Chemical Composition and Antioxidant Activities of *Russula griseocarnosa* sp. nov.

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Pileus and stipe of mushroom *Russula griseocarnosa* from South China were analyzed separately for chemical composition and antioxidant activities. The wild mushroom species proved to have antioxidant potential, using assays of reducing power, chelating effect on ferrous ions, scavenging effect on hydroxyl free radicals, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The mushroom contained very useful phytochemicals such as phenolics, flavonoids, ergosterol, and β-carotene. The phenolic composition of *R. griseocarnosa* was analyzed by high-performance liquid chromatography (HPLC). The major component in *R. griseocarnosa* was quercetin (95.82 μg/g). The combination of bioactive substances and rich nutritional composition (high contents in protein and carbohydrates, low content in fat) in the mushroom should be useful to consumers in encouraging them to utilize the nutritive potential of this edible wild mushroom.

INTRODUCTION

Wild mushrooms are becoming more and more important in our diet for their nutritional value, including high protein and low fat/energy contents. The fatty acid composition may also have beneficial effects on blood lipid profiles. Mushrooms have been used as food and food-flavoring materials in soups and sauces for centuries, due to their unique and subtle flavor. Recently, they have become attractive as functional foods and as a source of physiologically beneficial medicines, while being devoid of undesirable side effects (*1*). Mushrooms were found to be medically active in several therapies, such as anticancer, antiviral, immunopotentiating, and hypolipidemic activities (*2, 3*). Many of these biological functions have been attributed to their free radical scavenging and antioxidant activity. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen-derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis, and atherosclerosis as well as in degenerative processes associated with aging (*4*). Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols, and glutathione (*5*). When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur, resulting in diseases and accelerating aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage.

Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes, steroids, etc. Phenolic compounds can be classified as simple phenols and phenolic acids such as gallic acid, benzoic acid, syringic acid, chlorogenic acid, and other associates and polyphenols, which are classified into many groups such as flavonoids, tannins, and stilbenes. The antioxidant capacity of the phenolic compounds, especially gallic acid, catechin, caffeic acid, rutin, quercetin, tannic acid, and protocatechuic acid, in several mushrooms is well-known (*6−8*). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity. Phenolic compounds have significant biological and pharmacological properties, and some have demonstrated a remarkable ability to alter sulfate conjugation. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (*9*).

Russula griseocarnosa is a new species from China that was originally described by X. H. Wang in 2009. Fruit bodies of *R. griseocarnosa* have been reported as therapeutic foods, useful in preventing diseases such as anemia, dropsy, malnutrition, and excessive maternal bleeding (*10*). The cap is domed at first, later expands, and becomes concave. The cap is dark red or livid purple. The gills are adnexed. The stem is white and sometimes slightly pink. The context is grayish. Taste is mild. Subglobose basidiospores with high and acute warts. Spores white print. It grows in wooded habitats under deciduous trees. *R. griseocarnosa* is picked up every year if the weather condition is suitable for growth in South China. It is collected especially in June and September and marketed in China and abroad.

Although *R. griseocarnosa* is a well-known mycorrhizal fungus which is used as a food in China, there are no conclusive reports on chemical composition and antioxidant activities about this mushroom. Therefore, the aim of the present work is to evaluate the proximate chemical composition, amino acid and fatty acid profiles, and antioxidant activities of freshly harvested *R. griseocarnosa* fruit bodies from South China. The contents of potential

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Published on Web 04/29/2010

Article

J. Agric. Food Chem., Vol. 58, No. 11, 2010   **6967**

antioxidant components such as phenolics, flavonoids, carotenoids, and ascorbic acid and the phenolic composition of its extract by HPLC were also determined.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu's phenol reagent (FCR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT), *tert*-butylhydroquinone (TBHQ), and phenolic acid standards such as gallic acid, rutin, naringin, quercetin, tannic acid, caffeic acid, catechin, ferulic acid, gentisic acid, protocatechuic acid, hesperidin, epicatechin, and chlorogenic acid were purchased from Sigma-Aldrich (Germany). 3,5-Dinitrosalicylic acid (purity > 98.0%) and ascorbic acid (purity > 99.7%) were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade.

