Journal of Medicinal Plants Research Vol. 4(12), pp. 1141-1147, 18 June, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.184
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Ergothioneine accumulation in a medicinal plant *Gastrodia elata*

Eung-Jun Park*, Wi Young Lee, Seung Taek Kim, Jin Kwon Ahn and Eun Kyung Bae

Division of Forest Biotechnology, Korea Forest Research Institute, Suwon 441-847, Republic of Korea.

Accepted 2 June, 2010

Ergothioneine (ERG) has long been recognized as a potent and stable antioxidant. In nature, ERG is synthesized only in fungi and mycobacteria, not in plants. The lifecycle of *Gastrodia elata*, an achlorophyllous orchid plant, is completely dependent on the presence of symbiotic fungi, particularly *Armillaria mellea*. In this study, we report for the first time that *G. elata* accumulated ERG whose levels were correlated with the concentrations of ERG in *A. mellea*. The contents of ERG in *G. elata* were significantly higher in actively developing tissues, such as seed capsules and newly growing corms, than in mature rhizomes. The ERG levels in rhizomes were significantly correlated with antioxidant capacities and were quantitatively comparable to the previously established major pharmacological components in *G. elata*. These results indicated that ERG is a newly found functional substance in *G. elata*, which may be responsible for the beneficial health effects of *G. elata* on various human diseases.

Key words: *Gastrodia elata, Armillaria mellea*, ergothioneine, antioxidant.

INTRODUCTION

Ergothioneine (ERG) 2-mercaptohistidine trimethyl-betaine is a naturally occurring amino acid (Figure 1). In addition, it is a very stable antioxidant and is non auto-xidizable at physiologic pH (Hartman, 1990). ERG has several physiological benefits, including enhancement of metabolic energy, protection against formation of cataracts and molecular regulation of anti-inflammatory mechanisms in lungs (Shukla et al., 1981; Kawano et al., 1982; Rahman et al., 2003). Moreover, a recent study demonstrated that ERG depletion in mammalian cells causes severe oxidative stress, suggesting that ERG may represent a new vitamin due to its dietary origin and the toxicity associated with its depletion (Paul and Snyder, 2009). In nature, ERG biosynthesis is restricted to fungi and *Mycobacteria* (Melville et al., 1956; Genghof and Van Damme, 1964; Akanmu et al., 1991). ERG produced in these organisms is absorbed by plants and then passed on to animals and humans, where it accumulates at different concentrations in tissues and blood (Melville, 1958). We previously found that a number of fungal species accumulated ERG at different levels in their fruiting bodies and mycelia and that the addition of methionine to the culture medium increased the ERG content in the mycelial culture of *Ganoderma neo-japonicum* (Lee et al., 2009a; Lee et al., 2009b).

Although ERG have been found in a few plants (e.g., 0.017 mg/g in rolled oats, 0.014 mg/g in black turtle bean), the contents were very low compared to those found in fungal species (Ey et al., 2007). The rhizomes (also called corms or tubers) of *Gastrodia elata* have been used for centuries in Oriental countries as anticonvul-sants, analgesics and sedatives for the treatment of general paralysis, epilepsy, vertigo and tetanus (Hsieh et al., 2000; Kim et al., 2001; Ha et al., 2000; An et al., 2003). *G. elata*, which is an achlorophyllous orchid plant, must establish the symbiotic relationship with beneficial fungi, depending on its developmental stages such as seed germination and vegetative growth. After seed ger-mination, seed-derived protocorms should be associated with *Armillaria mellea* (Zhang and Li, 1980) to vegetative mature rhizomes. Since *A. mellea* accumulates ERG in its fruiting bodies (1.94 mg/g DW; Lee et al., 2009a), we assume that ERG may also be released into the cells of *G. elata*.

In this study, we showed for the first time that ERG was

*Corresponding author. E-mail. pahkej@forest.go.kr. Tel: +82-31-290-1167. Fax: +82-31-290-1020.

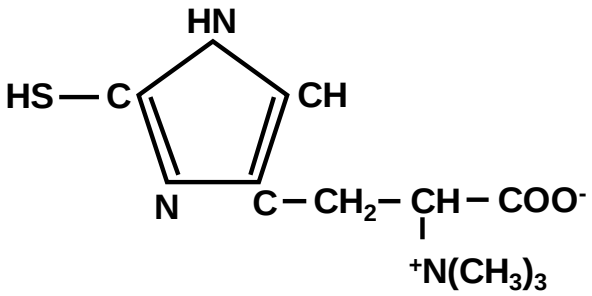

Figure 1. Chemical structure of ergothioneine.

accumulated in an achlorophyllous orchid plant, *G. elata*. In particular, we demonstrated that ERG is accumulated at higher levels in actively growing tissues compared to the mature rhizomes, suggesting that ERG may be transported from the mature rhizomes to actively growing tissues of *G. elata*. We also found that the levels of ERG were quantitatively comparable to those of other major pharmacological components in the mature rhizomes, suggesting that ERG in *G. elata* may be responsible for the beneficial health effects of *G. elata* on various human diseases.

