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Review

Comparison of the major chemical constituents and antioxidant effects in *Amauroderma rugosum* and *Ganoderma lucidum*

Renkai Li[1], Jingjing Li[1], Timothy Man-Yau Cheung[2], Bryan Siu-Yin Ho[2], George Pak-Heng Leung[1,*]

[1]*Department of Pharmacology and Pharmacy, The University of Hong Kong, Hong Kong, China;*
[2]*Tian Ran Healthcare Limited, Hong Kong*

Correspondence

Dr. George Pak-Heng Leung,

2/F, 21 Sassoon Road, Li Ka Shing Faculty of Medicine, Laboratory Block, Faculty of Medicine Building, Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong SAR, China.

Tel: +852 39176861

E-mail: gphleung@hku.hk

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Abstract

Aging is a major risk factor for many diseases, including cardiovascular diseases, neurological disorders, cancer and diabetes mellitus. Oxidative stress plays a key role in the aging process. *Amauroderma rugosum* is an edible mushroom that has rarely been studied. The aims of this study were to compare the major chemical constituents and to investigate the antioxidant effects of *Amauroderma lucidum* and *Ganoderma lucidum*. The water extract of *Amauroderma lucidum* contained a higher amount of total phenolic compounds than that of *Ganoderma lucidum*. The total polysaccharide and triterpene content in water extracts of *Amauroderma rugosum* and *Ganoderma lucidum* did not significantly differ. The water extract of *Amauroderma rugosum* demonstrated free radical scavenging capacity and could reduce doxorubicin-induced damage in H9c2 cardiomyoblasts. *Amauroderma rugosum* has stronger antioxidant and cellular protective effects than *Ganoderma lucidum*. *Amauroderma rugosum* may be beneficial in healthy aging, and further study should be encouraged.

Introduction

Aging is a progressive loss of tissue and organ functions over time. Although various hypotheses have been proposed to explain the cellular and molecular mechanisms of aging, many studies have clarified that age-associated functional loss is essentially due to the accumulation of oxidative stress-induced molecular damage [1].

The generation of free radicals and related reactive oxygen species is an inevitable consequence of aerobic life and serves many useful purposes; for instance, infectious disease might occur without them. However, when the oxidant/antioxidant balance is disrupted and tilts toward an oxidative status (a condition known as oxidative stress), harmful effects to cell survival may occur, including lipid peroxidation and oxidative modification of proteins and nucleic acids. Indeed, oxidative stress has been implicated in various pathologies including cardiovascular and neurodegenerative diseases, cancers, diabetes mellitus and cataracts, most of which are age-related.

The development of antioxidant therapeutic agents has received considerable interest, with the aim of treating or slowing the onset of aging-related diseases. Healthcare products containing edible and medicinal mushrooms such as *Ganoderma Lucidum* (also known as Lingzhi in Chinese) are very popular because they have been suggested to be beneficial in boosting the immune system. Studies on the antioxidant and anti-aging effects of *Ganoderma Lucidum* are emerging [2]. Although the beneficial effects of *Ganoderma Lucidum* have been widely studied, other similar species of mushroom might potentially be better than *Ganoderma Lucidum* or might have certain beneficial properties that *Ganoderma Lucidum* lacks.

Amauroderma rugosum is a dietary mushroom in the *Ganodermataceae* family. This mushroom has a black stipe and a white surface covered with numerous pores. A notable characteristic is that the mushroom's surface becomes red when it is scratched. Hence, it is also known as "blood Lingzhi" in Chinese. Although *Amauroderma rugosum* is commonly consumed by people in China and South Asia, few scientific studies have explored its medicinal or nutritional value. In this study, we sought to compare the major chemical constituents and antioxidant effects of *Amauroderma rugosum* and *Ganoderma Lucidum*.

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Materials and methods

Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin and 0.25% (w/v) trypsin containing 1 mM ethylenediaminetetraacetic acid were purchased from Invitrogen (Carlsbad, CA), and 6-OHDA, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), dimethyl sulfoxide and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) were purchased from Sigma Aldrich (St. Louis, MO). All chemicals were dissolved in appropriate solvents and stored at –20°C before use to maintain their chemical stability.

