancer patients. In addition, there is further evidence of *G. lucidum* inhibiting prostate cancer cell proliferation (Johnston, 2005). Pero et al. (2005) report on a combination of mushroom extracts (*Cordyceps sinensis*,

Grifola blazei, *Gr. frondosa*, *Trametes versicolor* and *G. lucidum*) into a formulation designed to optimise different modes of immunostimulatory action, and yet that would avoid metabolic anti-oxidant competition. Less than expected efficacious effects were obtained. However, despite this, the data were taken as strong evidence that the combination gave additive or synergistic effects to health. Furthermore, an extract from *G. lucidum* was screened by Müller et al. (2006) for anti-proliferative activity using a human cancer cell line. The results indicated that *G. lucidum* had a profound activity against leukemia, lymphoma and multiple myeloma cells and may be a novel adjunctive therapy for the treatment of haematologic malignancies. Kuo et al. (2006) obtained supportive evidence for the effect of dried mycelium of *G. lucidum* on the enhancement of innate immune response. Lin et al. (2006) investigated the effects of two *G. tsugae* supplement products and the results suggest that supplementation diets *might* alleviate bronchoalveolar inflammation via decreasing the infiltration of inflammatory cells and the secretion of inflammatory mediators into the lungs and airways. Overall, the results indicated a therapeutic application for *G. tsugae* in allergic asthma.

To continue, a methanol extract of *G. lucidum* demonstrated the strongest 5α-reductase inhibitory activity among 19 edible and medicinal mushrooms (Noguchi et al., 2005). A clinical trial was conducted to evaluate the safety and efficacy of the extract in men with mild symptoms of bladder outlet obstruction and significant reductions in the problem were observed. The extract of *G. lucidum* was well tolerated. Fujita et al. (2005) investigated the inhibitory effects of methanol extracts of 19 edible and medicinal mushrooms on 5α-reductase activity (see also Noguchi et al., 2005). The extract of *G. lucidum* Fr. Krast showed the strongest inhibitory activity. The treatment of the fruit body of the fungus or the extract prepared from it inhibited significantly the testosterone-induced growth of the ventral prostate in castrated rats. These results showed that *G. lucidum* might be a useful ingredient for the treatment of benign prostatic hyperplasia. Presumable the extracts were similar to those reported by Mau et al. below.

G. tsugae was available in the form of mature and baby Ling chih (note different spelling and construction), mycelia and fermentation filtrate (Mau et al., 2005a). The term "baby" is of course unscientific. From methanolic extracts the mature and baby Ling chih demonstrated high anti-oxidant properties. Total phenols were the major naturally occurring anti-oxidant components found in all extracts and in the range of 24.0–35.6 mg g^{-1}. Therefore phenols may not be the active component as the mycelium and filtrate were inactive. In hot water extracts from the same high anti-oxidant activities were again obtained (Mau et al., 2005b). At 20 mg/ml, the scavenging abilities on hydroxyl radicals were in the descending order of Ling chih > baby Ling chih > mycelia > filtrate. Again total phenols were the major naturally occurring anti-oxidant components found in hot water extracts and in the range of 40.86–42.34 mg/g. From EC_{50} values obtained, fruit bodies of *G. tsugae* (Ling chih and baby Ling chih) were

Table 2
General compounds and effects of *Ganoderma* reported in the literature until 2004

