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Chemical Composition and Antioxidant Activity of Two Edible Mycorrhizal Fungi from South China

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Two wild edible mycorrhizal fungi (*Russula virescens* and *Cantharellus cibarius*) from South China were analyzed for the chemical composition and antioxidant activity. The two mushrooms proved to have antioxidant potential, using reducing power, chelating effect on ferrous ions, scavenging effect on hydroxyl free radicals and 2,2-diphenly-1-picrylhydrazyl (DPPH) radical scavenging method. Both species contained very useful phytochemicals such as phenolics, flavonoids and β-carotene. The combination of bioactive substances and rich nutritional composition (high contents in protein and carbohydrates, low content in fat) in the two wild mushrooms should be useful to consumers in encouraging them to utilize the nutritive potential of the wildly growing edible mushrooms.

Key Words: Wild mushrooms, Chemical composition, Antioxidant activity.

INTRODUCTION

Wild mushrooms are becoming more and more important in human diet for their nutritional value, including high protein and low fat/energy contents[1,2]. The fatty acid composition may also have beneficial effects on blood lipid profiles. Mushrooms have been used as food and food-flavouring material in soups and sauces for centuries, due to their unique and subtle flavour. Recently, they have become attractive as functional foods and as a source of physiologically beneficial medicines, while being devoid of undesirable side-effects[3]. Mushrooms were also found to be medically active in several therapies, such as anticancer, antiviral, immunopotentiating and hypolipidemic activities[4,5]. Many of these biological functions have been attributed to their free radical scavenging and antioxidant activity.

South China, due to its climatic conditions and flora diversity, is one of the Asian regions with a high diversity of wild edible mushrooms, some of them with great gastronomic relevance. *Russula virescens* and *Cantharellus cibarius* are two of the most abundant wild edible mushrooms in South China. The two species are easy to recognize and they are collected in large quantities in pine woods between June and early September, but yields are closely tied to climate. The taste and size of their fruiting bodies and their abundance are important factors when considering

these mushrooms as potential important foods, with good prospects for industrial use.

Although there are many edible wild mushroom species growing in China, their consumption is mainly confined to some rural communities. Most of them being eaten only in the rainy season when they are abundantly available. Drying of fresh mushrooms is applied in some rural communities in order to preserve them for future use. In recent times, mushrooms have begun to assume greater importance in the diets of many urban dwellers in China. Because of increasing mushroom consumption, data on their nutritional value are needed. However, little information is available about comprehensive chemical composition and antioxidant activity of the two wild mycorrhizal fungi, *Russula virescens* and *Cantharellus cibarius*. Our objective is to evaluate the proximate chemical composition, amino acid and fatty acid profiles, antioxidant properties of the two wild mushrooms from South China. The contents of potential antioxidant components such as phenolics, flavonoids, carotenoids and ascorbic acid were also determined.

EXPERIMENTAL

Folin-Ciocalteu's phenol reagent (FCR), 2,2-diphenly-1-picrylhydrazyl (DPPH), 2,6-di-*tert*-butyl-4-methylphenol (BHT) and *tert*-butylhydroquinone (TBHQ) were purchased from Sigma-Aldrich (Germany). Rutin (purity > 99.8 %), 3,5-dinitrosalicylic acid (purity > 98.0 %) and ascorbic acid (purity > 99.7 %) were bought from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). All other chemicals and reagents were analytical grade.

Sample preparation: Samples of *Russula virescens* and *Cantharellus cibarius* were collected in Yunnan (South China), in summer 2008. A lot of samples grow in the same hill were analyzed for all the parameters. Each sample was oven-dried at 60 ºC to constant weight and then powdered to pass through a 40 mesh sieve.