Reflux extraction

Fruiting bodies of *Amauroderma rugosum* and *Ganoderma Lucidum* (Figure 1) were provided by Hong Kong Ganoderma Centre Limited (Hong Kong, China), an organic farm that had been granted an organic crop production certificate by the Hong Kong Organic Resource Centre. The samples were dried in an oven and ground into powder. A reflux system for the extraction process was used to prepare the crude extract. Two grams of powdered sample was extracted with 50 mL distilled water at 95 ± 2°C for 60 min. The crude extract was centrifuged at 4000 rpm for 20 min. Afterward, the supernatant was collected, and the sample residue was re-extracted twice via the steps described above. Subsequently, all extracts were pooled, filtered and concentrated to 80 mL with a rotary evaporator. The extract was stored at -20°C until further use.

Determination of total phenolic compounds, polysaccharides and triterpenes

To measure the total phenolic content of the extracts, we added 50 µL of 10% Folin-Ciocalteu phenol reagent to 50 µL extract and incubated the mixture in the dark at room temperature for 3 min. Afterward, 100 µL of 10% Na_2CO_3 was added to the mixture for 1 h. The absorbance at 750 nm was measured with a microplate absorbance reader. Gallic acid was used as a standard phenolic compound. All determinations were expressed as mg gallic acid equivalent per g (mg GAE/g).

Before measurement of total polysaccharides, 0.1 mL extract was precipitated with 1 mL of 95% ethanol overnight at 4°C. The precipitate was collected by centrifugation at 10,000 rpm for 10 min at 4°C. Then, the precipitate was dissolved in 50 µL water. The total polysaccharide content was measured by addition of 2.5 µL phenol (80%) and then 125 µL concentrated sulfuric acid. After incubation for 10 min, the mixture was shaken and incubated at 30°C for 20 min. The absorbance at 490 nm was measured with a microplate absorbance reader with glucose as a standard. The results were expressed as mg glucose equivalent per g (mg GE/g).

To measure the total triterpenes, we transferred 100 µL extract to a 15 mL tube and evaporated it to dryness using nitrogen flow. Then, 0.4 mL 5% vanillin–acetic acid solution and 1 mL perchloric acid were added into the tube, mixed and incubated at 60°C for 15 min. Afterward, 5 mL acetic acid was added and incubated at room temperature for 15 min. The absorbance at 549 nm was measured with a microplate absorbance reader. A solution of oleanolic acid was used as the standard. The results were expressed as mg oleanolic acid equivalent per g (mg OAE/g)

DPPH assay

The free radical scavenging capacity of the extract was measured with DPPH assays. Briefly, 5 µL extract (40 mg/mL) was mixed with 195 µL of DPPH solution (24 mg/L) in a 96-well plate. The reaction proceeded in the dark for 60 min. Afterward, the absorbance of the reaction mixture at 515 nm was measured with a microplate absorbance reader.