MATERIALS AND METHODS

Sample collection and preparation

The fresh mature rhizomes of *G. elata* (at least 10 cm in length) were obtained from three different cultivation areas in Korea: Muju, Boeun and Chungju. For flower induction, they were planted in square pots (90 x 90 x 90 cm) containing sand soil and maintained at 25±1°C in the dark. After flower induction, manual pollination was conducted prior to harvesting the flower stalks and seed capsules. Plant samples including rhizomes, flower stalks and seed capsules were harvested and stored at 80°C for later use.

Rhizomorphs of *A. mellea* were collected from the same area where the mature rhizomes were collected. However, we purchased fruiting bodies of *A. mellea* from a local market in Seoul, Korea, because they have never found in the cultivation area of *G. elata* rhizomes. The same fungal strain was collected from Korea Forest Research Institute and then freshly grown on the Potato Dextrose Agar (PDA) plates at 27°C for two weeks. Several 10 mm^2 agar discs from cultures grown on PDA plates were then transferred to 100 ml of the fungal growth medium (Lee et al., 2007). This culture was incubated in a 250 ml Erlenmeyer flask at 25°C on a rotary shaker incubator at 100 rpm for two to three weeks. Subsequently, the mycelial culture was harvested and stored at -80°C for further analysis.

Quantitative analysis of Ergothioneine, GA and Vanillyl alcohol

The procedures used for ERG analysis were previously described (Lee et al., 2009b). The levels of other major functional components in *G. elata*, such as gastrodin (GA, p-hydroxymethylphenyl-β-D-Glucopyranoside), Vanillyl alcohol (VA) and p-hydroxybenzyl alcohol (HBA), were determined as described by Liu et al. (2005) for the extraction procedure and by Ong et al. (2007) for the subsequent HPLC analysis with Luna C_{18} column (5 µm, 21.2 × 250 mm). Although the contents of HBA were also analyzed, the results were not included due to its very limited accumulation in *Gastrodia* rhizomes (< 5 ng/g DW).

Ergosterol measurement

Quantitative analysis of ergosterol (EST) was conducted as described by Zhao et al. (2005) with modifications. Approximately 100 mg of the freeze-dried samples were weighed and transferred into a 50 ml falcon tube. 50 ml of methanol was added and sonicated for 5 min. The contents were then centrifuged at 4000 rpm for 20 min. The supernatant was transferred into a 250 ml round bottom flask. 10 g of potassium hydroxide and 20 ml of ethanol were then added to the latter and refluxed for one hour to saponify the sterol esters.

After cooling, the contents were centrifuged at 500 rpm for 30 min. The supernatant was transferred to a separatory funnel; 25 ml of water was added and fractionated three times with 40 ml of hexane. The hexane fraction was concentrated in a rotary evaporator and re-dissolved in 3 ml of methanol by sonicating.

The re-dissolved hexane fraction in methanol was filtered through a 0.2 µm membrane filter and analyzed by HPLC with a Finnigan Surveyor system (Thermo Electron, MA, USA). Twenty µl of the sample were injected per analysis and applied on a LiChrospher 100RP-18S (5 µm, 4.6 X 250 mm) at a flow rate of 2 ml/min with methanol-water (98:2). The TSP UV 3000 HR detector (Thermo Fisher Scientific, Sao Jose, CA, USA) was set at 282 nm.

Determination of radical scavenging capacity

Various concentrations of the rhizome extracts (0.1 ml) were mixed with 0.9 ml of a methanolic solution containing 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals (60 µmol/l) (Hatano et al., 1988) and then incubated for 60 min in the dark. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The total antioxidant status of the extracts was also measured using the 2,2'-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay (Re et al., 1999). Absorbance at 734 nm was measured one minute after the addition of each extract at five different concentrations. The extract concentration that provided 50% inhibition (EC_{50}; mg/ml) was calculated from the graph of the radical scavenging capacity percentage against extract concentration. Gallic acid was used as a standard.

