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Potential role of host tree species in determining the composition of polysaccharides of *Ganoderma lucidum* (Fr.) Karst.(GLPS)

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Abstract

Ganoderma lucidum is a basidiomycetous white rot fungus which has been used for medicinal purposes for centuries particularly in countries such as China, Japan and Korea. Several classes of bioactive substances have been isolated and identified from basidiocarp, mycelia and spores of *G. lucidum*, such as polysaccharides, triterpenoids, nucleosides, sterols, fatty acids, protein and alkaloids. One of the major pharmacological properties of *G.lucidum* is antitumor activity and polysaccharides (GLPS) are the main component responsible for this property. In this paper, the possible role of host tree species in determining the composition of polysaccharides (GLPS) was examined. To date, a careful comparison of polysaccharides (GLPS) from different host tree species has not been performed. Fruiting bodies were collected from nine host tree species from different regions of India and examined for their polysaccharide composition. These fruit bodies were analyzed and compared on the basis of Gas liquid chromatography (GLC). The result showed the fruit body from *Dalbergia sissoo* was most potential for GLPS production, as it was composed of all six tested monosaccharides, including rhamnose, arabinose, xylose, fructose, glucose and mannose. It also showed the presence of intense host-dependent variability among isolates.

Key words – anti-tumor activity **–** *Dalbergia sissoo* – gas liquid chromatography – host dependent variability – medicinal mushroom

Introduction

Ganoderma lucidum (Fr.) Karst. (Ganodermataceae) is a medicinal mushroom, belonging to the family polyporaceae. It is used as a popular medicine in China (named Ling Zhi) and Japan (named Reishi, Mannentake) for hundreds of years as a health tonic to promote longevity. It has been used for the treatment of a wide range of ailments and chronic diseases, such as migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, diabetes, hypercholesterolaemia, nephritis, dysmenorrhoea, constipation, lupus erythematosis, hepatitis and cardiovascular problems. Importantly, it has been demonstrated by recent scientific studies to possess anticancer including leukemia, anti- ageing, anti- inflammatory and anti-microbial/viral activities, including anti-human immunodeficiency virus (HIV) activity (Gao et al. 2003).

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Pharmacological effect of *G. lucidum* is based on their powerful immune-modulating action and immune potential capability, which support and enhance the over all immune function, due to the presence of more than 200 active elements. Major elements include polysaccharide, triterpenoids, ptotrin, organic germanium, adenosine (Sanodiya et al. 2009).The highest attention is paid to polysaccharides from *G. lucidum*, which activate macrophages, lymphocytes, NK cells, proinflmmatory cytokines such as TNF or interleukins, essential for host survival from infection, and are also required for the repair of tissue injury (Gao et al. 2005).

Currently available data suggests that *G. lucidum* polysaccharides (GLPS), mostly β-glucanes, activate host's immune responses exerting anti-cancer functions, whereas anti-tumor effect of triterpenes seems to be related to their cytotoxic activity against the tumor cells directly (Wasser & Weis 1999, Lin & Zhang 2004, Kuo et al. 2006). It is also believed that *G.lucidum* acts as an immunological agent. It has been also used as a natural adjuvant for immunotherapy (Chan et al. 2005, Woźniak et al. 2012)

One of the most attractive properties of *G. lucidum* is anti-tumor effect which has been demonstrated to be mainly associated with its polysaccharides fraction by mediating immune system mechanisms (Sanodiya et al. 2009). Polysaccharides found in *G. lucidum* belong to β-glucan groups which according to Chan et al. (2008) is responsible for the stimulation of many kinds of immune response to prevent tumor formation

By keeping in view the medicinal importance, present study is focused on characterization of polysaccharides from different host tree species of *G. lucidum.* Increasing demand and nutraceutical significance of this fungus makes it necessary to explore the chemical diversity to open new prospects in natural product industry.

Materials & Methods

Fruiting bodies of *G. lucidum* from nine different tree host species (Table 1) were used for the chemical analysis. All the experiments were conducted with triplicate and obtained data were subjected to analysis of variance (ANOVA) using SPSS 16.0.

Drying of fruiting bodies

Fruiting bodies were dried at 55^{o}C for 4-5 h and then grounded to fine powder by using an electric grinder.