Sample Preparation. Fruit bodies of *R. griseocarnosa* were collected under beech in the Meizhou mountainous area (South China). Three lots of *R. griseocarnosa* that grew in the same sites at different times (within 4 months) were analyzed for all of the parameters. Each sample was oven-dried at 60 °C to constant weight and then powdered to pass through a 40 mesh sieve.

Chemical Composition. *Proximate Analysis.* Proximate analysis, including moisture, crude fat, fiber, ash, and crude protein (N × 4.38), was performed in triplicate, according to AOAC (1995) procedures. Moisture content was determined by further heating of the dried sample at 100 °C for 24 h, cooling in a desiccator, and weighing until a constant weight. Ash was determined by weighing the incinerated residue obtained at 550 °C after 3 h. Crude protein was determined by the Kjeldahl method, and a conversion factor of 4.38 was used to quantify the nitrogen percentage of the crude protein (*11*). Fat was extracted by Soxhlet extraction with petroleum ether. Crude fiber content was calculated by the ceramic fiber filter method. Reducing sugars were determined by the dinitrosalicylic acid (DNS) method. Total carbohydrates were calculated by difference.

Mineral Analysis. One gram of sample was placed in a porcelain crucible and ashed in an oven at 420−450 °C for 15−24 h. Ashed material was dissolved in 2 mL of concentrated HNO_3, evaporated to dryness, heated again to 450 °C for 3 h, dissolved in 1 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3, and diluted with distilled water up to 25 mL. A blank digest was carried out in the same way. For mineral analyses, a Hitachi Z-8000 atomic absorption spectrometer procedure reported by AOAC (1995) was used. Phosphorus content was measured by phosphorus molybdenum blue spectrophotometry. Pb and Cd in samples were determined by a HGA graphite furnace, using argon as the inert gas. Other measurements were carried out in an air/acetylene flame.

Fatty Acid Analysis. Sample (150 mg) plus heptadecanoic acid methyl ester (internal standard) was extracted with chloroform/methanol (2:1) at 60 °C for 1 h. The final extract was concentrated to 5 mL. Fatty acids in the extract were simultaneously hydrolyzed and derivatized as methyl esters with 1 mL of NaOH/methanol at 90 °C for 10 min, and then a complete derivation was assured with 1 mL of BF_3 at 90 °C for 10 min. The methyl esters were purified with 1 mL of hexane and 1 mL of water. Individual samples were passed through an anhydrous Na_2SO_4 column and then evaporated to dryness under a stream of nitrogen and redissolved in 100 μL of isooctane. The derivatized fatty acids were separated in a HP5890 Series II gas chromatograph equipped with a MS detector 5972 and a cross-linked (30 m × 0.2 mm × 0.25 μm) column with a stationary phase of 5% phenyl methyl silicone.

Amino Acid Analysis. A modified method of AOAC (1990) was used for amino acid analysis. Dry samples were hydrolyzed with 25 mL 6 N HCl at 110 °C for 24 h. Amino acid analysis was carried out by ion-exchange chromatography in an automatic amino acid analyzer (Hitachi L-8800).

Amino acid score (AAS) was calculated according to the procedure of Bano and Rajarathram (*12*).

Determination of Bioactive Compounds. For determination of phenolic compounds, protocatechuic acid was used to calculate the standard curve (absorbance = 0.0361 μg/mL protocatechuic acid + 0.0131; R^2 = 0.9989). For flavonoid content determination, rutin was used to calculate the standard curve (absorbance = 0.0164 μg/mL rutin − 0.0062; R^2 = 0.999). For ergosterol determination, ergosterol was used to calculate the standard curve (absorbance = 8.62 mg of ergosterol + 0.4862; R^2 = 0.9992). The content of ascorbic acid was calculated on the basis of the calibration

curve of ascorbic acid (absorbance = 6.689 mg of ascorbic acid − 0.6315; R^2 = 0.9928). Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = $-0.0458A_{663}$ + $0.204A_{645}$ + $0.372A_{505}$ − $0.0806A_{453}$; β-carotene (mg/100 mL) = $0.216A_{663}$ − $1.22A_{645}$ − $0.304A_{505}$ + $0.452A_{453}$.