Statistical analyses

Multiple comparison tests were performed to examine differences among the levels of ERG and EST using the Duncan's test at $P < 0.05$, while regression analysis was conducted to obtain a coefficient of determination (R^2) between the EC_{50} values and the contents of ERG in the different sizes of *G. elata* rhizomes. Statistical procedures were carried out using the SAS program (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Ergothioneine production in *A. mellea*

The entire lifecycle of *G. elata* is completely linked to symbiotic fungi, which is dependent on its developmental stages, where *Mycena osmundicola* is necessary for seed germination (Guo and Xu, 1990) and *A.*

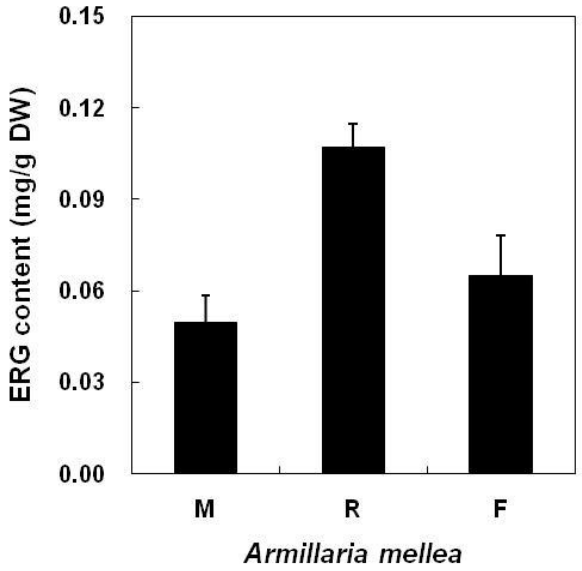

Figure 2. The ergothioneine (ERG) concentrations synthesized in the symbiotic fungus *A. mellea*. The data represents mean values ± S.D. (n = 6). *M. mycelia*; *R. rhizomorphs*; F, fruiting bodies.

(Zhang and Li, 1980) is required for vegetative rhizome growth. In this study, we used *A. mellea* (Strain number 69403; hereafter *A. mellea*), which is a main fungal strain that has been extensively used in Korea for the production of *G. elata*. First, we determined the ERG levels in various tissues of *A. mellea,* including mycelia, rhizomorphs and fruiting bodies (Figure 2). The ERG content in rhizomorphs was the highest among the tissues tested: 0.11 mg/g dry weight (DW) in rhizomorphs, 0.05 mg/g DW in mycelia and 0.07 mg/g DW in fruiting bodies (Figure 2).

This result indicated that ERG accumulated at different levels, depending on the tissues of *A. mellea*. We also previously showed that *Ganoderma* species contained at least ten times higher levels of ERG in the mycelia than in their fruiting bodies, which is in contrast to the *Lentinus* species, suggesting that the accumulated levels of ERG were different between either the tissues or the species of mushrooms (Lee et al., 2009a).

Ergothioneine accumulation in rhizomes of *Gastrodia elata*

The infection of seed-derived protocorms with *A. mellea* through its rhizomorphs is a prerequisite for their vegetative growth. After infection by rhizomorphs, the hyphae were digested and nutrients were released into cells of *G. elata* (Yang and Hu, 1990). Therefore, we assumed that ERG was also released upon hyphal digestion and the amount of ERG in *G. elata*, we determined the ERG contents in the rhizomorphs of *A. mellea* used for the production of *G. elata* at three different cultivation areas (Figure 3a). Consequently, we found that rhizomorphs contained a signifiant different amount of ERG depending on the cultivation areas: 0.09 mg/g DW in Boeun, 0.11 mg/g DW in Muju and 0.13 mg/g DW in Chungju (Figure 3a). The content of ERG was also measured in mature rhizomes (> 10 cm in length) from the corresponding areas. We found that a higher level of ERG was in rhizomorphs and more ERG was accumulated in mature rhizomes: 0.35 mg/g DW in Boeun, 0.50 mg/g DW in Muju and 0.70 mg/g DW in Chungju (Figure 3a). This result suggested that the ERG levels in mature rhizomes were dependent on the ERG contents in rhizomorphs. In previous studies, the ERG levels in either the mycelia or the fruiting bodies were altered by changing the cultivation conditions (Lee et al., 2009b; Dubost et al., 2007). Lee et al. (2009b) demonstrated that the addition of methionine into the culture medium significantly enhanced the ERG concentration in the mycelia of *Ganoderma neo-japonica*. The concentration of ERG in *Agaricus bisporus* also increased when the fungus was grown in a relatively dry substrate and when the substrate was fragmented and supplemented with the amino acid histidine, suggesting that ERG may be a stress-induced metabolite (Dubost et al., 2007). Taking together, these results indicated that the environmental conditions at different cultivation areas may stimulate ERG biosynthesis in *A. mellea* at different rates, resulting in significantly different amounts of ERG in the mature rhizomes of *G. elata*.

Since the development of *G. elata* is entirely dependent on the symbiotic fungal relationship, the effect of contamination on the ERG levels, which are imposed by *A. mellea*, must be investigated. EST is the most abundant sterol in the cell membranes of most filamentous fungi (Weete, 1989) and has been identified as a reliable indicator for active fungal biomass (Zhao and Lin, 2005). Therefore, the EST levels in both rhizomorphs and the rhizomes from three different cultivation areas were determined (Figure 3b).