Compound	Effect	Reference
Adenosine	Antiplatelet aggregation	Kawagishi et al. (1993), Shimizu et al. (1985)
Lectins	Mitogenic	Ngai and Ng (2004)
Polysaccharides	Antifibriotic	Park et al. (1997)
	Antiherpetic	Eo et al. (1999a,b, 2000), Kim et al. (2000), Oh et al. (2000)
	Anti-inflammatory	Ukai et al. (1983)
	Hepatoprotective	Zhang et al. (2002)
	Hypoglycaemic	Hikino et al. (1985, 1989), Hikino and Mizuno (1989), Tomoda et al. (1986), Zhang and Lin (2004)
	Immuno-modulatory – anti-tumour	Gao et al. (2000a,b), Li et al. (2000), Li and Zhang (2000), Ooi et al. (2002), Sasaki et al. (1971), Sone et al. (1985)
	Miscellaneous (radiation protection, DNA damage, anti-oxidant)	Kim and Kim (1999b), Lee et al. (2001)
Protein ("LZ-8")	Immunodulatory	van der Hem et al. (1995)
	Immunosuppressive	van der Hem et al. (1995)
Terpenoids and related compounds	Anti-bacterial	Smania et al. (1999)
	"Anti-complement"	Min et al. (2001)
	Anti-inflammatory	Kleinwächter et al. (2001)
	Anti-oxidant	Zhu et al. (1999)
	Antiplatelet aggregation	Shiao (1992)
	Antiviral	El-Mekkawy et al. (1998), Mothana et al. (2003)
	Cytotoxicity	Gao et al. (2002), Gonzalez et al. (2002), Kimura et al. (2002), Lin et al. (1991), Su et al. (2000), Wu et al. (2001)
	Enzyme inhibitors	Lee et al. (1998)
	Hepatoprotective	Chen and Yu (1999), Kim et al. (1999)
	Hypolipidemic (chloresterol inhibitors)	Komoda et al. (1989), Shiao (1992)
	Hypotensive	Morigiwa et al. (1986)

"good" in anti-oxidant properties, except for the chelating ability on ferrous ions. It is clear that the methanol and aqueous extracts were similar and one wonders why two papers were required to reveal this.

Lu et al. (2004) detected the chemo-preventive effects of *G. lucidum* using a unique in vitro human urothelial cell (HUC) model consisting of HUC-PC and other cells. Ethanol and water extracts of basidiocarps and spores of the *G. lucidum* were used. Results indicated that ethanol extracts had a stronger growth inhibition effect than water extracts. At non-cytotoxic (see Table 2) concentrations (40–80 μg/ml), these extracts induced actin polymerization, which in turn inhibited carcinogen 4-aminobiphenyl induced migration in both cell lines. Again there may be similarity in chemical composition to the extracts mentioned previously. In conclusion, preclinical and clinical studies are necessary for the validation of this natural product in the prevention and/or therapy of cancer (Sliva, 2006) and other applications. Also, the effects of isolated compounds require to be tested further as discussed subsequently.

6. Pure chemicals and biomedical applications

Gao et al. (2003) reviewed the "antiviral value" of the genus *Ganoderma*, which provided the major biologically active constituents and their effect or mode of action. The anti-cancer effects of *G. lucidum* were associated with triterpenes, polysaccharides, (and)/or immuno-modulatory proteins, via inhibition of DNA polymerase, inhibition of post-translational modification of the Ras oncoprotein, or the stimulation of cytokine production (Sliva, 2006). Active components of the fungus have been discovered in (a) liquid cultivated mycelia (Gao et al., 2000b), (b) culture medium (Song et al., 1998; Tasaka et al., 1988a) and (c) the spores (Min et al., 2001). Basidiocarps are well known to contain active compounds. A list of the biological activities and broad chemical groupings is provided in Table 2.

6.1. Triterpenoids and sterols

Terpenoids are comprised of four groups: the (a) volatile mono- and sesquiterpenes (essential oils) (C10 and C15), (b) less volatile diterpenes (C20), (c) involatile triterpenoids and sterols (C30), and (d) the carotenoid pigments (C40). Most investigations on *Ganoderma* concern the less volatile triterpenoid (triterpene) and sterol forms although there appears to be some confusion over names (see Lindequist, 1995). Increasingly other species to *G. lucidum* are now being investigated for triterpenoids and steroids. Kim and Kim (1999a) have summarised structural relationships in *G. lucidum*. The triterpene chemical structure is based on that of lanosterol, an important intermediate. Structural diversity is brought about by stereochemical rearrangements of this seminal compound. The physicochemical properties of over 130 lanostane-type triterpenoids have been described (Kim and Kim, 1999a) since ganodermic acid A and B were first described. The bitter components of "Lingzhi" are triterpenes/triterpenoids and have known pharmacological activities (Table 2; Huie and Di, 2004).

Interestingly, Nishitoba et al. (1986) suggested that triterpenes were specific to *G. lucidum*. However, they have been recorded subsequently in the following "species": *G. colossum*, *G. applanatum*, an unidentified *Ganoderma* sp., *G. tsugae*, *G. concinna, G. tropicum* and *G. pfeifferi* (Roberts, 2004). This indicates strongly that they are more closely related than first considered. The isolation of triterpenes from basidiocarps has been very well documented. Many have indicated biological activity as antitumour and antiviral agents: Spores are also sources. In fact, there are indications that spores have higher concentrations of ganoderic alcohols and acids than basidiocarps (Min et al., 1998). Cultivated mycelium has been reported to contain similar compounds (Lin et al., 2003).