Estimation of polysaccharides

Polysaccharide estimation and identification was carried by preparation of alditol acetate followed by Gas Liquid Chromatography analysis.

Hot water extraction

Fruiting bodies were grounded to a fine powder using an electric grinder. Ten g of the powder was boiled with 200 ml of distilled water at 100°C for 3 h. Mixture was cooled at room temperature and filtered through Whatman No.1 filter paper. Filtrate was then refluxed with two additional 100 ml portions of distilled water. Filtrates (crude polysaccharides) were dried and used further (Bao et al. 2002).

Complete hydrolysis

Complete hydrolysis of crude polysaccharides (hot water soluble) was carried out (Soni and Srivastava, 1992). 0.2 g of crude polysaccharides was heated with 10ml of 12M H_2SO_4 (2N) for 18 hr produced a mixture of monosugars. Resulting hydrolysate was converted into its alditol acetate.

Preparation of alditol acetates

For the quantitative estimation of sugars, hydrolysates were converted into its alditol acetate by the method of Jansson et al. (1976). Gas Liquid Chromatography was carried out with a BP5, 0.25 mm capillary column (30 mm x 0.25mm), range- bipolar, temp 190 – 240^{o}C, pressuse-2 bar and flow rate

1.5µl/min, run time 30 min. Alditol acetate of authentic sugar mixture was also run under the same conditions to obtain the values of relative retention times (Rt).

Results

Fruiting bodies were powdered and their hot water extract prepared to isolate crude polysaccharides. Crude polysaccharides were hydrolyzed into their respective monosaccharides and converted into their alditol acetate for GLC analysis. Experimental results of GLC are summarized in Table 2 and Figs 1, 2.

It was apparent; all isolates were considerably different from each other in respect to presence of monosaccharides and their percentage. The predominant monosaccharides in these samples were D-mannose, D-galactose and D-glucose. D-rhamnose was present only in five samples i.e. Gl-55, Gl-7, Gl-34, Gl-45 and Gl-31. Maximum quantity of D-rhamnose was found in isolate no.Gl-34 (33.32%) which was significantly superior to other isolates. D- arabinose was found in seven isolates Gl-15, Gl-40, Gl-25, Gl-55, Gl-34, Gl-45 and Gl-31. Maximum amount of D-arabinose was found in isolate no.Gl-25 (33.07%) which was statistically at par with isolate no.Gl-40 (32.67%) and Gl-34 (31.75%).

D-xylose was found in six isolates Gl-49, Gl-22, Gl-55, Gl-34, Gl-45 and Gl-31. Maximum quantity of D-xylose was found in Gl-22 (41.64%). which was significantly superior to all the isolates. D-mannose was isolated from nine isolates Gl-49, Gl-15, Gl-22, Gl-40, Gl-25, Gl-55, Gl-34, Gl-45 and Gl-31and was not found in one isolate Gl-7. Isolate no. Gl-15 (55.17 %) has the maximum amount of D-mannose which was statistically at par with isolate no. Gl-45 (39.19%). D-galactose was also found in nine isolates Gl-49, Gl-15, Gl-22, Gl-40, Gl-25, Gl-55, Gl-34, Gl-45 and Gl-31 and was absent in only one isolate no. Gl-7. D-glucose was present in eight isolates i.e. Gl-15, Gl-22, Gl-25, Gl-55, Gl-7, Gl-34, Gl-45 and Gl-31 and absent in two isolates i.e. Gl-49 and Gl-40. Maximum amount of D-glucose was found in isolate no. Gl-31 (61.89%), collected from *D.sissoo* which was statistically superior to other isolates.

In these isolates, some sugars were present and some were absent. There were only four isolates in which all the six sugars were present i.e. Gl-55, Gl-34, Gl-45 and Gl-31. Isolate no. Gl-55 was collected from *T. bellerica*. Isolate no. Gl-34 and Gl-31 were from *D. sissoo* and isolate no. Gl-45 was from *D. regia.* On the basis of above mentioned chemical analysis (Table. 2), it was found that the fruiting bodies collected from *D. sissoo* showed the best values for various sugars in comparison to fruiting bodies collected from other host tree species. Among these, isolate no. Gl-31, showed the maximum quantity of D-glucose (61.89%).