The EST content in rhizomorphs ranged from 0.65 to 0.66 mg/g DW while the rhizomes contained at least 10-fold lower levels of EST (0.061 ~ 0.064 mg/g DW) compared to those in rhizomorphs. However, as shown in Figure 3a, the ERG content in the rhizomes was at least 3.9-fold higher than those in rhizomorphs from the corresponding areas. This result suggested that the contamination effects of *A. mellea* on the ERG levels in *G. elata* were not significant. In addition, either rhizomorphs or rhizomes contained similar levels of EST regardless of cultivation area (Figure 3b). Based on the results shown in Figure 3a and 2b, ERG levels in the rhizomes were not considerably affected by contamination with active fungal biomass on the surface of the rhizomes, although there

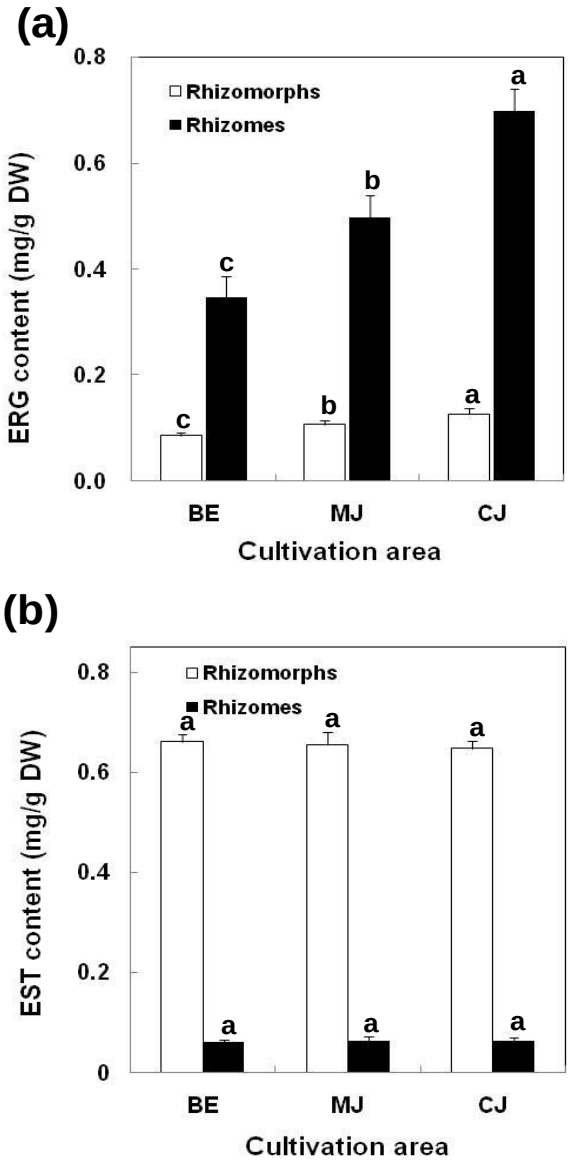

Figure 3. Quantitative analysis of both ergothioneine (ERG) in (a) and ergosterol (EST) in (b) from the rhizomorphs and rhizomes, respectively, which were collected from three different cultivation areas including Boeun (BE), Muju (MJ) and Chungju (CJ). The data represents mean values ± S.D. (n = 6). The same superscript letters indicate the corresponding components that do not differ statistically ($P < 0.05$; Duncan's test).

reasonable to suggest that ERG continuously accumulated in the rhizomes through rhizomorphs during rhizome development.

Higher accumulation of Ergothioneine in actively growing tissues

Humans accumulate ERG, which is a cell-membrane impermeative and thus requires a specific ERG receptor/ transporter OCTN1 for proper ERG function in tissues and blood (Gründemann et al., 2005). Therefore, we examined whether or not ERG is accumulated in the different tissues of *G. elata* other than rhizomes. We first observed that the highest levels of ERG were found in seed capsules, although there were differences in the ERG contents between these three cultivation areas (Figure 4a). Flower stalks also contained higher levels of ERG compared to rhizomes, indicating that ERG may be actively translocated to seed capsules through conductive organs such as flower stalks. The mature rhizome of *G. elata* is the sole source for flowering and contains all the nutrients required for flowering. We therefore assumed that during flowering, nutrients including ERG in the mature rhizome were continuously transferred through the flower stalk to seed capsules, resulting in the highest accumulation of ERG. However, since the economic values of rhizomes were severely hampered by flowering; that is rhizomes become hollow after flowering, tissues including flower stalks and seed capsules would not be used for practical sources of dietary.