Triterpenes are considered to be potential anti-cancer agents due to activity against growing tumours (Lin et al., 2003): they have direct cytotoxicity against tumour cells (Gonzalez et al., 2002) rather than enhancing the immune system as do polysaccharides. Basidiocarps of *G. lucidum* contain unique intensely-bitter compounds that may be related to activity (Mizuno, 1997). Furthermore, triterpenes inhibited HIV-1 protease (Min et al., 1998) and HIV-2 protease (El-Mekkawy et al., 1998). Hepatoprotective mechanisms of ganosporeric acid A from the spores of *G. lucidum* were observed by Chen and Yu (1999).

Also, triterpenoids from the basidiocarps of *G. lucidum* and *G. applanatum*, and malonate half-esters from the same *Ganoderma* species inhibitor tumour promotion (Chairul et al., 1991; Lin et al., 1991). However, and importantly, some esters showed toxicity at high concentrations (Chairul et al., 1990).

Su et al. (2000) examined the cytotoxic activity of lanostanoids from *G. tsugae* and found activity against three cancer cell lines. Lanostanoid and a sterol from *G. tsugae* caused cell death by apoptosis and suggested that it was the sterol that possessed the cell cycle inhibition activity (Gan et al., 1998a). Gonzalez et al. (2002) also observed apoptosis in human promyelocytic leukaemia HL-60 cells exposed to three lanostanoids isolated from *G. concinna*. In contrast, three triterpenoids from *G. concinna* inhibited calf and rat DNA polymerases implicated in DNA repair, recombination and DNA replication (Mizushina et al., 1999). Two terpenes, lucidenic acid O and lucidenic lactone, inhibited the HIV type 1 reverse transcriptase. Two cerebrosides from *G. lucidum* inhibited selectively the activities of replicative DNA polymerases in mammals (Mizushina et al., 1998) but had little effect on other polymerases, transferases, HIV reverse transcriptase, RNA polymerase, deoxyribonuclease I and ATPase. Morigiwa et al. (1986) observed that a methanol extract from the fruiting body of *G. lucidum* inhibited angiotensin-converting enzyme involved in the control of hypertension. The extract contained ten lanostane triterpenes, of which eight exhibited

activity. Furthermore, ganodermic acid S from *G. lucidum* induced platelet aggregation by stimulating the hydrolysis of phosphatidylinositol 4,5-bisphosphate (Shiao, 1992). From these, and the more recent work reported below, it is known now that triterpenes possess bioactivities, such as anti-oxidation, hepatoprotection, anti-allergy, anti-hypertension, cholesterol reduction, and inhibiting platelet aggregation. The effects are from inhibition of enzymes such as galactosidase, angiotension converting enzyme, cholesterol synthase, etc. (Huie and Di, 2004).

Hajjaj et al. (2005) report the isolation and identification of the 26-oxygenosterols ganoderol A, ganoderol B, ganoderal A, and ganoderic acid Y and they determined that the point of inhibition of cholesterol synthesis is between lanosterol and lathosterol. Interestingly, the 26-oxygenosterols could lead to novel therapeutic agents that lower blood cholesterol. Timo et al. (2005) isolated four sterols and 10 triterpenes from the fruiting bodies of *G. pfeifferi*, including the three new triterpenes: lucialdehyde D, ganoderone A, and ganoderone C. Ganoderone A, lucialdehyde B, (and ergosta-7,22-dien-3β-ol) were found to exhibit potent inhibitory activity against herpes simplex virus. Very significant activity was established. Lanostanoid triterpenes isolated from *G. amboinense* inhibited (a) the growth of numerous cancer cell lines, and (b) the activities of topoisomerases I and IIα (Li et al., 2005). One of the most potent triterpene was ganoderic acid X (GAX). The ability of GAX to inhibit topoisomerases and to sensitize the cancer cells toward apoptosis fulfils the feature of a potential anti-cancer drug. Liu et al. (in press) state that the ethanol extract of *G. lucidum* demonstrated inhibitory activity on both isozymes of 5α-reductase (see previously) and suppression effects of ventral prostate growth. Analysis suggested that the active principles in vivo were triterpenoids. These results indicate that the triterpenoids fraction of *G. lucidum* "might" be a useful ingredient in the treatment of benign prostatic hyperplasia. As mentioned previously, *G. lucidum* extracts containing 0.15% ganoderic acid C2 were used in demonstrating anti-cancer effects. However, it is uncertain if this amount of ganoderic acid could be responsible for the effect. In addition, different compounds can modulate unrelated signalling and therefore, can possess synergistic effect. The differing effects of triterpenes and polysaccharides are again noted.