Antioxidant Activity. *Reducing Power.* The reducing power was determined according to the method of Oyaizu (*13*). Various concentrations of mushroom methanolic extracts (2.5 mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10% trichloroacetic acid (TCA) (w/v) had been added, the mixture was centrifuged at 1000 rpm for 8 min. The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm. The extract concentration giving an absorbance of 0.5 (EC_{50}) was calculated from the graph plotting absorbance at 700 nm against extract concentration. BHT and ascorbic acid were used as standards. A higher absorbance indicates a higher reducing power.

DPPH Radical Scavenging Assay. The capacity to scavenge the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was monitored according to the method of Hatano et al. (*14*). Various concentrations of a methanolic extract of mushrooms (0.3 mL) were mixed with 2.7 mL of a methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and kept for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was measured by monitoring continuously the decrease of absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation % RSA = $100(1 - A_C/A_D)$, where A_C is the absorbance of the solution when the extract has been added at a particular level and A_D is the absorbance of the DPPH solution. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph plotting RSA percentage against extract concentration. BHT was used as standard.

Scavenging Effect on Hydroxyl Free Radicals. OH was generated by Fenton reaction and detected by its ability to salicylic acid as described by Smirnoff and Cumbes (*15*). The reaction mixture contained, in a total volume of 4 mL, 9 mM $FeSO_4$ (1 mL), 9 mM salicylic acid (1 mL), 8.8 mM H_2O_2 (1 mL), and varying concentrations of mushroom methanolic extract (1 mL). One milliliter of methanol instead of sample served as control, and the absorbance was measured spectrophotometrically at 510 nm. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of scavenging activity percentage against concentration. BHT was used for comparison.

Chelating Effect on Ferrous Ions. The chelating of ferrous ions of mushroom species was estimated by the method of Decker and Welch (*16*), with some modifications. Briefly, 0.5 mL of mushroom methanolic extracts was mixed with 0.05 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine. Total volume was diluted with 2 mL of methanol. Then, the mixture was shaken vigorously and kept at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine−Fe^{2+} complex formation was calculated using the formula:

$$\text{scavenging effect } (\%) = [A_{\text{control}} - A_{\text{sample}}/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of the ferrozine−Fe^{2+} complex and A_{sample} is the absorbance of the mushroom extract. BHT and TBHQ were used as standards.

Analysis of Phenolic Compounds. Five grams of dried mushroom powder was mixed with 30 mL of acetonitrile and 6 mL of 0.1 N hydrochloric acid and stirred for 2 h at room temperature. The suspension was filtered through No. 42 Whatman filter paper. The HPLC system consisted of a Shimadzu LC-10AT pump, a SPM-M10AVP detector, and a Hypersil BDS-C18 column (4.6 × 200 mm, 5 μm). The mobile phase was methanol (solvent A) and 0.1% phosphate (solvent B). The gradient was 0−60 min, 10%−80% A, 90%−20% B. The mobile phase was 0.1% phosphate/methanol at a flow rate of 1 mL/min, and UV detection was at 273 nm. Phenolic acid standards such as gallic acid, rutin, naringin, quercetin, tannic acid, caffeic acid, catechin, ferulic acid, gentisic acid, protocatechuic acid, hesperidin, epicatechin, and chlorogenic acid were used for the identification of phenolic acids present in the extract of

Table 1. Moisture Content and Proximate Composition of *R. griseocarnosa* (g/100 g DW)[a]

component	pileus	stipe
moisture	4.58 ± 0.13a	6.45 ± 0.02b
ash	7.84 ± 0.17a	6.59 ± 0.13b
crude fiber	8.85 ± 0.23a	11.72 ± 0.23b
crude fat	7.17 ± 0.13a	5.18 ± 0.11b
crude protein	32.31 ± 0.61a	19.11 ± 0.39b
carbohydrates	48.10 ± 0.92a	63.00 ± 0.51b
reducing sugars	2.11 ± 0.43a	1.97 ± 0.11a

[a] Each value is expressed as mean ± SD (*n* = 3). Means with different letters within a row are significantly different (*P* < 0.05).

