1,3-*β*-glucan Content of Local Medicinal Mushrooms from the Southern Region of Thailand

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Abstract

Local medicinal mushrooms were collected from the Songkhla, Phatthalung, Trang, and Satun provinces during the rainy season. There were 13 specimens identified among the collected samples. Among the samples, one species belonged to the genus of *Ganoderma* and exhibited a value for 1,3-*β*-glucan content that was significantly different ($p \leq 0.05$) from all other species of local medicinal mushrooms. The 1,3-*β*-glucan content was the highest in the strains of *Ganoderma calidophilum* (90.22 mg/g) and *Amauroderma rugosum* (89.24 mg/g). Some of the more efficacious compounds of *Ganoderma* were 1,6-branched 1,3-*β*-glucan, which have been reported to inhibit tumor growth by stimulating the immune system via the activation of macrophage, the balance of T helper cell populations, and subsequent effects on natural killer (NK) cells. Moreover, environmental factors, such as vegetation, soil characteristics, and forest stand, as well as microclimate, were found to be contributory to local medicinal mushroom habitats, and significantly accumulated with bioactive compounds or nutraceutical products, such as 1,3-*β*-glucan content.

Keywords: Medicinal mushrooms, mycelia extraction, nutraceutical, bioactive compounds, 1,3-*β*-glucan

Introduction

Medicinal mushrooms have been identified as remarkable therapeutic agents in traditional folk medicines and are important ingredients in popular culinary products all over the world. Various species of medicinal mushrooms have a long history of use for disease treatment in folk medicines, especially in countries such as China, India, Japan, Korea and Thailand [1-3]. Medicinal mushrooms have shown beneficial therapeutic activity against the development of many diseases, primarily because they contain a number of biologically-active compounds. They are also used in cosmetics because of their medicinal properties. This includes high-molecular weight compounds, such as polysaccharides, proteins, and lipids, as well as a number of low-molecular weight metabolites, such as lectins, lactones, and terpenoids, and also alkaloids, sterols, and phenolic substances [4-9].

β-glucans consist of a backbone of glucose residues that are usually joined by 1,3-*β* linkages, to which glucose side-chain residues are often attached. In some *β*-glucans, no side-chain substitution occurs, as with the bacterial *β*-glucan curdlan, which contains only 1,3-*β*-glucosidic linkages. No unbranched 1,3-*β* or 1,6-*β* fungal *β*-glucans are known, although the extent of the side-chain substitutions can vary considerably [10-13].

Some fungal *β*-glucans markedly stimulate the human immune system and protect people from attack by pathogenic microbes and the harmful effects of environmental toxins and carcinogens [14,15]. *β*-glucans are not synthesized by humans, so these compounds are recognized by the immune system as non-self-molecules, inducing both innate and adaptive immune responses. Most medicinal mushrooms, including *Agaricus bisporus*, *A. subrufescens*, *Cordyceps sinensis*, *Coprinus comatus*, *Ganoderma lucidum*, *Inonotus obliquus*, *Phellinus linteus*, *Pleurotus* spp, *Poria cocos*, and *Sparassis crispa*, have

been reported to have hypoglycemic effects on reducing blood glucose levels and anti-diabetic effects [16-20]. The presence or absence of fungal species is a useful indicator to assess the damage to, and maturity of, an ecosystem. In addition to knowledge of biodiversity at the community and species level, it is important for monitoring the effectiveness and effects of natural disturbances.

The purpose of the present survey was to identify local medicinal mushrooms in the southern region of Thailand, up to genus and species level, and record the 1,3-*β*-glucan content of extracts. Data concerning their diversity is important for nutraceutical production, and could be useful for local people and, industry in these areas to raise awareness about the importance of preserving local medicinal mushrooms, as well as the environments in which they live.