In summary, recent research has demonstrated that: (1) a triterpene fraction from the mycelia of *G. lucidum* inhibited growth of human hepatoma cells, (2) the anti-oxidative activities of *G. lucidum* extracts were examined and the triterpene fraction (ganoderic acids A, B, C and D, lucidenic acid B and ganodermanotriol) exhibited the highest effect by testing the ingredients against pyrogallol induced oxidation on erythrocyte membrane and Fe(II)-ascorbic acid induced lipid peroxidation in liver mitochondria, and (3) triterpenes isolated from the spores of *G. lucidum*, such as ganoderic acid, luciumol B, ganodermanodiol, ganodermanontriol and ganolucidic acid, showed significant anti-HIV-1 protease activity.

Sterols are related closely to triterpenoids and are found in *Ganoderma*. The basidiocarps and mycelium and have potent cytotoxic activity. Ergosterolperoxide, from *G. lucidum* enhances the inhibitory effect of linoleic acid on mammalian DNA polymerase (Mizushina et al., 1998). The effect of *Ganoderma* total sterol (GS) and its main components (GS_1) on rat cortical neuronal cultures exposed to hypoxia/reoxygenation (H/R) was studied in vitro (Zhao et al., 2005) and the results suggest that GS might be useful in treating H/R-induced oxidative stress and inflammatory response. In addition, pre-treatment with GS_1 significantly attenuated the decline of neuron viability and the formation of reactive oxygen species.

Anti-bacterial effects include those from steroidal compounds from the basiciocarps of *G. applanatum*, which were found to have broad spectrum activities and bactericidal effects (Smania et al., 1999). Ganomycins A and B, from *G. pfeifferi* exhibited anti-bacterial activity against Gram-negative and Gram-positive bacteria (Mothana et al., 2000). Sterols (Komoda et al., 1989) and oxygenated triterpenes (Shiao, 1992) have been shown to inhibit cholesterol synthesis in vitro.

6.2. Polysaccharides

Polysaccharides represent a structurally diverse class of biological macromolecules with a wide-range of physicochemical properties. The major bioactive *Ganoderma* polysaccharides species are β-1-3 and β-1-6-D glucans. The structure is β-1-3 D-glucopyronan with 1–15 units of β-1-6 monoglucosyl side chains.

Alternative anti-tumour compounds are glycoproteins (polysaccharides and proteins), heteropolysaccharides and ganoderans A, B and C (Lindequist, 1995). They have high molecular weights as a common feature which tends to increase water solubilities and result in more effective anti-tumour activity. However, some water insoluble polysaccharides also possess anti-tumour activity (Wang et al., 1993) and polysaccharide branching affects activity. Scientific investigations concerning the anti-tumour and immuno-modulating activities of *G. lucidum* were reported as early as in 1957 and, more recently, extensive studies on the anti-tumour ingredient(s), especially polysaccharides and protein/peptide bound polysaccharides, have been carried out. For example, several glucans that were isolated in the early 1980s from water and alkali extracts were found to be bioactive.

Bioactive polysaccharides have been isolated from the basidiocarps (Bao et al., 2002; Zhang and Lin, 2004) and from the mycelial biomass cultivated in liquid culture (Kim et al., 1993). Few have been isolated from the culture medium (Kim et al., 2003). Importantly, the water soluble polysaccharides of *G. lucidum* inhibited strongly the growth of Sarcoma 180 solid-type tumour, with an inhibition ratio of greater than 95%. Most of the anti-tumour glucans were reported to contain a branched glucan core and an average molecular weight of 1,050,000 Da. Polysaccharides are

believed to exert anti-tumour activities through an enhancement of host-mediated immunity rather than a direct cytotoxicity to the tumour cells. It should be noted that the amount of bioactive water-insoluble polysaccharides was found to be higher than that of water-soluble polysaccharides. More than 200 polysaccharides have been isolated from the fruiting bodies, spores, mycelia and cultivation broth of "Lingzhi" (Huie and Di, 2004).