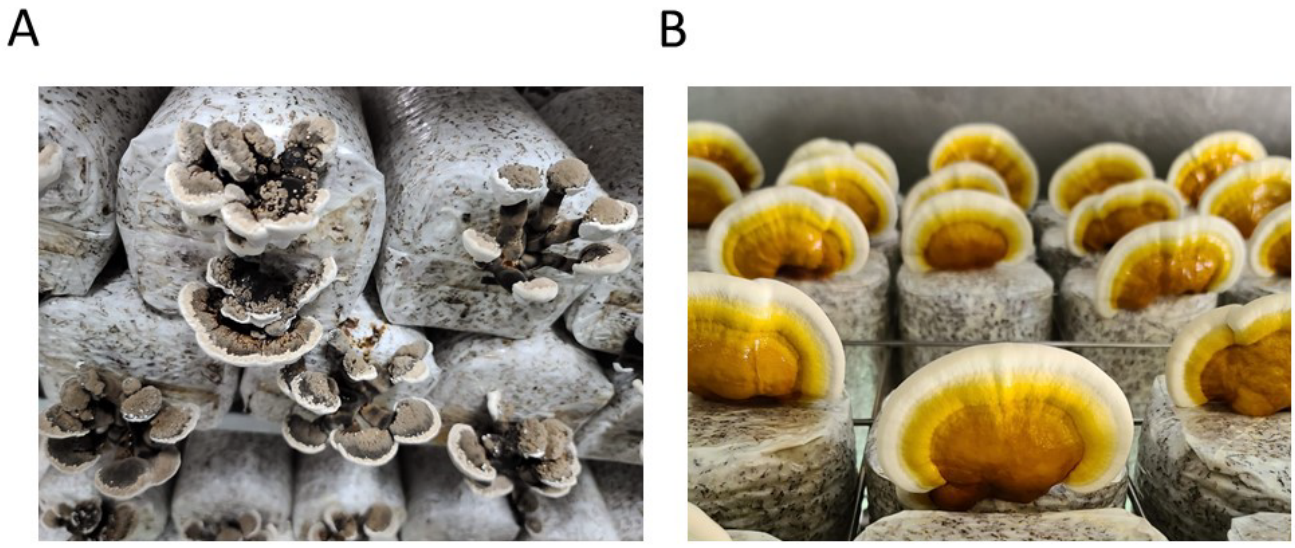

Figure 1. The typical appearance of (A) *Amauroderma Rugosum* and (B) *Ganoderma lucidum.*

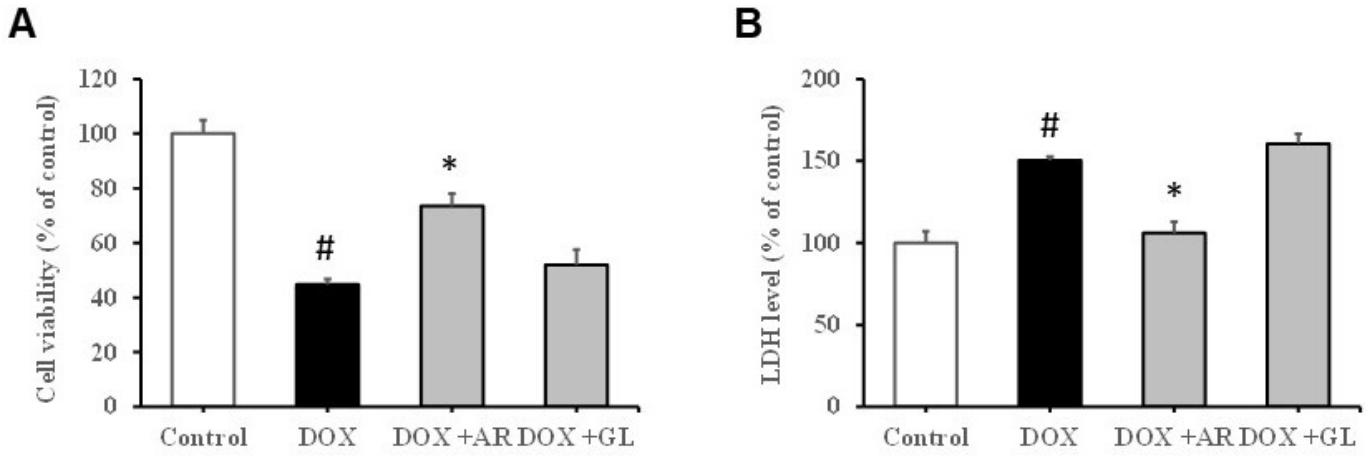

Figure 2. *Protective effects of water extract of Amauroderma rugosum and Ganoderma lucidum on H9C2 rat cardiomyoblasts under oxidative stress.* H9C2 cells were left untreated (control) or were treated with doxorubicin (Dox; 0.5 μM) in the absence or presence of 2 mg/mL water extract of Amauroderma rugosum (AR) or Ganoderma lucidum (GL) for 24 h. (A) Cell viability and (B) LDH levels were determined with MTT and biochemical assays, respectively. Values are means ± S.D. (n = 3). #p < 0.05 versus control; #p < 0.05 versus control; *p < 0.05 versus Dox.