Table 2. Mineral Composition of *R. griseocarnosa* (mg/kg DW)[a]

minerals	pileus	stipe
sodium	1340 ± 90a	530 ± 260b
potassium	19800 ± 50a	16800 ± 1870a
phosphorus	3420 ± 220a	1650 ± 120b
magnesium	570 ± 40a	380 ± 30b
calcium	850 ± 180a	3690 ± 1150b
iron	500 ± 36a	954 ± 35b
zinc	88 ± 8a	72 ± 11a
manganese	23 ± 2a	22 ± 3a
copper	48 ± 3a	34 ± 8a

[a] Each value is expressed as mean ± SD (*n* = 3). Means with different letters within a row are significantly different (*P* < 0.05).

R. griseocarnosa. Sample compounds were identified on the basis of the retention times of standard materials and were quantified by comparing their peak areas with those of standard curves.

RESULTS AND DISCUSSION

Chemical Composition. The proximate composition of *R. griseocarnosa* is shown in **Table 1**. The differences observed in the contents of proximate components seem to be related to mushroom parts. The moisture content in the mushroom was 4.58% in pileus and 6.45% in stipe. All of the following data are expressed in dry weight (DW). Carbohydrates, calculated by difference, were 49.10% in pileus and 62.68% in stipe. Reducing sugars were only a small part of carbohydrate content because polysaccharides such as chitin and starch were the most abundant mushroom carbohydrates (*17*). Ash contents of the pileus (7.84%) and the stipe (6.59%) were relatively low compared to those of *Tricholoma portentosum* (9.9%) and *Tricholoma terreum* (12.1%) reported by Díez and Alvarez (*18*). Crude fiber content of the studied mushroom was much higher in comparison to other edible mushrooms (*19*). Crude fat contents of the pileus (7.17%) and the stipe (5.18%) were close to data reported for *Russula lepida* (*20*), and most of them were constituted by unsaturated fatty acids. Crude protein contents of the pileus (32.31%) and the stipe (19.10%) seem to be well comparable with other edible mushroom species (*18*, *20*).

The knowledge of the levels of trace elements in mushrooms is necessary because of their effects on human health. Mushrooms were reported to be a good source of minerals. From the standpoint of those trace minerals that are necessary for human health, *R. griseocarnosa* seems to be a species that theoretically could provide nutritionally useful amounts of most of them, including potassium, calcium, copper, iron, magnesium, phosphorus, manganese, sodium, and zinc (**Table 2**). The levels of iron, manganese, and copper meet well the recommended dietary allowances of NRC/NAS (*21*).

The content of potassium was especially high in comparison to sodium, and the Na/K ratio is very low. This is considered to be an

Table 3. Fatty Acid Composition of *R. griseocarnosa*[a]

fatty acids	pileus	stipe
C16:0	19.30 ± 1.11a	30.87 ± 4.42b
C16:1	0.42 ± 0.04a	0.90 ± 0.11b
C16:3	0.38 ± 0.09a	2.04 ± 0.23b
C18:l	47.92 ± 6.47a	14.20 ± 2.06b
C18:2	28.40 ± 5.79a	50.22 ± 8.07b
C18:3	1.02 ± 0.11a	1.29 ± 0.16a
C20:4	2.55 ± 0.10a	0.48 ± 0.07b
total saturates	19.30	30.87
total unsaturates	80.69	69.13

[a] Values are expressed as percentage of total fatty acids. Each value is expressed as mean ± SD (*n* = 3). Means with different letters within a row are significantly different (*P* < 0.05).

advantage from the nutritional point of view, since the intake of sodium chloride and diets with a high Na/K ratio have been related to the incidence of hypertension. The contents of potassium and phosphorus were lower in *R. griseocarnosa* than in other mushrooms (*22*).

