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Research and Development of *Ganoderma lucidum Cultivated* in Thailand

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Abstract

Polysaccharides and triterpenes are the major pharmacologically active components in *Ganoderma lucidum* or Lingzhi, a medicinal mushroom well known for its immunomodulating activity. The *Ganoderma lucidum* used for this experiment were cultivars (MG1, MG2, and MG5) cultivated in Thailand collected from Muang Ngai Special Agricultural Project under the Patronage of Her Majesty Queen Sirikit, Chiang Mai province. The objectives of this study were to select the appropriate Lingzhi cultivars for cultivation, to investigate suitable wood logs as well as to study the appropriate harvest time. Quantitative determinations were performed using colorimetry and high performance liquid chromatography (HPLC). The results of this study indicated that the optimal harvest time of Lingzhi was 110 days after cultivation, the wood logs suitable for Lingzhi growth were paper mulberry, and longan wood logs, and the appropriate Lingzhi cultivar for cultivation in Thailand was MG2 since it produced the highest total polysaccharide content. The broken spore walls provide the highest percentage yield of major chemical compounds, especially triterpenoids.

Keyword: *Ganoderma lucidum*, Lingzhi, Triterpenes, Polysaccharides, Qualitative and quantitative analyses.

INTRODUCTION

Ganoderma lucidum (Fr.) Karst. (belonging to Polyporaceae family)[1] known as Lingzhi in China, one of the most famous mushroom, has been widely used for promoting health and longevity in China and other Asian countries for over 2,000 years. Evidence has accumulated concerning the medical application of Lingzhi in the treatment of various kinds of ailments such as bronchitis, anorexia, gastritis, hepatitis, nephritis, haemorrhoid, dysmenorrhoea, constipation, lupus erythematosis, and chronic diseases such as migraine, hypertension, arthritis, asthma, diabetes, hypercholesterolemia, cardiovascular problems including cancers[1-7].

In Thailand, Lingzhi was cultivated for a long time, but the information for their activities and chemical components were insufficient. So it is interesting to study in terms of qualitative and quantitative analyses for determining the chemical compounds from Lingzhi to select the Lingzhi cultivars, examine the suitable wood logs for cultivation and determine the appropriate harvest time.

The samples that used for these experiments including Muang Ngai-1 (MG1), Muang Ngai-2 (MG2), and Muang Ngai-5 (MG5). From the scientific reports, it was found that triterpenes and polysaccharides were the major pharmacologically active components in Lingzhi.

The triterpenes were bitter components that caused *G. lucidum* had remarkably strong bitter taste. Since ganoderic acids A and B

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were firstly discovered, more than 130 types of triterpenes had been isolated from the fruity bodies, mycelia, cultured media and spores of *G. lucidum* and they had been received considerable attention owing to their conspicuous pharmacological activities. The activities of their triterpenes were reported as anticholesterol, anti-complement, anti-hepatitis B, anti-histamine, anti-HIV-1, anti-HIV-1 protease, anti-hypertension, antinociceptive, and cytotoxic effect[1,8-12].

The polysaccharides also show an important role as functional food ingredients, because the alternative and complementary medicines are now paid increasing attention. The scientific elucidation of the role of the polysaccharides in phytomedicines is very important. The activities of polysaccharides were reported as anti-tumor, anti-hypertension, hypoglycemic in diabetes, and immune modulating[1,13-18].

This experiment was composed of the methods for analyzing chemical compounds both qualitatively and quantitatively by using colorimetric and high performance liquid chromatographic (HPLC) techniques. The objective of this study was to study the condition for cultivation Lingzhi in Thailand such as the appropriate harvest time of spore and fruity body, the suitable wood logs for cultivation and the appropriate Lingzhi cultivar.

MATERIALS AND METHODS

Plant materials:

The Lingzhi cultivars including Muang Ngai-1 (MG1), Muang Ngai-2 (MG2), and Muang Ngai-5 (MG5) were collected from Muang Ngai Special Agricultural Project under the Patronage of Her Majesty Queen Sirikit, Chiang Mai province (Fig. 1). The samples were identified and kept at the herbarium at the Faculty of Pharmacy, Mahidol University.

