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Antioxidant and antimutagenic of Thai *Ganodema lucidum*

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Abstract

Thai *Ganoderma lucidum* (G2), which has been grown in Thailand as part of the Royal Project since 1988 was studied for its safety and efficacy. The present study aimed at comparing the fruiting body (whole fruiting) and mycelium of *GL* hot water extracts with regards to their *in vitro* mutagenic and antimutagenic potential by bacterial reverse mutation assay, antioxidant activity by free radical scavenging activity (DPPH assay), and iron chelating activity. The results showed that both *GL* extracts ranging from 0.15 to 3.0 mg/ml was neither mutagenic nor antimutagenic in bacterial system. Higher antioxidant activity in term of DPPH scavenging free radicals was found in fruiting body extract when compared with the mycelium extract. Moreover, both *GL* extracts showed a slight effect of chelating activity on Fe^{2+}. Significantly, the antioxidant capacity correlated with the total phenolic content. While no tested concentrations of *GL* extracts were toxic to TA98 and TA100 *salmonella typhimurium,* the highest non-mutagenic concentration was cytotoxic to lung carcinoma cell lines (A549) as determined by Trypan blue assay. This is the first comparative study on the antimutagenic and antioxidant activities of Thai *Ganoderma* fruiting body and mycelium extracts. The information obtained from the study will provide useful information for safety profile of Thai *GL* and idea to select the *GL* parts to develop *GL* products that promote health and longevity or disease prevention.

Keywords: Thai *Ganoderma lucidum;* antioxidant; antimutagenic; Ames'test; cytotoxicity

Introduction

Recently there has been greater interest in investigating compounds originating from plants and their effects on DNA. The actions of these compounds may be involved in maintaining the balance between the consumption of mutagenic and antimutagenic substances, thus contributing to increases or reductions in the incidence of cancer in the population (Ames, 1971). Compounds from plants could act as protective agents with respect to human carcinogenesis, acting against the initiation, promotion or progression stages of this process or, perhaps, destroying or blocking the DNA-damaging mutagens outside the cells, thus avoiding cell mutations. On the other hand, it is known oxidative stress is involved in variety of disorders such as cancer; hypertension, neurodegenerative (Alzheimer's and Parkinson's disease) and autoimmune diseases, and thus many antioxidant ingredients from foods or other natural sources are being challenged for diseases protection and treatment.

Ganoderma lucidum (Lingzhi) is a woody mushroom, which is highly regarded in Asian traditional medicine and is widely consumed in the belief that it promotes health and longevity. Scientific evidences shown *G. Lucidum* therapeutic effects, which including anti-inflammatory, anti-tumor, anti-viral (e.g. Anti-HIV), anti-bacterial and anti-parasitic, blood pressure regulation, cardiovascular disorder, kidney tonic, nerve tonic, sexual potentiator, chronic bronchitis, proteinuria in nephoresis. These benefits are thought to arise partly from *GL*'s role as an antioxidant. A variety of chemical ingredients of *GL* includes triterpenes, polysaccharides, nucleosides, steroids, fatty acids, alkaloids, proteins, peptides, amino acids,

and inorganic elements, which vary among geographic cultivation, species and part of the mushroom.

In this study, Thai *Ganoderma lucidum* (G2 species), which has been grown in Thailand as part of the Royal Project since 1988 was studied for its safety and efficacy. The purpose of this study was to evaluate the mutagenic and antimutagenic potential, cytotoxic, antioxidant activities and total polyphenol contents of hot water extracts from fruiting body and mycelium of Thai *GL*.

Material and methods

Fruiting body and mycelium of *GL* obtained from the Royal Project were extracted with hot water and dried by lyophilizer.

Determination of mutagenic/antimutagenic potential

The bacterial reverse mutation test (Ames' test) was used to detect mutagenicity of *GL* extracts. Five concentrations of *GL* (0.15, 0.3, 1.0, 1.5 and 3.0 mg/plate) were tested, with and without S9 supernatant, the number of revertant colonies were counted and compared to negative and positive control plates. Antimutagenicity was determined against known mutagenic substances (4-oxide-1-nitroquinoline, and 3,4 benz(a)pyrene), using the modified. Ames'test.

Determination of antioxidant activity and total polyphenol content

The DPPH radical scavenging activity of *GL* extracts was determined by spectrophotometer in terms of hydrogen donating or radical scavenging ability. The change in color was measured at 515 nm on an automated micro-plate reader. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50 % (EC 50 value). The chelating activity on Fe^{2+} was measured after the *GL* extract was reacted with $FeSO_4$ and ferrozine at 562 nm. Chelating activity was calculated as % = [(A562 nm of blank - A562 nm of sample)/A562 nm of blank] x 100. The effective concentration value (EC50) is the plot extrapolated concentration at which ferrous ions were chelated by 50 %.

The amount of total phenolics in *GL* extracts was determined with the Folin-Ciocalteu reagent using the method of Singleton and Rossi, 1965. *GL* extract was reacted with Folin-Ciocalteau's reagent and Na_2CO_3. The absorbance of sample was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

Determination of cytotoxicity

The *GL* extracts were evaluated for their cytotoxic potential using lung carcinoma cell lines (A549). Cells ($5x10^4$ cells per well) were incubated at 37 ºC for 24 h. before exposed to different concentration of *GL* extracts for 9 h. Viability of cells were determined by Trypan blue assay.

Statistical analysis

All data are presented as mean ±SD from three or more independent experiments. Statistical comparison between different groups was done by one-way ANOVA. Differences were considered significant at $p<0.05$.

Results and Discussion

No mutagenic activity was found for base-pair substitution (TA100) and frame-shift mutations (TA98) in *GL* fruiting body and mycelium hot water extracts studied. However, the extracts of these two parts against the mutagen 4NQO and Benz(a)pyrene were unable to inhibit mutagenic activity. The anti-oxidative effectt of plants is mainly due to phenolic components and the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals,

quenching singlet and triplet oxygen, or decomposing peroxides. The amount of total phenolics varied in *GL* parts and ranged from 6.65 to 31.77 mg GAE/g of dry material. In this study, higher antioxidant activity in term of DPPH scavenging free radicals was found in fruiting body extract when compared with the mycelium extract. Moreover, both *GL* extracts showed a slight effect of chelating activity on Fe^{2+}. The correlation between free radical scavenging capacity and total phenolic contents of *GL* extracts was observed. Both *GL* extracts ranging from 0.15 to 3.0 mg/ml were not cytotoxic to TA98 and TA100 *Salmonella typhimurium*, while the highest non-mutagenic concentration was cytotoxic to lung carcinoma cell lines (A549) as determined by Trypan blue assay.

Conclusion

For 4000 years *G. lucidum* has been used as a part of Chinese and Japanese medicine especially for the treatment of most of the human ailments. *Ganoderma lucidum* (G2 species) was selected to cultivate by the Royal Project because of its pharmaceutical functionality. This is the first comparative study on the antimutagenic and antioxidant activities of Thai *GL* fruiting body and mycelium extracts. The results revealed that *GL* hot water extracts showed antioxidant activity, with no mutagenicity/antimutagenicity. The present study also showed that hot water extract of *GL* fruiting body, which are often present in many *GL* products, are stronger radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial use.

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