Materials and methods

Details of the experiment methods and process conditions are explained in **Figure 1,** below.
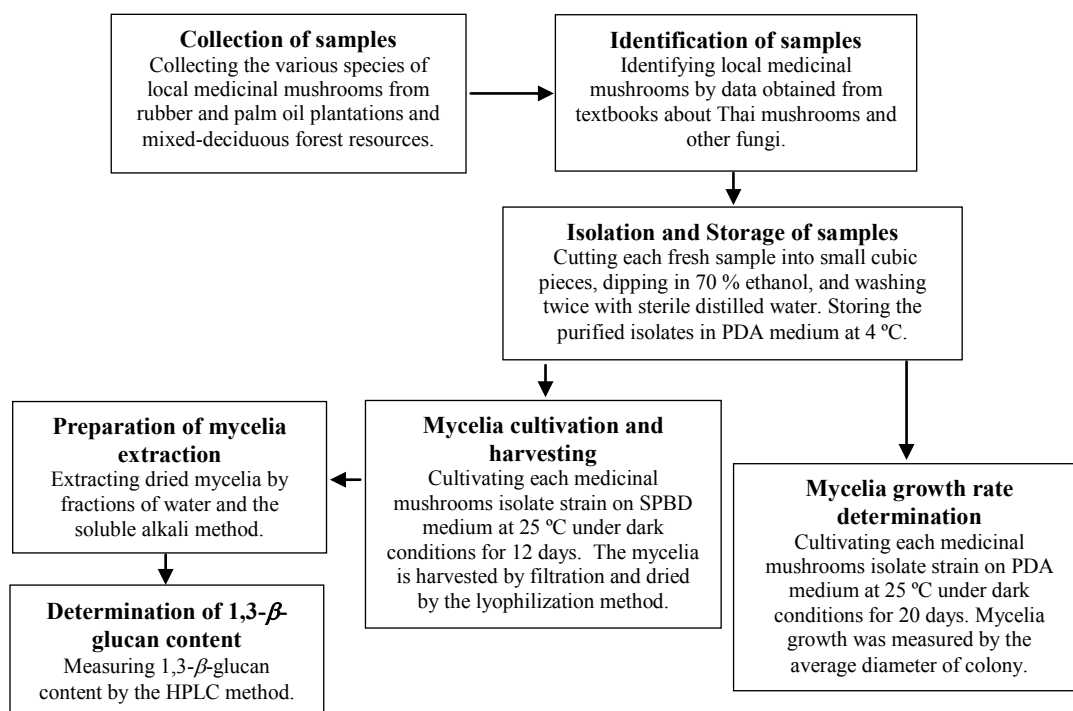

Figure 1 Flowchart diagram of experiment methods and process conditions for determination of 1,3-*β*-glucan content of local medicinal mushrooms from the southern region of Thailand.

Collection of samples

Various species of local medicinal mushrooms were collected from rubber and palm oil plantations and mixed-deciduous forest resources in several provinces of southern Thailand, particularly, Songkhla, Phatthalung, Trang, and Satun. The samples were collected during the rainy seasons between September - November of 2013 and May - July of 2014. The number of samples collected in each province was of at least 2 - 3 species, depending on the type of fungus found in each area. Every sample comprised complete mushroom fruiting bodies (cap, gills, tubes, and stipe). Each sample was carved out and placed into individual plastic bags for transport back to the lab for further identification and preparation. The macroscopic description notes included size, shape, color, and texture. The local medicinal mushrooms

were identified by data obtained from textbooks about Thai mushrooms and other fungi [21], as well as a checklist of mushrooms (Basidiomycetes) in Thailand [22].

Isolation and storage of the samples

Each fresh sample was cut into small cubic pieces and dipped in 70 % ethanol for a few seconds for surface disinfection. Subsequently, each sample was washed twice with sterile distilled water. The sample was then placed in a PDA (potato infusion 200 g, dextrose 20 g, agar 20 g, and distilled water 1000 ml) medium and incubated in darkness at 25 °C. The single hypha of any resulting fungal mycelia growing on the plate was transferred to a new plate. The purified isolates were stored at 4 °C for further experimentation [23,24].

Mycelia growth rate determination

PDA medium was used for determination of mycelia growth rate. Cultivation on solid media was carried out in Petri dishes (9 cm diameter). The dishes were inoculated with mycelia plugs using a cork borer (4 mm diameter) cut from the peripheral region of a mycelium colony. Cultivation on solid media was carried out at 25 °C under dark conditions for 20 days. Mycelia growth was measured with a caliper gauge along 2 diameters at right angles to one another. The average diameter for each plate was calculated. The mean mycelia growth was then calculated from 6 replicates of each treatment [25,26].

Mycelia cultivation and harvesting

Each isolate strain from the medicinal mushrooms was cultivated on a SPBD (sweet potato 25 g, peptone 1 g, vitamin B_6 0.5 g, $Cacl_2$ 1.3 g, dextrose 20 g, agar 20 g, and distilled water 1000 ml) medium by the static cultivation method [27]. Mycelia agar discs from the margin of 8-day-old colonies were isolated from the PDA medium. Two mycelia agar pellets were transferred to 100 ml SPBD media broth in 500 ml flasks and incubated in dark conditions at 25 °C for 12 days. The mycelia were harvested and separated from the media by filtration through Whatman No.1 filter paper. About 100 ml of distilled water was used to wash the filtered mycelia 3 times [28]. The filtered mycelia were dried overnight by lyophilization. The dried mycelia were kept at −20 °C until use.