In this analysis, it was found that D-mannose and D-galactose were most frequent sugars, present in 9 isolates, whereas D-glucose was found in eight isolates only. At the same time, D-arabinose was present in 7 isolates and D-xylose was present in 6 isolates. The least occurring monosaccharide was D-rhamnose, present only in 4 isolates.

On this basis, isolate no.Gl-31 from *D. sissoo* which has the maximum quantity of glucose (61.89%) as well as also contains all the six monosugars was the most potent host tree species for polysaccharide (GLPS) production. From the above analysis (Table 2) it can be conferred that nature of host tree species affect the sugar composition of *G. lucidum.*

Discussion

Chemical diversity in context of host tree considered in present work was mainly focused on polysaccharides which attributed for the anticancerous properties of *G.lucidum,* reported by many researchers (Wasser 2002, Zhang et al. 2002, Zhang & Lin 2004, Li et al. 2007, Zhang et al. 2007).Presence of different monosaccharides predominantly, D-mannose, D-galactose and D-glucose in fruiting bodies of *G.lucidum* was in conformity with the results mentioned by Zhang et al. (2002).

In the current study, *D. sissoo* was found the most suitable host tree species for production *G. lucidum* polysaccharide (GLPS). It contains maximum quantity of glucose (61.89%) as well as all other monosugars. The morphological and culture characters of all isolates were same but their polysaccharide composition was noticeably varies. The plausible reason for this difference was

Table 1 List of fruiting bodies used for chemical study

S. No.	Isolate No.	Host tree species
1	Gl-49	*Mangifera indica* L.
2	Gl-15	*Ficus religiosa* L.
3	Gl-22	*Cassia siamea* Lam.
4	Gl-40	*Cassia fistula* L.
5	Gl-25	*Albizia lebbek* Benth.
6	Gl-55	*Terminelia bellerica* Roxb.
7	Gl-7	*Acacia nilotica* L.
8	Gl-34	*Dalbergia sissoo* Roxb.
9	Gl-45	*Delonix regia* Raf.
10	Gl-31	*Dalbergia sissoo* Roxb.

climatic and nature of the host tree. The host tree on which the fungus grows saprophytically contributed in its chemical diversity. This fact was also supported by the findings Fernando 2008.

Observable variability was found in composition as well as in quantity of monosaccharide. This diversity was due to the effect of host on growth pattern of fruiting bodies. Isolates were selected according to their host tree species and it was observed that isolates from same host irrespective of their climate and location were less variable in polysaccharide contents, implying the presence of intense host-dependent variability. Similar observations were confirmed by Zakaria et al. (2009). It is evident from the above analysis that host tree plays a mojor role in determining the chemical composition. This study concludes that *D. sissoo* is the most preferable host of *G. lucidum* among the tested host tree for the production of polysaccharide (GLPS).

Table 2 Monosaccharides in the fruiting bodies of *Ganoderma lucidum*

Isolates	D-rhamnose	D-arabinose	D-xylose	D-mannose	D-galactose	D-glucose
Gl-49	--	--	11.64 (4.1)	16.20 (7.8)	69.85 (88.12)	--
Gl-15	--	12.25 (4.6)	--	55.17 (67.3)	20.92 (12.87)	22.91 (15.2)
Gl-22	--	--	41.64 (44.2)	24.13 (20.0)	10.85 (3.56)	32.18 (29.5)
Gl-40	--	32.67 (29.1)	--	25.30 (18.5)	43.28 (47.0)	--
Gl-25	--	33.07 (29.8)	--	27.29 (21.6)	37.74 (37.47)	40.98 (43.0)
Gl-55	23.74 (15.11)	20.01 (12.6)	21.89 (14.1)	22.34 (15.0)	20.25 (13.38)	35.90 (35.1)
Gl-7	26.18 (20.87)	--	--	--	--	40.23 (41.72)
Gl-34	33.32 (30.18)	31.75 (28.2)	17.47 (11.4)	29.56 (24.4)	19.28 (11.45)	21.25 (14.3)
Gl-45	23.18 (15.38)	15.42 (7.1)	3.83 (0.5)	39.19 (42.8)	20.71 (12.50)	40.98 (43.0)
Gl-31	6.18 (1.16)	20.94 (14.1)	15.18 (8.4)	29.19 (42.8)	15.67 (7.53)	61.89 (77.7)
CD at 5 %	3.02	9.53	11.19	16.93	6.91	11.75
SEM	4.47	5.44	7.70	8.07	10.48	8.05

-- not present
Original values are given in parenthesis.