Table 1. *Total content of phenolic compounds, polysaccharides and triterpenes in water extracts of Amauroderma rugosum and Ganoderma lucidum.* Values are means ± S.D. (n = 5). *p < 0.05 compared with Ganoderma lucidum.

	Total Contents (mg/g)		
	Phenolic compounds	**Polysaccharides**	**Triterpenes**
Amauroderma rugosum	5.529 ± 0.111*	1.119 ± 0.230	3.196 ± 0.136
Ganoderma lucidum	3.995 ± 0.076	1.352 ± 0.193	3.423 ± 0.185

Cell culture and treatment

H9c2 rat cardiomyoblasts were obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin, and then incubated at 37°C in a humidified atmosphere with 5% CO_2. For the cell viability and LDH assays, H9c2 cells in DMEM with low serum (0.5% FBS) were seeded in 96-well plates. The cells were incubated with *Amauroderma rugosum* and *Ganoderma Lucidum* extracts (40 mg/mL) for 2 h, and then treated with 0.5 μM doxorubicin for 24 h.

Cell viability assay

Cell viability was measured with MTT assays according to the manufacturer's protocol. In brief, the culture medium was discarded, and the cells were incubated with MTT solution (at a final concentration of 0.5 mg/mL) for 4 h at 37°C. Dimethyl sulfoxide was then added to lyse the cells and dissolve the violet formazan crystals that had formed inside the cells. The absorbance at 570 nm was measured with a microplate absorbance reader.

LDH assay

Cellular injury was determined by measurement of the LDH released into the culture medium. LDH activity was measured with a detection kit according to the manufacturer's instructions. The absorbance at 490 nm was measured with a microplate absorbance reader.

Data and statistical analysis

Data are expressed as the mean ± standard deviation (SD) of at least three independent experiments. Statistical analyses were performed with one-way ANOVA followed by Tukey's multiple comparison test (for two or more groups) in GraphPad Prism 6.0 software (GraphPad Software Inc., San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

Chemical content of *Amauroderma rugosum* and *Ganoderma Lucidum* extract

The major chemical composition of *Amauroderma rugosum* and *Ganoderma Lucidum* extract, including total phenolic compounds, polysaccharides and triterpenes, was measured with chemical assays (Table 1). The content of total phenolic compounds of water extracts of *Amauroderma rugosum* was 5.529 ± 0.110 mg GAE/g dry weight. This value was 38.4% higher than that of *Ganoderma Lucidum*. The total polysaccharide content of water extract of *Amauroderma rugosum* was 1.119 ± 0.230 mg GE/g dry weight, which was not statistically significant different from that of *Ganoderma Lucidum*. The total triterpene content of water extract of *Amauroderma rugosum* was 3.196 ± 0.136 mg OAE/g dry weight, which was also not statistically significantly different from that of *Ganoderma Lucidum*.

Antioxidant capacity of *Amauroderma rugosum* and *Ganoderma Lucidum* extracts in DPPH assays

The antioxidant capacity of water extracts of *Amauroderma rugosum* and *Ganoderma Lucidum*, was studied with DPPH assays. The scavenging ability of *Amauroderma rugosum* extract and *Ganoderma Lucidum* extract was 59.2 ± 1.8 and 39.4 ± 1.5%, respectively.

Protective effects of *Amauroderma rugosum* and *Ganoderma Lucidum* extracts on H9c2 cardiomyoblasts

H9c2 cells were used as a model to compare the cellular protective effects of *Amauroderma rugosum* and *Ganoderma Lucidum* extracts. The MTT assays showed that the viability of H9c2 cells decreased by 55.2% in the presence of doxorubicin but by only 26.4% when the cells were pre-incubated with *Amauroderma rugosum* extract (Figure 2A). LDH was used as a marker of cellular damage. The LDH level increased by 50.5% under doxorubicin treatment, whereas this increase was abolished by *Amauroderma rugosum* extract (Figure 2B). *Ganoderma Lucidum* extract had no effect on both the cell viability and LDH level in H9c2 cells. .