Preparation of mycelia extraction

Fractions of water and soluble alkali were extracted from the dried mycelia of medicinal mushrooms (**Figure 2**). This method had been modified from Suwanno *et al*. [28]. One gram of freeze-dried mycelia was ground by mortar and pestle, with silicon dioxide (Sigma, USA) added 2 times. The mycelia powders were extracted using 5 ml of distilled water at 120 °C for 2 h. The extracts were centrifuged at 4000 rpm for 20 min, and the supernatant was collected. The mycelia residue was washed with 5 ml of distilled water, and centrifuged again. The supernatant was combined with the previously mentioned water-soluble fraction. The precipitate was extracted with 5 ml of 4 % NaOH. The samples were autoclaved at 120 °C for 2 h, followed by centrifuging at 4000 rpm for 20 min. Then, the supernatant was collected. The residue was washed with 5 ml of 4 % NaOH. The supernatant was removed by centrifugation and the sample combined with the previously mentioned alkaline-soluble fraction.

Determination of 1,3-*β*-glucan content

1,3-*β*-glucan content in the mycelia extract was determined from water and alkali soluble fraction by HPLC (Hewlett Packard, Germany) separation with a LiChrospher 100 RP-18 column (250×4 mm^2, mesh particle size 5 μm). The mobile phase was 100 % de-ionized water with a flow rate of 0.7 ml/min. Injection volume for the sample and standard solution was 20 μl. An RI detector was used for detection. Quantification was completed by comparisons with a standard solution of *β*-1,3-glucan from *Euglena gracilis* (Sigma, USA), with concentrations ranging between 100 and 5000 μg/ml used [29].

Statistical analysis

The means of the results were evaluated using analysis of variance (ANOVA). The test was used to compare the differences ($p \leq 0.05$) in *β*-1,3glucan content among the 14 samples. SPSS 22.0 software (IBM, Version 22.0, Armonk, NY, USA) was used for statistical analysis.
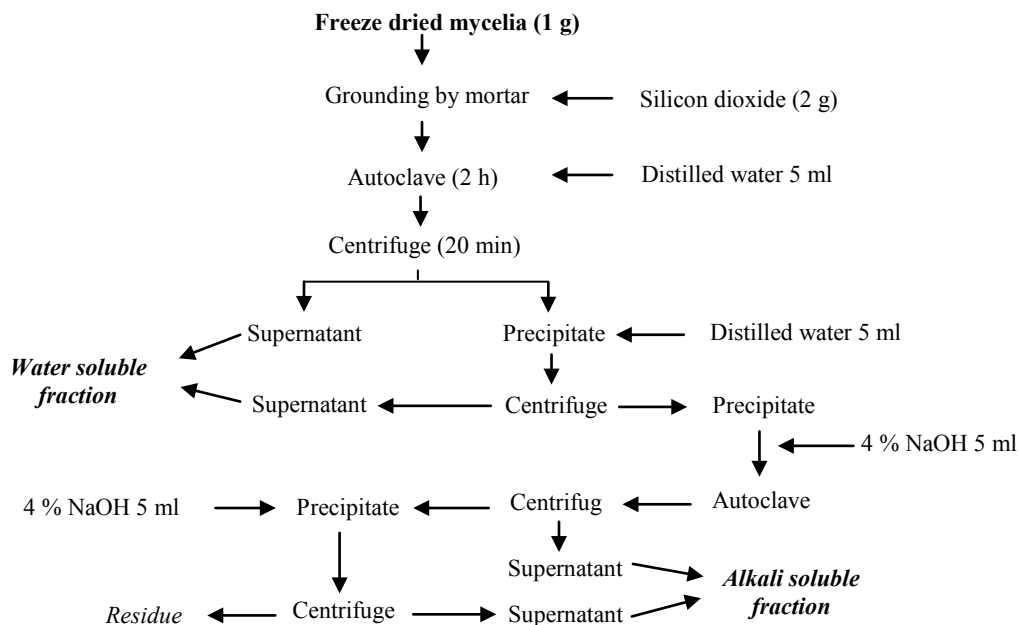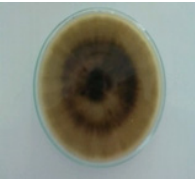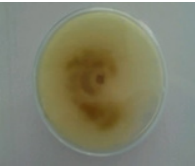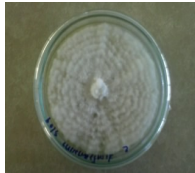

Figure 2 Scheme of isolation for polysaccharide fractions from dried mycelia of medicinal mushrooms.
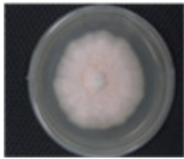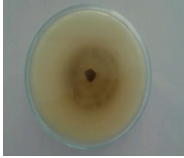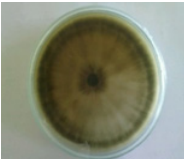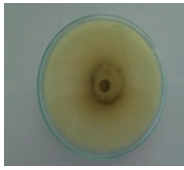
Results and discussion
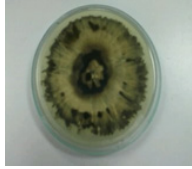
Morphology of local medicinal mushrooms