In addition, polysaccharides from *G. lucidum* demonstrated activity (Kim et al., 1999; Zhang et al., 2002). Similar fractions from *G. lucidum* exhibited activity against HSV-1 and HSV-2 (Eo et al., 1999b, 2000; Kim et al., 2000; Oh et al., 2000). Unsurprisingly, high concentrations can be immunosuppressive and so it would be important to use safe concentrations for any proposed treatment.

Fractions from *G. lucidum* have been shown to inhibit tumour growth through activation of host-mediated immune responses by stimulating the production of inducing cytokines (or cytotoxic T lymphocytes) from mononuclear leukocytes (Lieu et al., 1992) and promoting the production of interleukin 2 (Lei and Lin, 1992; Ooi et al., 2002). The activity and mechanism of these polysaccharides was a major area of research at that time. It has also been observed that the host mediated anti-tumour effect is increased by the attachment of polyol groups to glucans (Sone et al., 1985). Anti-tumour activity against transplanted Sarcoma 180 in mice has been exhibited by the β-D-glucan polysaccharides from the basidiocarps of *G. applanatum* (Sasaki et al., 1971), *G. lucidum* (Sone et al., 1985) and the culture fluid of *G. lucidum* (Sone et al., 1985). Ooi et al. (2002) also observed the suppression of growth of Sarcoma 180 solid tumour in mice and a marked increase in the expression levels of immuno-modulatory cytokines in the presence of a hot-water-extracted *G. lucidum* polysaccharide. In contrast, there was no cytotoxic effect observed for a water-insoluble glucan from *G. japonicum* (Ukai et al., 1983) or for the mycelial extract from *G. lucidum* on oral tumour cells in vitro (Chen et al., 1991).

Six water-soluble polysaccharides were extracted sequentially from the mycelium of *G. tsugae* (Peng et al., 2005). They were heteropolysaccharide–protein complexes. GTM3 and GTM4 contained (1 → 3)-β-D-glucans and (1 → 4)-α-D-glucans, while GTM5 and GTM6 were mainly a (1 → 6)-branched (1 → 3)-β-D-glucan. With the progress of isolation, the content of a fraction decreased from 90.2% to 57.1%, and accompanied with enhanced anti-tumour activity. The polysaccharides GTM1, GTM2 and GTM3 had significantly higher anti-tumour activity against solid tumour Sarcoma 180 with an inhibition ratio higher than 50%. The results suggested that the effects on the improvement of anti-tumour activities of polysaccharides "could not be negligible". Polysaccharides have been shown to enhance the activity of DNA polymerases (Lei and Lin, 1993). These authors observed the activity of DNA polymerase to increase in the splenocytes of mice, after they had administered intraperitoneally *Ganoderma* polysaccharides (GL-B).

Zhang and Lin (2004) and Hikino et al. (1985) observed that polysaccharides from the basidiocarps of *G. lucidum* possessed a hypoglycaemic effect in mice. However, studies by Tomoda et al. (1986) found that they were not glycans but peptidoglycans. An unusual report is available on a chitin membrane used as a wound dressing known as "sacchachitin"and developed from the residue of the fruiting body of *G. tsugae* (Su and Hsu, 1995).

In the most recent reports, Chen et al. (2006) present results indicating that *G. lucidum* may have potential immuno-modulating effect in patients with advanced colorectal cancer. More recent research into polysaccharides includes:

(1) Water soluble polysaccharides extracted from *G. lucidum* were effective in preventing DNA from strand breakage indicating the anti-tumour and immunomodulating activities of "Lingzhi" are strongly related to its anti-oxidative property.
(2) The anti-oxidant property of *Ganoderma* polysaccharide peptide decreased the oxidation of low density lipoprotein and exhibited anti-oxidant effect by scavenging reactive oxygen species in mice.
(3) A proteoglycan having a carbohydrate/protein ratio of 11.5 to 1 – isolated from *G. lucidum*, was found to stimulate the proliferation of mouse spleen lymphocytes, resulting in a three to fourfold increase in the (a) percentage of B cells, (b) secretion of immunoglobulin, (c) production of interleukin 2 and (d) expression of protein kinase.
(4) It was found that the polysaccharide extracts from the mycelium of *G. lucidum* exhibited anti-tumour effects against fibrosarcoma in male and female mice and inhibited the metastasis of a tumour to the lung. The bioactive polysaccharides could stimulate blood mononuclear cells to increase cytokines, tumour necrosis factor, interferon and interleukin production, and the lifespan of tumour-implanted mice was found to increase significantly due to the administration of GLP.
(5) Two protein bound polysaccharides, a neutral protein bound polysaccharide (NPBP) and an acidic protein bound polysaccharide (APBP), were isolated from water soluble substances of *G. lucidum* APBP was found to be more potent than NPBP as an antiviral agent against herpes simplex viruses (HSV). The antiviral activity of APBP appeared to be related to its binding with HSV-specific glycoprotein at the cell membrane (Huie and Di, 2004). *G. lucidum* polysaccharides (Gl-PS) have shown a variety of immune modulating effects. Results confirmed that Gl-PS was a promising biological response modifier and immune potentiator (Zhu and Lin, 2006).

The extraction procedures for polysaccharides involve standard methodology (see Huie and Di, 2004). However, the extraction of water insoluble polysaccharides from

"Lingzhi" can be carried out by a cellulase hydrolysis reaction. This method is based on the attack of the enzyme on the polysaccharide substrates, composed of cellulose and lignin. However, lignin in the fungal preparation is very surprising and is probably a false assumption. This may have arisen from referring to the fungi as herbs as they may contain lignin. For information, it has been reported that enzyme hydrolysis reaction can be enhanced by ultrasonic waves.

6.3. Proteins, peptides and amino acids

Bioactive proteins have been isolated from various "Lingzhi" species and characterized by chromatographic/electrophoretic techniques. For example, a new immunomodulatory protein, known as Ling Zhi-8 (LZ-8), was isolated from the mycelia of *G. lucidum*. LZ-8 is a polypeptide consisting of 110 amino acid residues with an acetylated amino terminus and has a molecular mass of 12 kDa (Tanaka et al., 1989). Mitogenic activity and also has been reported (e.g. van der Hem et al., 1995).

Ngai and Ng (2004) isolated a lectin from *G. capense* that exhibited potent mitogenic activity toward mouse splenocytes and anti-proliferative activity toward leukaemia cells and hepatoma cells in vitro. The lectin exhibited more potent mitogenic activity than that of concanavalin A. It was found that three kinds of bioactive proteins from the fruiting body and spores of "Lingzhi" showed obvious mitogenic activity. Proteins in the form of enzymes have also been isolated and characterized by various types of column chromatography as well as electrophoretic techniques. For example, DEAE-Sephadex column (gel filtration) and Con A-Sepharose (affinity chromatography) were employed for the purification of galactosidase from the fruiting body of *G. lucidum*. In addition, a variety of amino acids have been found in Lingzhi. Oxidative stress has been linked with the pathogenesis of many human diseases including cancer, aging, and atherosclerosis (Sun et al., 2004). Their study investigated the potent anti-oxidant activities of peptides isolated from *G. lucidum*. Polysaccharides, polysaccharide–peptide complex (and phenolic components) of *G. lucidum* have been proposed to be responsible for this anti-oxidant effect. However, research has shown that the *G. lucidum* peptide is the major anti-oxidant component. In another investigation of *G. lucidum* polysaccharide peptide (Gl-PP) demonstrated some effects as anti-tumours compounds in mice and potential anti-angiogenesis (Cao and Lin, 2006). They elucidated the possible mechanism of Gl-PP action on anti-angiogenesis of tumours. Inducing cell apoptosis by Gl-PP might be the mechanism of inhibiting proliferation. Human lung carcinoma cells when exposed to high dose of Gl-PP in hypoxia for 18 h resulted in a decrease in an important chemical indicator of cancer. Taken together, these findings support the hypothesis that the key attribute of the anti-angiogenic potential of Gl-PP is that it may directly inhibit vascular endothelial cell proliferation or indirectly decrease growth factor expression of tumour cells.