The content of ERG in rhizomes of different lengths were also measured (Figure 4b). In this analysis we found that longer rhizomes had lower levels of ERG. The smallest rhizomes (less than 0.5 cm in length), that is newly growing terminal corms, contained the highest levels of ERG: 1.9 mg/g DW in Boeun, 2.2 mg/g DW in Muju, and 2.7 mg/g DW in Chungju (Figure 4b). Once the rhizomes grew to the size of Group III (> 1.0 cm in length), the ERG levels no longer changed. Consequently, the rhizomes in Group I contained 3.9 to 5.4 times higher levels of ERG compared to the mature rhizomes (Group V). These results suggested that ERG in the rhizomes of *G. elata* may be translocated to actively growing tissues, such as seed capsules and newly growing terminal corms.

Significant correlation of Ergothioneine contents with radical scavenging capacity

ERG, a water-soluble thiol compound, has been implicated in the intracellular antioxidant thiol defense system along with glutathione (Brummel and Stegink, 1993). Various antioxidant activities have been proposed for ERG, which involve the removal of cell toxic radical species or chelating of metal ions (Akanmu et al., 1991; Aruoma and Spencer, 1999; Franzoni et al., 2006). Furthermore, a recent study showed that ERG depletion caused severe oxidative stress on mitochondrial components, which suggests that ERG may represent a new treatment with physiologic roles that include antioxidant cytoprotection (Paul and Snyder, 2009).

To evaluate the beneficial health effects of rhizome-extracts containing ERG, the total antioxidant power was measured using two different chromogenic redox reagents with different standard potentials, DPPH and

ABTS. Figure 5a and 5b showed the scavenging

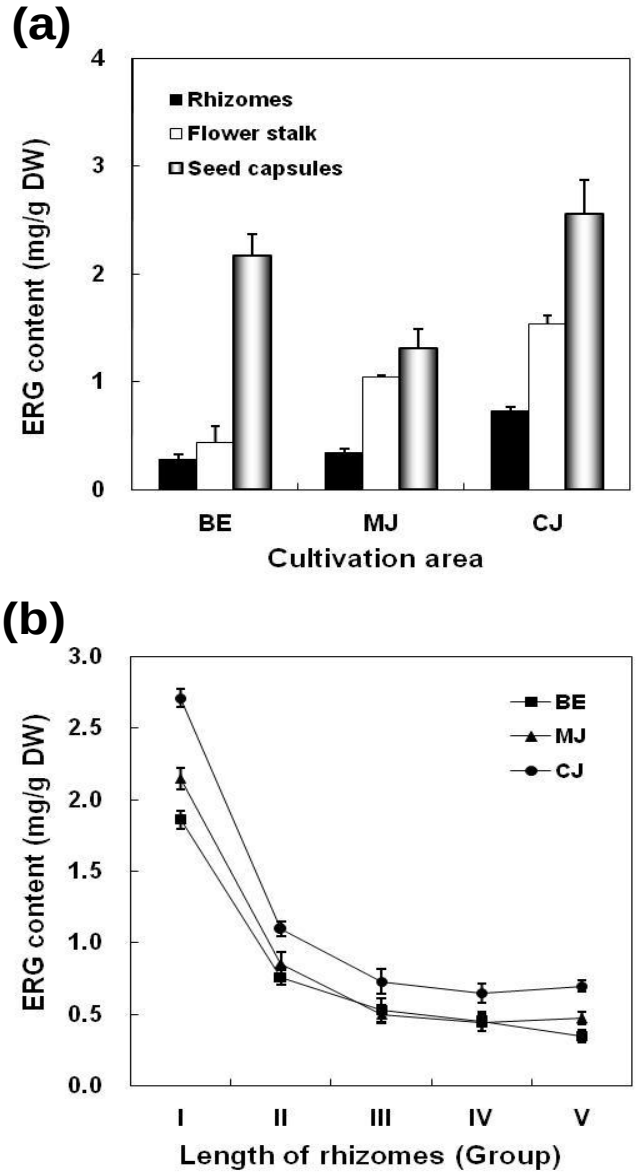

Figure 4. Ergothioneine (ERG) levels in various tissues of *G. elata* from the three different cultivation areas: Boeun (BE), Muju (MJ), and Chungju (CJ). (a) ERG contents in the mature rhizomes, flower stalks and seed capsules, (b) ERG contents in rhimozomes of different lengths from *G. elata* (Group I, < 0.5 cm; Group II, 0.5 - 1.0 cm; Group III, 1.0 - 2.0 cm; Group IV, 2.0 - 4.0 cm; Group V, > 4.0 cm). The data represents mean values ± S.D. (n = 6).

capacities of extracts obtained from rhizomes of different lengths (Group I - Group V). The radical scavenging capacity (RSC) values were expressed as the ratio percentage of sample absorbance decrease and the absorbance of either DPPH or ABTS solution without the extracts at 517 nm. The DPPH and ABTS scavenging capacities were the highest in Group I (80.3% at 4 mg/ml for DPPH and 92.3% at 1.6 mg/ml for ABTS). The RSCs for Group I on DPPH and ABTS were 21.7 and 29.3%