The medicinal mushroom specimens collected from the southern region of Thailand were assigned to 3 different groups, based on recognition as outlined by Thai Mushrooms and other Fungi [21] and a Checklist of Mushrooms (Basidiomycetes) in Thailand [22], for macrofungi as follows: the polypores or bracket fungi was the dominant group of the medicinal mushrooms, and included *Ganoderma calidophilum*, *Ganoderma lucidum*, *Ganoderma* sp., *Ganoderma dahlia*, *Amauroderma amoiense*, *Amauroderma rugosum*, and *Ganoderma valesiacum*; the second group included club and coral fungi. These fungi make beautiful coral-like mushrooms, including *Ramariopsis pulchella*, *Ramaria concolor*, and *Clavulina cinera*. *Xylaria* and *Daldinia* fungi are the third group of local medicinal mushrooms found in various southern areas of Thailand. The conditions for the explored locations of all medicinal mushrooms were such that the mushrooms tended to grow primarily on dead wood or brown loamy sand, used as food sources. The environmental conditions included average temperatures between 24 - 29 °C and humidity at 59.5 - 68.5 % [30]. The morphology of the medicinal mushrooms with different habitation is shown in **Table 1**. The *Ganoderma* genus of mushrooms was assessed in terms of their economic importance and usefulness in the traditional medicine of local people [31,32]. Moreover, the biodiversity of macrofungi in the southern region of Thailand indicates the presence of degradable, organic plant waste remains available for these fungi colonies, meaning that they have a major role in the decomposition of organic matter in this region. Hence, decreased mushroom diversity may be the result of increased human activities [33].

Table 1 Morphological data for local medicinal mushrooms collected from the southern region of Thailand.

Medicinal mushroom	Sampling area	Morphology and Utilization	Mycelia
 Ganoderma calidophilum	Hatyai, Songkhla	Pileus, kidney-shaped 3.2-3.9 cm diameter, reddish brown to dark brown surface. Stipe eccentric, 6-10 mm thick near the apex, light gray. Gray to black-tinged with yellowish, bruising pore-surface. A common medicinal mushroom.	
 Ganoderma lucidum	Hatyai, Songkhla	Pileus, kidney-shaped, reddish-brown to dark purple surface, with lacquer-like luster. The individual cap is 3.5-5.5 cm in diameter, with a thickness of about 2 cm, thickness 0.5-1.0 cm. white or light brown pore-surface. A common medicinal mushroom.	
 Ramariopsis pulchella	Rattaphum. Songkhla	The fruiting body is a widely expanding bush-shaped variety similar to coral, 4.5-8.0 cm tall and 3.5-7.0 cm wide. It is frail and easily broken. When scratched, it does not change color. Three to four sub-branches extend from the stipe and divide 2 times into more branches. The color is purplish-pink. Used as traditional medicine in some areas.	
 Ramaria concolor	Rattaphum. Songkhla	The fruiting body is a widely expanding, bush, 7-10 cm tall and 5-15 cm wide, It is shaped similar to coral, slightly wavy and with a sticky surface. When scratched, it does not change color. It has a single, creamy-white or yellowish-white, thick and fat stalk. Used as traditional medicine in some areas.	
 Clavulina cinera	Rattaphum. Songkhla	The fruiting body is grayish-brown, with narrow cylindrical stalks extensively branching into antler-like tufts, shaped similar to coral. It is frail and easily broken. When scratched, it does not change color. It is an inedible mushroom but is used as traditional medicine in some areas.	

Medicinal mushroom	Sampling area	Morphology and Utilization	Mycelia
Ganoderma dahlia	Khao Chaison, Phatthalung	Pileus is rigid, corky, dimidiate to fan-shaped, 8-16 cm diameter, serrated edge, stipe 2-3 cm long, surface caps glossy-brown to black, while the lower surface of caps turn white to gray. Used as traditional medicine in some areas.	
Amauroderma amoiense	Pa Bon, Phatthalung	Asidiocarps annual to perennial, with a short stipe-like base, attached centrally. Pileus up to 20 cm wide, woody, flabelliform or circular, upper surface almost buff dark gray to black, minutely hairy, lower surface turns white to gray. Used as traditional medicine in some areas.	
Daldinia concentrica	Pa Bon, Phatthalung	Fruiting bodies are ball-shaped hard, and friable at 2-6 cm wide. The surface pilei are smooth, at first reddish-brown, soon becoming black and somewhat shiny, perithecia small, crowded, in a single layer beneath the thin crust. Used as traditional medicine in some areas.	
Fomitopsis feei	Khuan Don, Satun	Pileus surface is pale rose-brown on young specimens and at the margin on older ones, becoming pale wood brown, glabrous, smooth to shallowly sulcata, margin acute, narrowly sterile below, rose-colored to rose-brown pore surface with age, caps 3-16 cm in diameter. Used as traditional medicine in some areas.	
Amauroderma rugosum	Muang, Satun	Pileus is rigid, corky, ladle-shaped, 3-15 cm in diameter, brown to dark brown surface, stipe eccentric, 6-10 cm long near the apex, light gray, gray to white pore-surface. A common medicinal mushroom.	
Daedaleopsis confragosa	Khuan Don, Satun	Pileus is 5 - 15 cm, broadly convex to more or less flat, fan-shaped or nearly round in outline, dry, smooth or minutely hairy, pale grayish to brown or reddish-brown, typically with zones of color. White pore surface, becoming dingy brownish with age. Used as traditional medicine in some areas.	