Discussion

Ganoderma Lucidum is the best-known and most popular edible and medicinal mushroom in Asia. In traditional Chinese medicine, it is used to promote health and longevity [3]. Numerous studies have demonstrated that *Ganoderma Lucidum* exerts significant beneficial effects in neurodegeneration, diabetes mellitus, cardiovascular diseases and tumor development [4]. These promising pharmacological effects of *Ganoderma Lucidum* are at least partly attributed to its antioxidant and free radical scavenging activity. For instance, *Ganoderma Lucidum* extract has been found to ameliorate MPTP-induced Parkinsonism and protect dopaminergic neurons against oxidative stress via regulating mitochondrial function, autophagy and apoptosis [5]. In a rat model, pre-administration of *Ganoderma Lucidum* has been found to prevent mitochondrial dysfunction and apoptosis of hippocampal neurons by alleviating oxidative stress [6]. Other dietary mushrooms in the *Ganodermataceae* family might also potentially exhibit beneficial effects for the prevention or treatment of oxidative stress-related diseases. *Amauroderma rugosum* is a species in the *Ganodermataceae* family whose chemical constituents and pharmacological effects have rarely been explored. Only two studies have described the chemical constituents of *Amauroderma rugosum*; these studies have reported that ethanolic extract of *Amauroderma rugosum* contains phenolic compounds [7,8]. However, no information was reported about polysaccharides and triterpenes, which are well-known active ingredients in *Ganoderma Lucidum*. Moreover, the major chemical contents of aqueous extract of *Amauroderma rugosum* had not been explored. This information is important because water decoction, but not ethanol extraction, is the traditional and most common method of consumption of those mushrooms.

In addition to chemical analysis, pharmacological studies associated with *Amauroderma rugosum* are also very rare. Only one publication has reported lipid modulating effects [9], one has reported antioxidant effects [10], one has reported anti-inflammatory effects [8], one has reported morphological assessment [11], one has reported the identification of lignin peroxidase [12], and one has reported nutritional composition [7]. In addition, one report described an acute toxicological study [13] demonstrating that *Amauroderma rugosum* is biologically safe. In that study, oral administration of a dose of *Amauroderma rugosum* powder (2000 mg/kg) had no adverse effects on the growth rate or hematological and clinical biochemical parameters in an animal model. Histological studies also showed that the treatments did not induce any pathological changes in the organs.

In the present study, the results of chemical assays demonstrated that aqueous extract of *Amauroderma rugosum* contains phenolic compounds, polysaccharides and triterpenes. The content of total polysaccharides and triterpenes of *Amauroderma rugosum* extract was comparable to that of *Ganoderma Lucidum* extract. Interestingly, the content of total phenolic compounds of *Amauroderma rugosum* extract was unexpectedly much higher than that of *Ganoderma Lucidum* extract. Indeed, numerous studies have shown that phenolic content is highly correlated with antioxidant activity [14]. Therefore, we sought to investigate whether *Amauroderma rugosum* extract might potentially have antioxidant activity. Ethyl acetate extract of *Amauroderma rugosum* mycelium has been reported to have antioxidant and anti-inflammatory effects in lipopolysaccharide-stimulated RAW 264.7 cells [7]. Our results in this study demonstrated that the aqueous extract of *Amauroderma rugosum* also exerted significant antioxidative activity in DPPH assays. In agreement with the higher phenolic content of *Amauroderma rugosum*, the antioxidant capacity of *Amauroderma rugosum* extract was stronger than that of *Ganoderma Lucidum* extract. Moreover, the antioxidant effect of *Amauroderma rugosum* extract was further examined in an *in vitro* cell model involving doxorubicin. Although doxorubicin is a highly effective anticancer drug, its clinical use is limited by its cardiotoxicity, which is due to doxorubicin-induced oxidative stress. Doxorubicin induces death of H9c2 rat cardiomyoblasts. However, this detrimental effect of doxorubicin decreased when the cells were treated with *Amauroderma rugosum* extract. The protective effect of *Amauroderma rugosum* was also stronger than that of *Ganoderma Lucidum*.