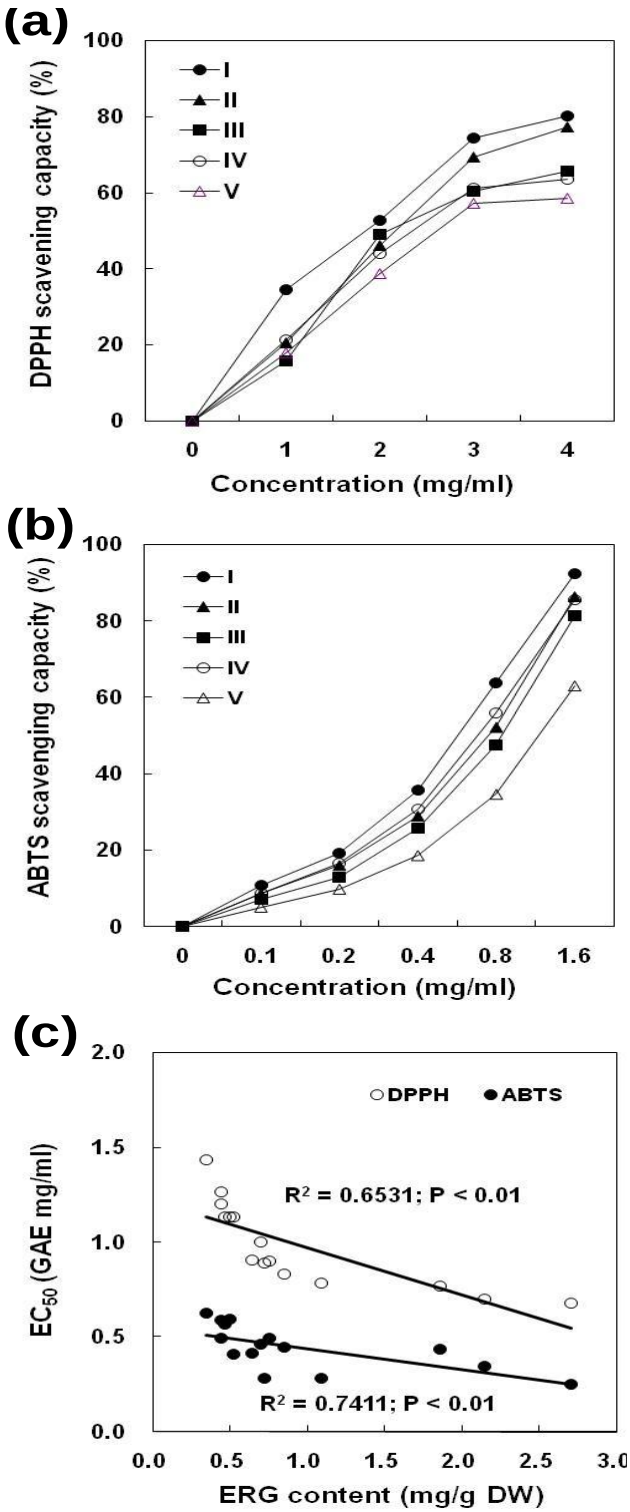

Figure 5. Antioxidant activities of the rhizome extracts containing ERG. (a) and (b) Scavenging capacities of the extracts obtained from rhizomes of different lengths (Group I, < 0.5 cm; Group II, 0.5 - 1.0 cm; Group III, 1.0 - 2.0 cm; Group IV, 2.0 - 4.0 cm; Group V, > 4.0 cm) on DPPH (A) and ABTS (B), (c) Correlation between ERG content in the rhizomes of *G.*

elata and scavenging capacity (EC_{50}) of DPPH and ABTS radicals. The data values indicate the mean ± S.D. from three independent experiments.

Table 1. Contents of three major pharmacological components in the mature rhizomes of *G. elata* collected from three different cultivation areas.

Cultivation site	Content (µg/g DW)		
	Ergothioneine	Gastrodin	Vanillyl alcohol
Boeun	472.5 ± 81.2[a]	1091.8 ± 181.7[b]	131.2 ± 8.1[c]
Muju	359.6 ± 12.3[b]	1981.0 ± 259.3[a]	151.2 ± 5.8[b]
Chungju	385.7 ± 16.7[ab]	1597.5 ± 347.0[ab]	189.4 ± 7.8[a]

The mature rhizomes of *G. elata* (> 10 cm in length) were collected from the corresponding cultivation sites. The data represents mean values ± S.D. (n = 6). The same superscript letters indicate the corresponding components that do not differ statistically ($P < 0.01$; Duncan's test).

higher than the corresponding values for Group V, respectively. Figure 5C showed the EC_{50} values obtained in the antioxidant capacity assays of the rhizome extracts containing different amounts of ERG. Significant negative linear correlations were observed between the ERG contents in the rhizome extracts and EC_{50} values of both the DPPH and ABTS scavenging capacities (determination coefficient 0.6531 for DPPH and 0.7411 for ABTS; $p < 0.01$) (Figure 5C). These negative linear correlations proved that the samples with the higher ERG levels showed higher antioxidant capacity and lower EC_{50} values.