Medicinal mushroom	Sampling area	Morphology and Utilization	Mycelia
Xylaria polymorpha	Muang, Satun	Fruiting bodies 3-10 cm tall, up to 2.5 cm across, tough, shaped like a finger but occasionally flattened, usually with a rounded tip. The fungus produces a cluster of large, hard, black stromata. Occurrence on the wood of decaying deciduous trees. Used as traditional medicine in some areas.	
Ganoderma valesiacum	Khuan Don, Satun	Pileus is 5 - 10 cm, broadly convex to more or less flat, fan-shaped, upper surface cap tangerine, lower surface is orange colored to orange-brown with age. Occurrence on the wood of decaying deciduous trees. Used as traditional medicine in some areas.	

Mycelia growth rate

Variation in colony diameter for the 13 specimens of the local medicinal mushrooms collected from the southern region of Thailand is presented in **Figure 3**. The results showed that the best growth rates for mycelia obtained in this study for PDA medium were from *Daldinia concentrica*, *Ramaria concolor*, *Fomitopsis feei*, *Amauroderma amoiense*, *Ramariopsis pulchella*, and *Daedaleopsis confragosa*. The colony sizes varied from 6.9 - 9.0 cm in diameter at the 9th - 15th days of cultivation time. Specimens presented mycelia morphology of a cottony texture with high density, abundant growth, and differences in pigmentation (**Table 1**). Conversely, other species showed a slower rate of growth. The mycelia morphology of the species is classified into 2 types. In the first type, the characteristics of mycelia morphology showed a cottony texture, regular density, and regular growth. The isolate of this group included *Clavulina cinerea*, *Xylaria polymorpha*, and *Ganoderma* sp. The second type showed a floccose texture, with the characteristics of mycelia morphology presenting scarce growth and low density, such as for *Ganoderma dahlia*, *Ganoderma lucidum*, *Ganoderma valesiacum*, *Amauroderma rugosum*, and *Ganoderma calidophilum*. Mycelia growth rate for both groups was stopped on the 12th - 20th days of incubation time. The diameter of mycelia colonies varied from 2.3 to 6.9 cm. According to Setliff and Eudy [34], the growth of isolated fungi could be classified into high, medium, and slow growth rate. The results suggest that high growth rate of fungi cannot be assumed to indicate the ability to utilize lignin. White-rot fungi, belonging mostly to the basidiomycetes, are characterized by their ability to quickly and efficiently degrade the lignin moiety of woody tissues. Different fungal species can either modify or completely degrade all the major components of wood. Lignin biodegradation is an oxidative process, involving enzymes such as lignin peroxidase (LiP), MnP, VP, and laccase [35]. One of the most important aspects of medicinal mushrooms is related to the use of their ligninolytic systems for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for bioactive compounds and other food products and the use of their ligninolytic enzymes for the biodegradation of organopollutants [36-38]. These results suggest that the slow growth rate of local medicinal mushrooms has potential for the improvement of lignocellulosic wastes, as well as the production polysaccharides, mainly *β*-glucans.
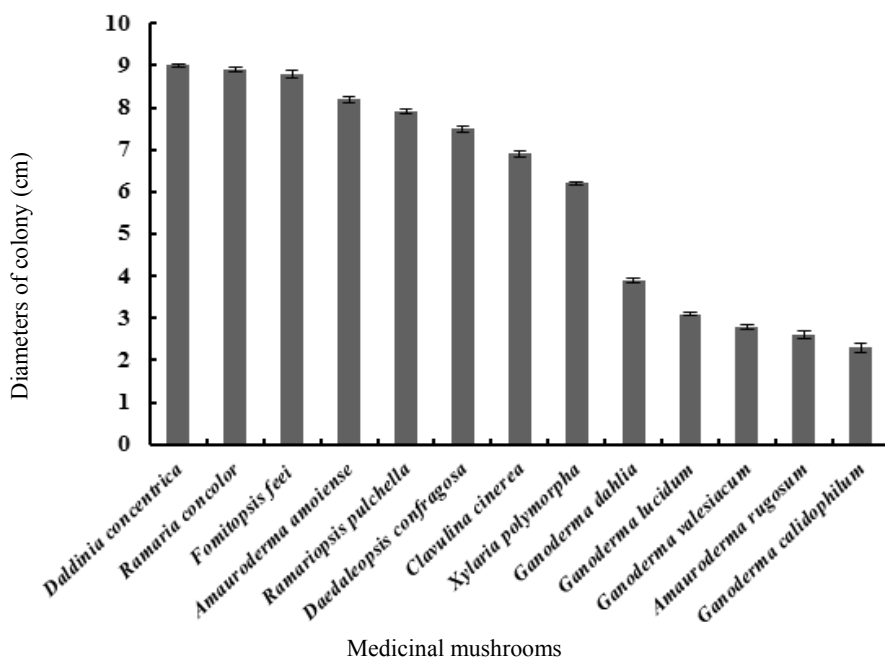
Values are given as mean ± SD from 6 replicates determination.