In *R. griseocarnosa*, there is a higher level of iron in the stipe (954 mg/kg DW) than pileus (500 mg/kg DW). Calcium content for the pileus and stipe was 850 and 3690 mg/kg DW, respectively. Calcium is important for bone growth and muscle and neurologic function, whereas iron is a component of hemoglobin, myoglobin, and the cytochrome pigments of the respiratory chain of mitochondria. Magnesium is not only reported to be very abundant in many mushrooms, it is also very abundant in some vegetables. Dietary deficiency of magnesium which is linked with ischemic heart disease (*23*) could possibly be overcome, or prevented, by regular consumption of mushrooms. The divalent cations magnesium and manganese are cofactors for many enzymes. *R. griseocarnosa* which contained large amounts of magnesium in the pileus (570 mg/kg DW) than stipe (380 mg/kg DW) could be a good source of magnesium. Manganese level was very similar in pileus (23 mg/kg DW) and stipe (22 mg/kg DW).

Zinc, a trace mineral that is especially important for the normal functioning of the immune system, was relatively abundant in pileus (88 mg/kg DW) and stipe (72 mg/kg DW). Zinc levels are in agreement with previous studies (*24*).

R. griseocarnosa contained a lot of copper, both in pileus (48 mg/kg DW) and in stipe (34 mg/kg DW). Copper is an essential component of numerous enzymes that catalyze oxidation−reduction reactions and is required for collagen synthesis and iron mobilization. In this study, lead content of both parts was very similar and close to 2.7 mg/kg DW. Cadmium content was 0.9 mg/kg DW in pileus and 0.7 mg/kg DW in stipe. The lead and cadmium levels are below the maximum levels for lead (3.0 mg/kg DW) and cadmium (2.0 mg/kg DW) established by the Commission of the European Communities (*25*).

Table 3 shows the results of fatty acid composition of the species examined. C18:l, C18:2, and C16:0 were the main fatty acid constituents, as occurs in many other species (*26*). Essential fatty acids common to all species included C18:2 and C18:3. These two fatty acids constituted 29.4% in pileus and 51.5% in stipe of total fatty acids. Other fatty acids, for example, C16:1, C16:3, C18:3, and C20:4, were found only in minor amounts.

The data presented in **Table 3** are characterized by a high concentration of unsaturated fatty acids, with a proportion above 65% of total fatty acids. This is consistent with the observation that, in mushrooms, unsaturated fatty acids predominate over the saturated (*18*, *26*). The high concentration of unsaturated fatty acids in the wild edible mushroom is very significant from a nutritional standpoint (*11*).

The amino acid compositions of pileus and stipe proteins are shown in **Table 4**. The results showed that *R. griseocarnosa*

contained 17 known amino acids. The percentage of the essential amino acids (EAAs) in total amino acids was 48.6% in pileus and 54.9% in stipe, and the ratio of EAAs to non-EAAs was 0.9 in pileus and 1.2 in stipe, which meets well the reference values of

Table 4. Amino Acid Composition of *R. griseocarnosa* Fruiting Body Proteins (mg/g of Protein)

amino acid	pileus	stipe
glutamate	114	142
proline	100	88
alanine	46	45
glycine	46	46
arginine	49	45
aspartate	73	76
serine	36	39
histidine	24	30
lysine	60	64
cysteine + methionine	30	60
isoleucine	38	38
threonine	44	46
leucine	55	53
tyrosine + phenylalanine	66	81
valine	84	150

Table 5. Total Bioactive Compounds of *R. griseocarnosa*[a]

	pileus	stipe
flavonoids (mg/g)	8.40 ± 0.20a	3.35 ± 0.42b
ascorbic acid (mg/g)	ND[b]	ND[b]
total phenols (mg/g)	4.76 ± 0.01a	6.25 ± 0.02b
ergosterol (mg/g)	7.14 ± 0.24a	6.58 ± 0.18b
β-carotene (μg/g)	31.96 ± 2.25a	31.18 ± 0.31a
lycopene (μg/g)	ND[b]	ND[b]

[a] Each value is expressed as mean ± SD (n = 3). Means with different letters within a row are significantly different ($P < 0.05$). [b] ND: not determined.