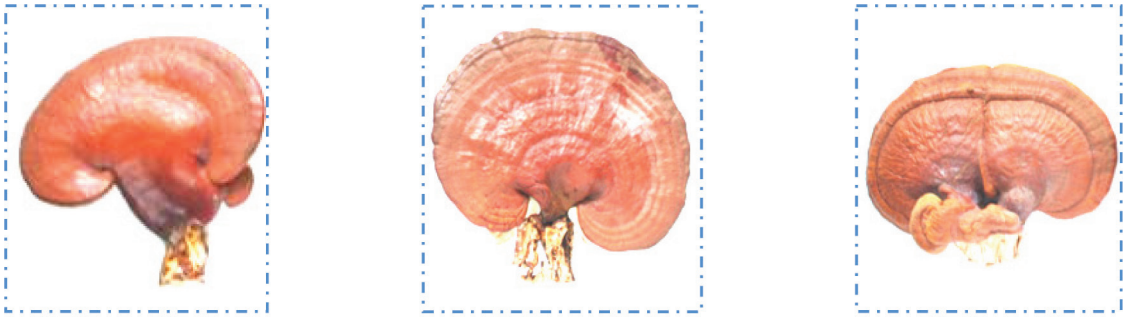

Figure 1. The sample of Lingzhi cultivars; MG1 = Muang Ngai-1, MG2 = Muang Ngai-2, MG5 = Muang Ngai-5

Extraction procedures:

Extraction method for the determination of total triterpenes was performed by sonicating 1 g of powder of Lingzhi with 20 ml of 95% methanol in an ultrasonic bath at 60ºC for 30 minutes, then filtered, the residue was washed with methanol for 3 times and adjusted volume with methanol. For the determination of polysaccharides and uronic acid was prepared by extraction with hot water (95-100ºC) three times for 1 hour and drying by lyophilization to give a water extract of Lingzhi.

Quantitative analysis by using colorimetric method

Total polysaccharides and uronic acids contents were determined by forming color with phenol-H_2SO_4 reagent[19] and *m*-hydroxybiphenyl reagent[20], respectively, and examined under colorimeter, using glucose and glucuronic acid as reference standards.

Triterpenes content was measured by colorimetric method by reacting with 5% vanillin/glacial acetic acid and perchloric acid, using ursolic acid as reference standard[21].

Quantitative analysis of ganoderic acids using HPLC method[22]

Preparation of samples for HPLC: A 50.0 mg of dried powder was extracted with 1.0 ml 95% methanol in ultrasonic bath for 1 hr. This solution was centrifuged at 14,000 rpm. The supernatant liquid was collected, filtered with nylon filter and kept in a well-closed injection vial.

Preparation of ganoderic acids reference standard (GA, GF and GH) for HPLC: Every standard compound was accurately weight and dissolved in 95% methanol to prepare a concentration of 2.5 mg/5 ml. The internal standard (cortisone 21-acetate) solution was prepared at a final concentration of 20 µg/ml in 95% methanol

Condition for HPLC analysis: The method uses a Inertsil® ODS-3 (250×4.0 mm, 5 µm) column as the stationary phase, a gradient mobile phase of acetonitrile (CH_3CN), perchloric acid ($HClO_4$), ammonium acetate (CH_3COONH_4), deionized water, and methanol, and UV detection at 252 nm. as described in Table 1.

Table 1. HPLC conditions for the analysis of ganoderic acids content

Analytical column : Inertsil® ODS-3 (250×4.0 mm, 5 µm)
Guard column : Inertsil® ODS-3 (10×4.0 mm, 5 µm)
Flow rate : 1 ml/min
Temperature : 55 °C
Detector : UV 252 nm
Injection volume : 10 µl
Mobile phase :

Mobile phase	CH_3CN (ml)	$HClO_4$ (M)	CH_3COONH_4 (M)	DI-H_2O (ml)	CH_3OH (ml)
A	150	0.019	0.020	250	1.50
B	60	0.019	0.020	250	1.50

RESULTS AND DISCUSSION

1. *Selection of Lingzhi cultivars*

The Lingzhi cultivars including Muang Ngai-1 (MG1), Muang Ngai-2 (MG2), and Muang Ngai-5 (MG5) were determined the contents of total polysaccharides, total uronic acids and total triterpenes by using colorimetric method and the content of total ganoderic acids was performed by using high performance liquid chromatographic (HPLC) method compared with Chinese cultivars (G5 and G9). The result in Table 2 showed that Chinese cultivars composed of total triterpenes content more than cultivars cultivated in Thailand. Comparing to Thai cultivars, MG5 (stalk) contained the highest content of total triterpenes and total ganoderic acids. The result in Table 3 showed that the content of polysaccharides (% w/w) in broken spores, non-broken spores, fruity bodies and stalk in MG2 were 4.77, 4.21, 3.06 and 1.72 %w/w, respectively, and the stalks of MG2 contained the highest content of uronic acid (% w/w). The highest content of uronic acid in Chinese cultivars was broken spore of G5 (Table 4).

2. Studying of suitable wood logs for Lingzhi cultivation

To evaluate the triterpenes content and composition among the fruity bodies cultivated on different mushroom-growing logs, a single strain of *G. lucidum* (MG5) was cultivated on Rain tree, Paper mulberry, Tamarind, Mango, Longan, and Neem wood logs. The result showed that MG5 cultivated on the paper mulberry wood log

contained the highest triterpenes content, and longan wood log was suitable for the production of polysaccharides (Table 5).