Figure 3 Diameter of mycelia growth rates for local medicinal mushrooms collected from the southern region of Thailand.

1,3-*β*-glucan content

Beta glucan extraction from the dried mycelia of 13 species of local medicinal mushrooms was quantified by HPLC. The amounts of 1,3-*β*-glucan content in the local medicinal mushrooms from the southern region of Thailand are shown in **Table 2**. The results showed that the values of 1,3-*β*-glucan content exhibited significant differences ($p \leq 0.05$) among all species of the local medicinal mushrooms. The 1,3-*β*-glucan content was the highest in the strains of *Ganoderma calidophilum* (90.22 mg/g) and *Amauroderma rugosum* (89.24 mg/g). However, *Ganoderma dahlia*, *Ganoderma* sp., *Ganoderma valesiacum*, and *Daedaleopsis confragosa* mushrooms were the species that contained the highest amounts of 1,3-*β*-glucan in the present work, with values for 1,3-*β*-glucan of 70.64, 58.11, 55.54, and 51.0 mg/g, respectively. Baral and Adur [39] reported that *Ganoderma* is number one among medicinal mushrooms, and has been considered the "king of medicinal mushrooms". These fungi have a long history of more than 2000 years of use. They are called the "mushrooms of immortality" in the traditional medicines of China, Japan, Korea, and other Asian nations including Thailand [3-5,11,16-19]. Some of the more efficacious compounds of *Ganoderma* are 1,6-branched and contain 1,3-*β*-glucan, which have been reported to inhibit tumor growth by stimulating the immune system via the activation of macrophage, via the balance of T helper cell populations, and subsequent effects on natural killer (NK) cells, as well as via cytokine production [3,4,15-18]. Furthermore, this work found several new specimens of local medicinal mushrooms that exhibited high amounts of 1,3-*β*-glucan content, such as in *Daedaleopsis confragosa* (51.0 mg/g). The authors [40] found that the concentration of 1,3-*β*-glucan varied from 27.0 - 89.0 mg/g in dehydrated mushrooms. These values are lower than those obtained in the present study, in which the highest 1,3-*β*-glucan concentrations were 89.24 - 94.22 mg/g. Different strains may have different amounts of 1,3-*β*-glucan. Moreover, environmental factors such as vegetation, soil

characteristics, forest stand, and microclimate, were found to be contributory to local medicinal mushrooms habitats, and accumulated significantly with bioactive compounds, including polysaccharides and *β*-glucan [41]. Nevertheless, the majority of local people lack requisite knowledge to utilize medicinal mushrooms for health-promoting benefits. This is mainly because they do not recognize medicinal mushrooms, and are unaware of their health benefits [7,30]. Therefore, more research is needed for the development of nutraceutical products to produce bioactive compounds, such as 1,3-*β*-glucan, for pharmaceutical applications.

Table 2 1,3-*β*-glucan content in local medicinal mushrooms collected from the southern region of Thailand.

Medicinal mushrooms	1,3-*β*-glucan (mg/g-dry wt.)
Daldinia concentrica	41.18±6.82[g]
Ramaria concolor	14.60±2.72[d]
Fomitopsis feei	23.88±1.91[c]
Amauroderma amoiense	25.07±2.23[b]
Ramariopsis pulchella	12.61±1.37[a]
Daedaleopsis confragosa	51.01±9.03[i]
Clavulina cinerea	40.38±9.58[f]
Xylaria polymorpha	37.33±3.03[e]
Ganoderma dahlia	70.65±11.6 [k]
Ganoderma lucidum	42.56±8.03 [h]
Ganoderma valesiacum	55.58±4.67 [j]
Amauroderma rugosum	89.24±1.43[l]
Ganoderma calidophilum	90.22±7.73[m]

Values are given as mean ± SD from triplicate determination.
Within a column, mean values with different superscripts are significantly different ($p \leq 0.05$)