Ergothioneine is a major pharmacological ingredient in rhizomes of *Gastrodia elata*

The major components in *G. elata* have previously shown to be GA, VA, and HBA (Ong et al., 2007). Each component has been shown to have a number of pharmacological effects on Alzheimer's disease (Liu et al., 2005), neuronal damage (Kim et al., 2001), memory consolidation and retrieval (Hsieh et al., 1997), and epileptic seizures (Hsieh et al., 2000). In order to determine if the ERG content is comparable to their levels, we verified their contents in mature rhizomes from three different cultivation areas (Table 1). Although the HBA levels were determined, the data values were not included in Table 1 due to the very limited level of accumulation (< 5 µg/g DW). Liu et al. (2005) also found that the HBA contents in *G. elata* tuber samples from three different places were 130 - 260 times lower than the GA contents. GA was the most dominant ingredient, which accumulated up to a concentration of 1983 µg/g DW, while the content of VA was significantly lower than the GA levels. ERG levels were 2.0 to 3.6 times higher than the VA levels but lower than the GA levels, indicating that ERG may be one of major components responsible for the antioxidant activity and beneficial effects of *G. elata* on human health.

Although no ERG biosynthesis has been detected in higher plants, some plants contained ERG (Ey et al., 2007). Among ERG-accumulating plants, the black turtle bean appeared to contain the maximum level of ERG (up to 13.49 µg/g wet weight), while other plants had very little or no ERG at all (Ey et al., 2007). It has been suggested that the incorporation of ERG in plants may result from the absorption of the ERG produced by microorganisms in the soil through the root system and that it is enriched in animals through ERG-containing plant and animal food (Melville et al., 1956). The highest ERG level found in the rhizomes of *G. elata* (Chungju) was 0.7 mg/g DW, which is approximately a 52-fold higher level than that in black turtle bean. This means that thus far, *G. elata* contained the highest amount of ERG compared to any other plants analyzed for their ERG contents.

In conclusion, this study showed for the first time that ERG was accumulated in *G. elata* and that its level was dependent on the level of ERG synthesized in the symbiotic fungus, *A. mellea*. The ERG levels in *G. elata* were significantly higher in actively growing tissues such as seed capsules and newly growing terminal corms, compared to mature rhizomes, suggesting possible translocation of ERG from rhizomes to actively growing tissues. Furthermore, the ERG levels in the rhizomes were quantitatively comparable to the previously established pharmacological components (such as GA, VA and HBA), indicating that ERG is a newly found functional component in *G. elata* that may be responsible for the proven antioxidant activities and beneficial effects of *G. elata* on various human diseases.

REFERENCES

Akanmu D, Cecchini R, Aruoma OI, Halliwell B (1991). The antioxidant action of ergothioneine. Arch. Biochem. Biophys., 288: 10-16.

An SJ, Park SK, Hwang IK, Choi SY, Kim SK, Kwon OS, Jung SJ, Baek NI, Lee HY, Won MH, Kang TC (2003). Gastrodin decreases immunoreactivities of gamma-aminobutyric acid shunt enzymes in the hippocampus of seizure-sensitive gerbils. J. Neurosci. Res., 71: 534-543.

Aruoma OI, Spencer JP, Mahmood N (1999). Protection against oxidative damage and cell death by the natural antioxidant ergothioneine, Food Chem. Toxicol., 37: 1043-1053.

Brummel MC, Stegink LD (1993). Ergothioneine (ERT) in foods and

human erythrocytes (RBC). FASEB J., 7: A741.
Dubost NJ, Beelman RB, Royse DJ (2007). Influence of selected cultural factors and postharvest storage on ergothioneine content of common button mushroom *Agaricus bisporus* (J. Lge) Imbach (*Agaricomycetideae*). Int. J. Med. Mushroom., 9: 163-176.