40% and 0.6 recommended by FAO/WHO (1973). According to the amino acid scores, the first limiting amino acid is leucine (amino acid score 83). The levels of the other essential amino acids isoleucine, threonine, and valine are present in relatively large quantities when compared with the reference values recommended by FAO/WHO/UNO (1985).

Table 5 presents phenol, flavonoid, ascorbic acid, ergosterol, and carotenoid concentrations in the mushroom extract. Phenolics exhibit a wide range of biological effects including antibacterial, antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic, and vasodilatory actions (27). Phenols and flavonoids were the major antioxidant components found in the extracts. β-Carotene was found in only vestigial amounts (<0.04 mg/g); ascorbic acid and lycopene were not determined. Ergosterol contents of the pileus (7.14 mg/g) and the stipe (6.58 mg/g) were relatively high compared to other edible mushroom species (2.96−4.89 mg/g) reported by Mattila et al. (28). Ergosterol is a biological precursor (a provitamin) to vitamin D_2. It is turned into viosterol by ultraviolet light and is then converted into ergocalciferol, which is a form of vitamin D.

Antioxidant Activity. The antioxidant properties were evaluated using the pileus and stipe of the mushroom extracts, which is a complex mixture of phytochemicals with additive and synergistic effects. To screen the antioxidant properties, several chemical and biochemical assays were performed: reducing power (measuring the conversion of a Fe^{3+}−ferricyanide complex to the ferrous form), scavenging activity on DPPH radicals (measuring the decrease in DPPH radical absorption after exposure to radical scavengers), metal chelating (by measuring color reduction in the Fe^{2+}−ferrozine complex), and scavenging effect on hydroxyl free radicals (measured by the color intensity of the Fenton reaction system).

Figure 1a shows the reducing power of the mushroom methanolic extracts. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the
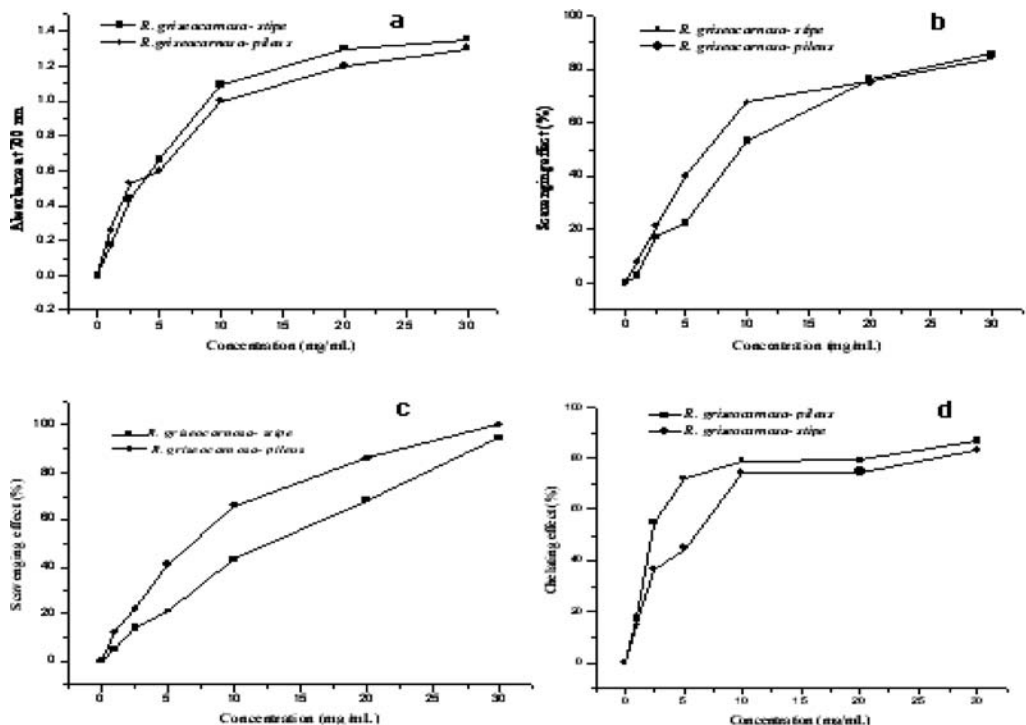