Conclusions

The study conducted the survey and collection of local medicinal mushrooms between September-November of 2013 and May-July of 2014 from the Songkhla, Phatthalung, Trang, and Satun provinces in southern Thailand. The study period coincided with the local rainy season, when the temperature ranges between 24 - 29 °C and humidity ranges between 59.5 - 68.5 %. The 13 specimens of local medicinal mushrooms were identified and measured for values of 1,3-*β*-glucan content. The results showed that the values of 1,3-*β*-glucan content were significantly different ($p \leq 0.05$), from 14.6 to 90.22 mg/g for all species. It is apparent that local medicinal mushrooms have played an important role in several aspects of human existence, particularly for the local population. This is due to the properties of 1,3-*β*-glucan being recognized as a source of development for medicines and nutraceuticals. Today, nutraceuticals are a growing health care industry worldwide. As occurrence of chronic disease and awareness of preventative strategies increase, so too will the demand for nutraceuticals [12]. Such increasing demand for these nutraceutical products will raise the level of awareness among local people to preserve the local medicinal

mushrooms that grow in the mixed-deciduous forests of the southern region of Thailand. Furthermore, the local medicinal mushrooms have the potential to be used for bioconversion of lignocellulosic residues in large quantities in places where agricultural residues present a disposal problem of deterioration of the environment and loss of potentially valuable material.

Acknowledgements

References

[1]   U Kűes and Y Liu. Fruiting body production in basidiomycetes. *Appl. Microbiol. Biot*. 2000; **54**, 141-52.

[2]   JL Mau, HC Lin and CC Chen. Antioxidant properties of several medicinal mushroom. *J. Agr. Food Chem*. 2002; **50**, 6072-7.

[3]   MF Moradali, H Mostafavi, S Ghods and GA Hedjaroude. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). *Int. Immunopharmacol*. 2007; **7**, 701-24.

[4]   M Zhang, SW Cui, PCK Cheung and Q Wang. Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends Food Sci. Tech*. 2007; **18**, 4-19.

[5]   S Sing, NSK Harsh and S Chandra. Morphological studies of *Ganoderma lucidum* from different host tree species. *Int. J. Inst. Pharma. Life Sci*. 2014; **4**, 40-9.

[6]   AC Ruthes, FR Smiderle and M Iacomini. d-Glucans from edible mushrooms: A review on the extraction, purification and chemical characterization approaches. *Carbohydr. Polym*. 2015; **117**, 753-61.

[7]   A Villares, L Meteo-Vivaracho and E Guillamón. Structure features and healthy properties of polysaccharides occuring in mushrooms. *Agriculture* 2012; **2**, 452-71.

[8]   GCF Chan, WK Chan and DMY Sze. The effects of *β*-glucan on human immune and cancer cells. *J. Hematol. Oncol*. 2009; **10**, 25.

[9]   JE Rambeg, ED Nelson and RA Sinnott. Immunomudulatory dietary polysaccharides: A systematic review of the literature. *Nutr. J*. 2010; **9**, 54.

[10] C Grönndemann, M Garcia-Käufer, B Sauer, R Scheer, S Merdivan, P Bettin, R Huber and U Lindequist. Comparative chemical and biological investigations of *β*-glucan-containing products from shiikake mushroom. *J. Funct. Foods* 2015; **18**, 692-702.

[11] E Guillamón, A García-Lafuente, M Lozano, M Ďarrigo, MA Rostagno, A Villares and JA Martínez. Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia* 2010, **81**, 715-23.

[12] KS Bishop, CHJ Kao, Y Xu, MP Glucina and RRM Paterson. From 2000 years of *Ganoderma lucidum* to recent developments. *Phytochemistry* 2015; **114**, 56-65.

[13] J Chen and R Seviour. Medicinal importance of fungal *β*-(1→3),(1→6)-glucans. *Mycol. Res*. 2007; **111**, 635-52.

[14] A Hesham, E El and HK Rajni. Mushroom immunomodulators: Unique molecules with unlimited applications. *Trends Biotecnol*. 2013; **31**, 668-77.

[15] MS Mantovani, MF Bellini, JPF Angeli, RJ Oliveira, AF Silva and LR Ribeiro. *β*-glucans in promoting health: Prevention against mutation and cancer. *Mutat. Res*. 2008; **658**, 154-61.

[16] JE Smith, NJ Rowan and R Sullivan. Medicinal mushrooms: A rapidly developing area of biotechnology for cancer therapy and other bioactivities. *Biotechnol. Lett*. 2002; **24**, 1839-45.

[17] P Roupas, J Keogh, M Noakes, C Margetts and P Taylor. The role of edible mushrooms in health: Evaluation of the evidence. *J. Funct. Foods* 2012; **4**, 687-709.

[18] F Zhu, B Du, Z Bian and B Xu. Beta-glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *J. Food Compos. Anal*. 2015; **41**, 165-73.

[19] DD de Silva, S Rapior and KD Hyde. Medicinal mushrooms in prevention and control of diabetes mellitus. *Fungal Divers*. 2012; **56**, 1-29.

[20] SP Wasser. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Appl. Microbiol. Biotechnol*. 2002; **60**, 258-74.

[21] P Ruksawong. *Thai Mushrooms and Other Fungi*. National Science and Technology Capability. Bangkok, Thailand, 2001.