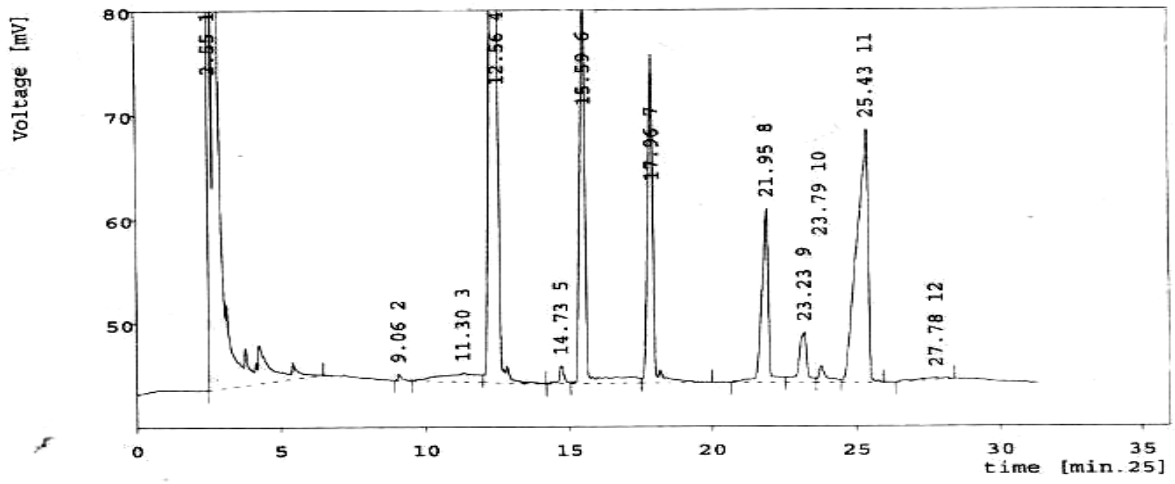

Fig.1–GLC graph of alditol acetate of six different standard sugars

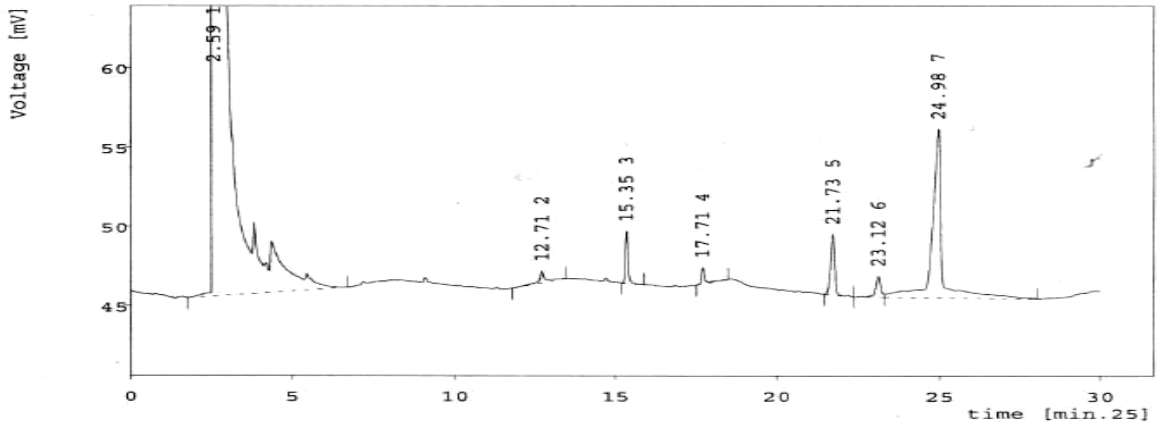

Fig. 2–GLC chromatograph of alditol acetate of isolate no. 31 of *G.lucidum* on *D. sissoo*

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