Figure 1. Antioxidant activity of methanolic extracts from *R. griseocarnosa.* (**a**) Reducing power of methanolic extracts from *R. griseocarnosa.* (**b**) Scavenging activity of methanolic extracts from *R. griseocarnosa* on DPPH radicals. (**c**) Scavenging effect of methanolic extracts from *R. griseocarnosa* on hydroxyl free radical. (**d**) Chelating effect of methanolic extracts from *R. griseocarnosa* on ferrous ions.

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reducing power of each compound. The presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}−ferricyanide complex to the ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor the Fe^{2+} concentration. Reducing powers of methanolic extracts from the mushroom were excellent and increased steadily with the increased concentrations. At 30 mg/mL, the reducing power was higher than 1.3, and the methanolic extract of *R. griseocarnosa* stipes showed a slightly higher reducing power value than *R. griseocarnosa* pileus. The reducing power of the methanolic extract of the mushroom was 0.60−0.67 at 5 mg/mL and 0.17−0.27 at 1 mg/mL. The reducing power of BHT at 0.6 mg/mL and ascorbic acid at 2.5 mg/mL was 0.58 and 0.77, respectively. Huang (*29*) reported that the methanolic extract from Chang-chih showed an excellent reducing power of 0.96−0.97 at 10 mg/mL, whereas that from Brazilian mushrooms showed a reducing power of 0.86 at 10 mg/mL.

DPPH·, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical scavenging. Scavenging effects of methanolic extracts from the mushroom on the DPPH radical increased with the increased concentrations (**Figure 1b**). At 0−20 mg/mL, the extracts obtained from the mushroom pileus scavenged DPPH radicals by higher percentages than extracts from the stipe. At 30 mg/mL, scavenging effects were 84.4% for pileus and 85.8% for stipe. However, the scavenging effect of BHT at 0.8 mg/mL was 75.9%.

The ·OH scavenging activity of mushroom extracts was assessed by its ability to compete with salicylic acid for ·OH radicals in the ·OH generating/detecting system. As shown in **Figure 1c**, as concentrations of the mushroom methanolic extracts increased, their ·OH scavenging activity increased. The scavenging effects of methanolic extracts from *R. griseocarnosa* pileus and *R. griseocarnosa* stipe on hydroxyl free radicals were highest (>90%) at 30 mg/mL. At 20 mg/mL, scavenging effects for the mushroom stipe and the mushroom pileus were 68.0% and 85.6%, respectively. In addition, at 1 mg/mL, the scavenging effect of methanolic extracts from the mushroom pileus on hydroxyl radicals was 12.2%, whereas the mushroom stipe scavenged hydroxyl radicals by 5.4%. However, the scavenging effect of BHT at 1 mg/mL was 6.1%.

The binding of ferrous ions by methanolic extracts from *R. griseocarnosa* was estimated by the method of Decker and Welch (*16*). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction therefore allows estimation of the chelating activity of the coexisting chelator.