Wang and Ng (2006) isolated a 15-kDa antifungal protein, designated ganodermin, from *G. lucidum*. Ganodermin inhibited the mycelial growth of *Botrytis cinerea*, *Fusarium oxysporum* and *Physalospora piricola*. However, it was devoid of haemagglutinating, deoxyribonuclease, ribonuclease and protease inhibitory activities.

6.4. Nucleosides, nucleotides and RNAs

Yu and Zhai in an obscure journal (see Huie and Di, 2004) were the first to report the isolation of adenine, adenosine, uracil and uridine from the mycelia of a *Ganoderma* species, *G. capense*. Among these, nucleosides, uridine and uracil were found to be capable of lowering the serum aldolase level of mice suffering from experimental myotonia. Adenosine has also been shown to inhibit platelet aggregation (Shimizu et al., 1985). In contrast, Gau et al. (1990) found that the administration of crude *Ganoderma* extracts, known to have a high content of adenosine, had no effect on platelet aggregation in haemophiliac patients who were HIV positive. An epimer of 5′-deoxy-5′-methylsulphinyladenosine was found to inhibit platelet aggregation in vitro (Kawagishi et al., 1993). However, why these compounds should be different in any way than those from other sources is not clear (to me).

6.5. Alkaloids, vitamins, essential minerals, flavours, and fatty acids

The compounds cyclooctasulfur (Tasaka et al., 1988b) and oleic acid (Tasaka et al., 1988a), isolated from the culture broth of *G. lucidum*, were both found to inhibit histamine release which is an important activity for treatment of inflammation, allergies, and anaphtlactic shock. The alkaloids, choline and betaine were isolated from the spores of *G. lucidum*. Furthermore, two novel pyrrole alkaloids (ganoine and ganodine) and a novel purine alkaloid (ganoderpurine) were discovered from the mycelia of *G. capense*. Vitamins (including β-carotene) and essential elements have been isolated from various "Lingzhi" "species". Aroma compounds were detected from *G. applanatum* which may have novel biotechnological applications. Over 120 volatile flavour compounds, mostly alcohols, aldehydes, ketones, esters, and phenols, have been identified (Huie and Di, 2004). Finally, very-long-chain fatty acids with more than 23 carbon atoms have been reported in *G. applanatum* at trace levels (1–2%).

7. Toxicity

It is obvious that this issue has received little attention in the literature, despite it being crucial for the success of any proposed medical treatments. This may reflect a certain

lack of balance in reporting only the potential benefits. However, indications of toxicity are provided in Table 2.

8. Conclusions

The first thing to say is that the activity data on isolated compounds mentioned herein is very convincing. This review summarised important areas of investigation being performed on *Ganoderma* species around the world, with particular emphasis on chemicals of biomedical relevance. There is abundant evidence that these are effective, and more clinical trials are now in order. Searching for other secondary metabolites from *Ganoderma* may prove fruitful especially if the taxonomy was usefully revised.

However, unlike "Western medicine" in which therapeutic effects usually derive from a single chemical substance within the drugs, the pharmacologically activities of "herbal" medicines invariably arise from a mixture of "active" ingredients within the materials. In fact, in many cases, the biological activities of the active components administrated or consumed alone were found to be lower than those obtained from the original mixture of active ingredients present in herbs, such as "Lingzhi". Thus, the combined, synergistic effects of a mixture of active components that are present in "Lingzhi" on biological activities need to be thoroughly assessed. A factor which is not considered by those who appear to advocate using *Ganoderma* is the possibility of toxic components in the fungus and preparations, and more work is required in this area. Some combinations of compounds are more toxic than the individual parts while others "potentiate" usually non-toxic compounds. This is why a good chemotaxonomy is required of the genus and employing the bioactive components for this purpose. It is clear that many issues are raised of a systematic nature – not least as they relate to finding fungi with active but not toxic ingredients. Crucially, we need to know what the non-Latinised names actually represent – past and present.

In the search for active compounds from *Ganoderma* species, the majority of research has been performed on extracts from the fruiting body and there have been fewer studies on extracts from the liquid cultivated mycelium. It appears that there are a number of biologically active compounds to be found in the mycelium and the benefits of liquid cultivation over solid cultivation include: the ability to manipulate the cultivation medium to optimise mycelial growth; a shorter cultivation time; and less contamination. In fact, the reason that some of the *Ganoderma* preparations are not yet available as medicines may be from difficulties relating to mass production (Smith et al., 2002).

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