Oxidative stress is widely believed to be involved in the pathogenesis of many age-related diseases, such as

neurodegenerative diseases, cardiovascular diseases and cancer. Some antioxidants have been demonstrated to be effective in the prevention or treatment of oxidative stress-related diseases [15]. The most extensively studied antioxidants are vitamin A and its precursor β-carotene, vitamin C, and vitamin E. Several large observational studies have been conducted on the effects of the intake of different vitamins on the risk of cardiovascular diseases [15]. However, systematic reviews and meta-analyses conducted by Cochrane group investigators studying the effects of vitamins on all-cause mortality have indicated many conflicting results. In some trials, vitamins did not appear to significantly affect mortality, but in several other trials, they were administered alone or in combination and were associated with a significant increase in all-cause mortality [16]. Although the reason for these disappointing results is unclear, these findings led to the conclusion that vitamins cannot be used as effective antioxidant therapeutic agents.

N-acetylcysteine is a precursor of glutathione. Although N-acetylcysteine is considered a safe substance, the results of clinical trials have sometimes been controversial or incomplete [17]. Coenzyme Q_{10} (CoQ_{10}) is an endogenous lipid that participates in mitochondrial respiratory chain reactions. Numerous pathological processes are associated with primary and secondary CoQ_{10} deficits, including mitochondrial diseases, fibromyalgia, cardiovascular diseases, neurological disorders, cancer and diabetes mellitus. Although CoQ_{10} levels decreased with aging in humans, this effect is not seen in all species or all tissues [18]. In addition, whether lower CoQ_{10} levels have a part in aging or are merely an inconsequential cellular response to aging is unknown. Despite the current public interest in supplementation with CoQ_{10}, current evidence is insufficient to recommend CoQ_{10} supplementation as an anti-aging antioxidant therapy [18]. The effects of CoQ_{10} on specific cardiovascular risk factors, such as blood pressure, dyslipidemia, and glycemic control, are less impressive. Therefore, current evidence does not support the routine use of CoQ_{10} in patients with coronary heart disease.

Our findings in this study have significant implications. *Amauroderma rugosum* may be beneficial in healthy aging because of its excellent antioxidant and cellular protecting effects. Although the active ingredients in *Amauroderma rugosum* have not yet be defined, phenolic compounds are most likely to be involved. Polyphenols are secondary metabolites of plants, and are widely found in fruits, vegetables, cereals and beverages. An epidemiological study on French people has indicated a low incidence of cardiovascular diseases in this population despite high dietary consumption of saturated fat [19]. This paradox might be attributed to high wine consumption in this population, which provides high amounts of polyphenols, particularly resveratrol, which decreases platelet aggregation [20]. Wine and/or resveratrol can also decrease the incidence of other pathologies, such as neurodegenerative conditions, cancer and osteoporosis. Nevertheless, polyphenols are characterized by their very low bioavailability: <1% of the ingested amount reaches the plasma [21]. Large amounts of wine/polyphenols must be consumed to produce significant antioxidant effects. Therefore, the pharmacokinetic and pharmacodynamic properties of bioactive ingredients in *Amauroderma rugosum* warrant further study. Development of *Amauroderma rugosum* as a functional food product is promising, because *Amauroderma rugosum* grows quickly under cultivation conditions, and thus the production cost is relatively low.

In conclusion, our results provide the first evidence that the total phenolic compounds and antioxidant capacity of *Amauroderma rugosum* extract are higher than those of *Ganoderma Lucidum*. We propose that *Amauroderma rugosum* extract may potentially decrease the occurrence or slow the progress of oxidative stress-associated disorders.

Conflict of Interest

The authors declare no conflict of interest.

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