As shown in **Figure 1d**, the formation of the Fe^{2+}−ferrozine complex was prevented by methanolic extracts of the mushroom. The absorbance of the Fe^{2+}−ferrozine complex was decreased in a linear dose-dependent manner (1, 2.5, 5, 10, 20, 30 mg/mL). At 30 mg/mL, the percentage of chelating capacity of methanolic extracts from *R. griseocarnosa* pileus and stipe was found to be 86.9% and 83.1%, respectively. At 10 mg/mL, chelating effects for the mushroom stipe and the mushroom pileus were 74.4% and 79.2%, respectively. In addition, at 2.5 mg/mL, the chelating effect of methanolic extracts from the mushroom pileus on ferrous ions was 54.9%, whereas the mushroom stipe chelated ferrous ions by 36.4%. However, at 2 mg/mL, the chelating effect of BHT and TBHQ showed 5.3% and 1.6%, respectively. The data obtained from **Figure 1d** reveal that metanolic extracts of the wild edible mushroom in this study demonstrated a

Table 6. EC_{50} Values (mg/mL) of Mushroom Extracts in the Antioxidant Activity Evaluation Assays

	pileus	stipe
reducing power (EC_{50}^{a})	2.05	2.60
DPPH (EC_{50}^{b})	11.65	13.88
hydroxyl free radicals (EC_{50}^{c})	7.13	11.80
ferrous ions (EC_{50}^{d})	2.33	5.99

^{a} EC_{50} (mg/mL): effective concentration at which the absorbance is 0.5. ^{b} EC_{50} (mg/mL): effective concentration at which 50% of DPPH radicals are scavenged. ^{c} EC_{50} (mg/mL): effective concentration at which 50% of hydroxyl free radicals are scavenged. ^{d} EC_{50} (mg/mL): effective concentration at which 50% of ferrous ions are chelated.

marked capacity for iron binding, suggesting that their action as a peroxidation protector may be related to its iron binding capacity.

Table 6 shows the EC_{50} values for the antioxidant activity assays obtained from the mushroom methanolic extracts.

Overall, *R. griseocarnosa* pileus revealed better antioxidant properties than *R. griseocarnosa* stipe (lower EC_{50} values), which is in agreement with the higher content of phenols and flavonoids found in the mushroom pileus. A relationship between the reducing power, DPPH·-scavenging activity, hydroxyl free radicals, and Fe^{2+}-chelating extent was found, indicating that the mechanisms of action of the extracts for the antioxidant activity may be identical, being related to the contents of total phenols and flavonoids. Though other antioxidants were probably present in these mushroom extracts, the amounts of ascorbic acid, β-carotene, and lycopene found in the mushroom extract were very low, which emphasizes the idea that phenolic compounds and flavonoids could make a significant contribution to the mushrooms' antioxidant activity.

Phenolic Composition of *R. griseocarnosa*. In the present study, the phenolic composition of *R. griseocarnosa* was reported for the first time. Thirteen components were analyzed, and three phenolic components were determined among them in *R. griseocarnosa*. The three components were identified as protocatechuic acid, caffeic acid, and quercetin. The analysis showed that quercetin (95.82 μg/g) was the major component in *R. griseocarnosa*. Quercetin is a strong antioxidant, as has been demonstrated by several methods to determine its free radical scavenging capacity, such as those using the reaction with the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (*30, 31*). Protocatechuic acid has been documented that this compound possesses antioxidative, antibacterial, and antimutagenic activities (*32−34*). Caffeic acid was described before in other mushroom species such as *Cantharellus cibarius*, *Agaricus bisporus*, *Lentinus edodes*, and *Pleurotus ostreatus* (*35, 36*). Also, Ribeiro et al. determined caffeic acid as a major component of the aqueous extract of *Fistulina hepatica* (*37*).

In quantitative and qualitative terms, *R. griseocarnosa* was found to be an excellent source of protein. Its amino acid composition met favorably with the World Health Organization (WHO) protein standard. It also contained considerable amounts of the two fatty acids that are essential in humans (linoleic and linolenic) and a number of minerals including iron, magnesium, and copper. The mushroom contained very useful phytochemicals such as phenolics, flavonoids, ergosterol, and β-carotene and revealed interesting antioxidant activity. Phenolic compounds and flavonoids seem to be the main compenents responsible for the antioxidant activity of the mushroom extracts. Thus, the combination of bioactive compounds and rich nutritional composition (high contents in protein and carbohydrates, low content in fat) in the mushroom makes it appropriate for functional food or as nutritional supplements.

Article

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