Ey J, Schömig E, Taubert D (2007). Dietary sources and antioxidant effects of ergothioneine. J. Agric. Food Chem., 55: 6466-6474.
Franzoni F, Colognato R, Galetta F, Laurenza I, Barsotti M, Di Stefano R, Bocchetti R, Regoli F, Carpi A, Balbarini A, Migliore L, Santoro G (2006). An *in vitro* study on the free radical scavenging capacity of ergothioneine: comparison with reduced glutathione, uric acid and trolox. Biomed. Pharmacother., 60: 453-457.
Genghof DS, Van Damme O (1964). Biosynthesis of ergothioneine and hercynine by mycobacteria. J. Bacteriol., 87: 852-862.
Gründemann D, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E (2005). Discovery of the ergothioneine transporter. Proc. Natl. Acad. Sci. U. S. A., 102: 5256-5261.
Guo SX, Xu JT (1990). Studies on the cell ultrastructure in the course of *Gastrodia elata* digesting *Mycena osmundicola* Lange and *Armillaria mellea* Fr. Mycosystema, 9: 218-225.
Ha JH, Lee DU, Lee JT, Kim JS, Yong CS, Kim JE, Ha JS, Huh K (2000). 4-Hydroxybenzaldehyde from *Gastrodia elata* Bl. is active in the antioxidation and GABAergic neuromodulation of the rat brain. J. Ethnopharmacol., 73: 329-333.
Hartman PE (1990). Ergothioneine as an antioxidant. Methods Enzymol., 186: 310-318.
Hatano T, Kagawa H, Yasuhara T, Okuda T (1988). Two new flavonoids and other counstituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bull., 36: 2090-2097.
Hsieh CL, Chang CH, Chiang SY, Li TC, Tang NY, Pon CZ, Hsieh CT, Lin JG (2000). Anticonvulsive and free radical scavenging activities of vanilyl alcohol in ferric chloride-induced epileptic seizures in Sprague-Dawley rats. Life Sci., 67: 1185-1195.
Hsieh MT, Wu CR, Chen CF (1997). Gastrodine and ρ-hydroxybenzyl alcohol facilitate memory consolidation and retrieval, but not acquisition on the passive avoidance task in rats. J. Ethnopharmacol., 56: 45-54.
Kawano H, Higuichi F, Mayumi T, Hama T (1982). Studies on ergothioneine. VII. Some effects of ergothioneine on glycolytic metabolism in red blood cells from rats. Chem. Pharm. Bull., (Tokyo). 30: 2611-2613.
Kim HJ, Moon KD, Oh SY, Kim SP, Lee SR (2001). Ether fraction of methanol extracts of *Gastrodia elata*, a traditional medicinal herb, protects against kainic acid-induced neuronal damage in the mouse hippocampus. Neurosci. Lett., 314: 65-68.
Lee WY, Park EJ, Ahn JK, Ka KH (2009). Ergothioneine Contents in Fruiting Bodies and Their Enhancement in Mycelial Cultures by the Addition of Methionine. Mycobiol., 37: 43-47.
Lee WY, Park EJ, Ahn JK (2009). Supplementation of methionine enhanced the ergothioneine accumulation in the *Ganoderma neo-japonicum* Mycelia. Appl. Biochem. Biotech., 158: 213-221.
Lee WY, Park Y, Ahn JK, Ka KH, Park SY (2007). Factors influencing the production of endopolysaccharide and exopolysaccharide from *Ganoderma applanatum*. Enzyme Microb. Tech., 40: 249-254.
Melville DB (1958). Ergothioneine. Vitam. Horm., 17: 155-204.
Melville DB, Genghof DS, Inamine E, Kovalenko V (1956). Ergothioneine in microorganisms. J. Biol. Chem., 223: 9-17.
Paul BD, Snyder SH (2009). The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. Cell Death Differ. DOI 10.1038/cdd.2009.163.
Rahman I, Gilmour PS, Jimenez LA, Biswas SK, Antonicelli F, Aruoma OI (2003). Ergothioneine inhibits oxidative stress- and TNF-α-induced NF-κB activation and interleukin-8 release in alveolar epithelial cells. Biochem. Biophys. Res. Commun., 302: 860-864.
Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.
Shukla Y, Kulshrestha OP, Khuteta KP (1981). Ergothioneine content in normal and senile human cataractous lenses. Indian J. Med. Res., 73: 472-473.
Zhang WL, Li BF (1980). Biological relationship of *Gastrodia elata* and *Armillaria mellea*. Acta Bot. Sinica., 22: 57-62.
Liu ZH, Hu HT, Feng GF, Zhao ZY, Mao NY (2005). Protective effects of gastrodine on the cellular model of Alzheimer's disease induced by Abeta 25-35. Sichuan Da Xue Xue Bao., 36: 537-540.
Ong ES, Heng MY, Tan SN, Yong JWH, Koh H, Teo CC, Hew CS (2007). Determination of gastrodin and vanillyl alcohol in *Gastrodia elata* Blume by pressurized liquid extraction at room temperature. J. Sep. Sci., 30: 2130-2137.
Weete JD (1989). Structure and function of sterols in fungi. Adv. Lipid Res. 23: 115-167.
Yang Z, Hu Z (1990). A preliminary study on the chitinase and β-1,3-glucanase in corms of *Gastrodia elata*. Acta Bot. Yunnanica., 12: 421-426.
Zhao XR, Lin Q, Brookes PC (2005). Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biol. Biochem., 37: 311-317.