[22] ONEP. *Checklist of Mushrooms (Basidiomycetes) in Thailand*. Office of Natural Resources and Environmental Policy and Planning (ONEP). Bangkok, Thailand, 2011.

[23] P Stamets. *Growing Gourmet and Medicinal Mushrooms*. Ten Speed Press, Berkeley California, USA, 2000. p. 17-9.

[24] AJ Chang, J Fan and X Wen. Screening of fungi capable of highly selective degradation of lignin in rice straw. *Int. Biodeter. Biodegr*. 2012; **72**, 26-30.

[25] HJ Weitz, AL Ballard, CD Campbell and K Killham. The effect of culture conditions on the mycelial growth and luminescence of naturally bioluminescent fungi. *FEMS Microbiol. Lett*. 2001; **202**, 165-70.

[26] E Kalmis and F Kalyoncu. Mycelial growth rate of some morels (*Morchella* spp.) in four different microbiological media. *Am. Eur. J. Agr. Environ. Sci*. 2008; **3**, 861-4.

[27] S Suwanno and S Mulang. Enhancement of mycelial and polysaccharide production from *Schizophyllumm commune* by optimization of culture medium (*in Thai*). *J. Sci. Tech. MSU.* 2012; **31**, 335-42.

[28] S Suwanno, K Nakamura, Y Amano, M Shida and I Horiuchi. Development of the method for efficient disruption and rapid extraction on the *β*-1,3-glucan determination from the mycelium of *Ganoderma lucidum*. *Japanese Soc. Mushroom Sci. Biotechnol*. 2005; **13**, 83-93.

[29] AM Përez-Vendrell, J Guasch, M Francesch, JL Molina-Cano, J Brufau. Determination of *β*-(1-3), (1-4)-D-glucans in barley by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* 1995; **718**, 291-7.

[30] C Phutphat and S Suwanno. Biodiversity of medicinal mushrooms in the community forestry of upper southern part of Thailand as utilization for pharmacy. *In*: Proceedings of the 35th the IIER International Conference, Bangkok, Thailand, 2015, p. 11-4.

[31] MY Kim, P Seguin, JK Ahn, JJ Kim, SC Chun, EH Kim, SH Seo, EU Kang, SL Kim, YJ Park, HM Ro and IM Chung. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *J. Agr. Food Chem*. 2008; **56**, 7265-70.

[32] N Kalogeropoulos, AE Yanni and M Aloupi. Bioactive microconstituents and antioxidant properties of wild edible mushrooms from the island of Lesvos, Greece. *Food Chem. Toxicol*. 2013; **55**, 378-85.

[33] H Pushpa and KB Purushothama. Biodiversity of mushrooms in and around Bangalore (Karnataka), India. *Am. Eur. J. Agr. Environ. Sci*. 2012; **12**, 750-9.

[34] EC Setliff and WW Eudy. *Screening White-Rot Fungi for Their Capacity to Delignify Wood*. *In*: TIC Kirk, T Higuchi and HM Chang. (eds.). Lignin Biodegradation: Microbiology Chemistry, and Potential Applications, Vol. I. CRC Press, Boca Raton (Fia), 1980, p. 135-49.

[35] R Cohen, L Persky and Y Hadar. Biotechnological applications and potential of wood-degrading mushrooms of the genus Pleurotus. *Appl. Microbiol. Biot*. 2002; **58**, 582-94.

[36] N Silanikove, O Danai and D Levanon. Composted cotton straw silage as a substrate for *Pleurotus* sp. cultivation. *Biol. Wastes* 1988; **25**, 219-26.

[37] J Burakorn, TB Ngueyn and R Praphruet. Biological activities of a Thai luminescent mushroom. *Walailak J. Sci. Tech*. 2015; **12**, 505-14.

[38] PA Karas, S Makri, ES Papadopoulou, C Ehaliotis, U Menkissoglu-Spiroudi and DG Karpouzas. The potential of organic substrates based on mushroom substrate and straw to dissipate fungicides contained in effluents from the fruit-packaging industry–Is there a role for *Pleurotus ostreatus*? *Ecotox. Environ. Safe*. 2016; **124**, 447-54.

[39] SP Baral and A Adur. Extraction of mushroom *β*-glucan and its immunomodulatory effects. *Int. J. Pharm. Biol. Sci.* 2014; **3**,160-71.

[40] RCC Toledo, MA Carvalho, LCO Lima, EVB Vilas-Boas and ES Dias. Measurement of *β*-glucan and other nutritional characteristics in distinct strains of *Agaricus subrufescens* mushrooms. *Afr. J. Biotechnol.* 2013; **12**, 6203-9.

[41] J Boonyanuphap and C Hansawasdi. Spatial distribution of Beta glucan containing wild mushroom communities in subtropical dry forest, Thailand. *Fungal Diver.* 2011; **46**, 29-42.