3. Determination of optimal harvest time The fruity bodies and spores of MG5 were studied at different period of harvest time at 90, 100, 110, and 120 days. The result showed that the contents of total triterpenes and total polysaccharides were the highest in fruity bodies at 110 days, the content of polysaccharides in spores was higher than fruity bodies about two times. Therefore, the optimal harvest time for fruity bodies and spores of Lingzhi was 110 days after cultivation (Table 6).

Table 2. Content of total triterpenes in different Lingzhi cultivars

Cultivars	% Total triterpenes			% Total ganoderic acids		
	UV			HPLC		
	FB	SP(B)	Stalk	FB	SP(B)	Stalk
MG1	1.37	1.32	2.08	0.064	-	0.164
MG2	1.42	1.46	2.53	0.121	0.013	0.348
MG5	2.65	1.80	3.57	0.877	0.023	1.178
G5	-	3.69	-	-	-	-
G9	-	2.53	-	-	-	-

*MG1 = Muang Ngai-1, MG2 = Muang Ngai-2, MG5 = Muang Ngai-5, G5 and G9 = Lingzhi cultivars from China, FB = fruity body, SP(B) = broken spore

Table 3 Content of total polysaccharide in different Lingzhi cultivars

Cultivars	% Total polysaccharide			
	FB	SP(NB)	SP(B)	Stalk
MG1	2.16	3.62	4.18	1.83
MG2	3.06	4.21	4.77	1.72
MG5	1.73	3.62	4.67	1.80
G5	-	2.55	2.92	-
G9	-	2.45	3.57	-

*MG1 = Muang Ngai-1, MG2 = Muang Ngai-2, MG5 = Muang Ngai-5, G5 and G9 = Lingzhi cultivars from China, FB = fruity body, SP(B) = broken spore, SP(NB) = non broken spore

Table 4. Content of total uronic acid in different Lingzhi cultivars

Cultivars	% Total uronic acid			
	FB	SP(NB)	SP(B)	Stalk
MG1	0.06	0.03	0.02	0.10
MG2	0.05	0.04	0.05	0.18
MG5	0.07	0.03	0.05	0.13
G5	-	0.18	0.31	-
G9	-	0.15	0.24	-

*MG1 = Muang Ngai-1, MG2 = Muang Ngai-2, MG5 = Muang Ngai-5, G5 and G9 = Lingzhi cultivars from China, FB = fruity body, SP(B) = broken spore, SP(NB) = non broken spore

Table 5. Content of total polysaccharide, uronic acid and triterpenes for studying suitable wood logs for cultivation of Lingzhi

Type of wood logs	% Total triterpenes		% Total polysaccharides	% Total uronic acid
	UV	HPLC		
Rain tree	0.319	0.141	1.96	0.06
Paper mulberry	0.771	0.362	1.94	0.05
Tamarind	0.499	0.120	1.86	0.05
Mango	0.155	0.047	1.43	0.06
Longan	0.645	0.347	2.24	0.03
Neem	0.617	0.371	2.16	0.08

Table 6. Contents of total polysaccharide, uronic acid and triterpenes for determination the optimal harvest time of spore and fruity body

MG5 (Days)	% Total triterpenes		% Total polysaccharides		% Total uronic acid	
	UV	HPLC	FB	SP(NB)	FB	SP(NB)
90	1.01	0.182	0.77	1.03	0.03	0.01
100	1.28	0.404	0.92	2.33	0.05	0.02
110	1.51	0.380	1.23	2.34	0.07	0.03
120	1.49	0.398	1.21	2.47	0.07	0.03

* MG5 = Muang Ngai-5, FB = fruity body, SP(NB) = non broken spore

CONCLUSION

It could be summarized from the result of this study into three topics as followings. First, to estimate the optimal harvest time between 90, 100, 110, and 120 days of MG5FB, it was shown that the optimal harvest time of Lingzhi was at 110 days after cultivation because of its highest content of total triterpenes and total polysaccharides. Moreover, the broken-walled spores exhibited almost 2 folds of total polysaccharides content than its fruity bodies. Second, to examine the suitable wood logs for Lingzhi growth between rain tree, paper mulberry, tamarind, mango, longan, and neem, it was concluded that the suitable wood logs were paper mulberry and longan wood logs due to its higher content of polysaccharides. Third, to select the appropriate Lingzhi cultivars between MG1, MG2 and MG5 cultivars for cultivation in Thailand, it was found that MG2 was the appropriate Lingzhi. Since MG2 cultivar was shown the highest content of polysaccharides among all cultivars.

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