Chemical composition

Proximate analysis: Proximate analysis, including moisture, crude fat, fibre, ash and crude protein (N × 4.38), were performed carried out in triplicate, according to AOAC[6] procedures. Moisture contents were determined by further heating of dried samples at 100 ºC for 24 h, cooling in a desiccator and weighing them until a constant weight. Ash was determined by weighing the incinerated residue obtained at 550 ºC 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[7]. Fat was extracted by Soxhlet extraction with petroleum ether. Crude fibre content was calculated by the ceramic fibre filter method. Reducing sugars were determined by 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 ºC for 15-24 h. Ashed material was dissolved in 2 mL of concentrated HNO_3, evaporated to dryness, heated again to 450 ºC 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[6] was used. Phosphorus content was measured by phosphorus molybdenum blue spectrophotometry. Lead and cadmium in samples were determined by HGA graphite furnace, using argon as 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 ºC for 1 h. The final extract was concentrated to 5 mL. Fatty acids in the extract were simultaneously hydrolyzed and derived to their methyl esters with 1 mL of NaOH/methanol at 90 ºC for 10 min and then a complete derivation was assured with 1 mL of BF_3 at 90 ºC for 10 min. The methyl esters were purified with 1 mL of hexane and 1 mL of water. Individual samples were passed through 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 Serie 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[8] was used for amino acid analysis. Dry samples were hydrolyzed with 25 mL 6 N HCl at 110 ºC for 24 h. Amino acid analysis was carried out by ion-exchange chromatography in an automatic amino acid analyzer (Hitachi L-8800).

Determination of bioactive compounds: For phenolic compounds determination, protocatechuic acid was used to calculate the standard curve (absorbance = 0.0049 µg protocatechuic acid + 0.0083; R^2 = 0.9907). For flavonoid contents determination, rutin was used to calculate the standard curve (absorbance = 0.4894 mg rutin - 0.0121; R^2 = 0.9977). The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (absorbance = 6.689 mg 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) = -0.0458 A663 + 0.204 A645 + 0.372 A505 - 0.0806 A453; β-carotene (mg/100 mL) = 0.216 A663 - 1.22 A645 - 0.304 A505 + 0.452 A453.

Antioxidant activity

Sample preparation: The samples (*ca.* 20 g) were extracted by stirring with 100 mL of methanol at 25 ºC at 150 rpm for 24 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 mL portions of methanol, as described earlier. The combined methanolic extracts were evaporated at 40 ºC to dryness and redissolved in methanol.

Reducing power: The reducing power was determined according to the method[9]. Various concentrations of mushroom methanolic extracts (2.5 mL) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min.

After 2.5 mL of 10 % trichloro acetic 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 providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration. BHT and L-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 reported method[10]. Various concentrations of methanolic extracts from mushrooms (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and left to stand for 1 h 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 discolouration using the equation: RSA % = $100 \times (1-A_C/A_D)$, 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 of radical scavenging activity percentage against extract concentration. BHT was used as standard.

Scavenging effect on hydroxyl free radicals: $OH^{\bullet}$ was generated by Fenton reaction and detected by its ability to salicylic acid as described by Smirnoff and Cumbes[11]. The reaction mixture contained, in a total volume of 4 mL, 9 mmol/L $FeSO_4$ (1 mL), 9 mmol/L salicylic acid (1 mL), 8.8 mmol/L H_2O_2 (1 mL) and varying concentrations of mushroom methanolic extracts (1 mL). 1 mL 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 extract concentration. BHT was used for comparison.

Chelating effect on ferrous ions: The chelation of ferrous ions of mushroom species was estimated by the known method[12], with some modifications. Briefly, 0.5 mL of mushroom methanolic extracts was mixed with 0.05 mL 2 mM $FeCl_2$ and 0.2 mL 5 mM ferrozine. Total volume was diluted with 2 mL methanol. Then, the mixture was shaken vigorously and left standing 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 given below:

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

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

RESULTS AND DISCUSSION

Chemical composition: The proximate composition of the two wild mushrooms is shown in Table-1. The moisture content in the mushrooms was 4.11-5.25 %, which is in line with those obtained for other mushrooms[2]. Carbohydrates, calculated by difference, were an abundant macronutrient and accounted for 51.33 % in *Russula virescens* and 35.23 % in *Cantharellus cibarius*. Reducing sugars were only a small part of carbohydrates content because polysaccharides such as chitin and starch were the most abundant mushroom carbohydrates[13]. The ash contents of *Russula virescens* and *Cantharellus cibarius* compared favourably with other mushrooms such as *Schizophyllum commune* (8 %) and *L. entinus edodes* (6 %) reported[2]. The crude fiber contents of the studied mushrooms were much higher in comparison to some edible tropical species of mushrooms reported[14]. The crude fat contents of *Russula virescens* (1.94 %) and *Cantharellus cibarius* (2.59 %) were lower than the reported for *Tricholoma portentosum* (5.77 %) and *Tricholoma terreum* (6.64 %)[1]. Finally, the protein contents of *Russula virescens* and *Cantharellus cibarius* were 18.88 and 15.34 %, respectively, both of them higher than those obtained for other mushrooms[15].

TABLE-1
PROXIMATE COMPOSITION OF *Russula virescens* AND *Cantharellus cibarius* (g/100 g DRY WEIGHT)

Component	*R. virescens*	*C. cibarius*
Moisture	5.25 ± 0.22	4.11 ± 0.14
Ash	9.82 ± 0.23	10.39 ± 0.12
Crude fiber	10.53 ± 0.30	11.14 ± 0.14
Crude fat	1.94 ± 0.09	2.59 ± 0.38
Crude protein	18.88 ± 1.89	15.34 ± 1.93
Carbohydrates	51.33 ± 0.74	35.23 ± 1.16
Reducing sugars	8.22 ± 0.43	3.29 ± 0.36

Mineral content: Mineral concentrations varied with fungal species (Table-2). Potassium, ranging from 21900-28900 mg/kg, was the most abundant mineral found in the two mushroom samples. The contents of K were especially high in comparison to Na and Na/K ratio is very low, which is considered to be an 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. *Russula virescens* had the higher phosphorus content (4940 mg/kg) and *Cantharellus cibarius* contained 4180 mg/kg.

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. *Russula virescens* contained 3400 mg/kg calcium and 1758.5 mg/kg iron. However, *Cantharellus cibarius* contained relatively little calcium (3380 mg/kg) and much iron (2906 mg/kg).

TABLE-2
MINERAL COMPOSITION OF *Russula virescens* AND *Cantharellus cibarius* (mg/kg DRY WEIGHT)

Trace mineral	*R. virescens*	*C. cibarius*
Phosphorus	4940 ± 500	4180 ± 700
Sodium	1410 ± 140	1430 ± 320
Potassium	21900 ± 1700	28900 ± 1200
Calcium	3400 ± 700	3380 ± 200
Magnesium	1050 ± 40	1280 ± 200
Iron	1758.5 ± 51.9	2906 ± 91.4
Zinc	96.6 ± 7.9	82.8 ± 4.3
Manganese	41.0 ± 5.3	55.2 ± 7.1
Copper	43.8 ± 3.4	39.0 ± 3.8

Copper is an essential component of numerous enzymes that catalyze oxidation-reduction reactions and is required for collagen synthesis and iron mobilization. Zinc is especially important for the normal functioning of the immune system. The contents of copper and zinc were little higher in *Russula virescens* than in *Cantharellus cibarius*.

Magnesium is not only reported to be very abundant in many mushrooms, it is also very abundant in some vegetables[16]. Dietary deficiency of magnesium which is linked with ischemic heart disease[17] could possibly be overcome and or prevented by regular consumption of the mushrooms. The divalent cations magnesium and manganese are cofactors for many enzymes. The magnesium contents obtained in this study were generally in accordance with previous publications[18,19]. The higher manganese content was 55.2 mg/kg, for the species *Cantharellus cibarius*, whereas manganese content was 41.0 mg/kg, for the species *Russula virescens*. Manganese contents are in good agreement with other studies[18,20,22].

Fatty acid composition: Table-3 shows the results of fatty acid composition of the two species examined. Palmitic acid, oleic acid and linoleic acid were the main fatty acid constituents, as occurs in many other species[2,22,23]. Essential fatty

TABLE-3
FATTY ACID COMPOSITION OF
Russula virescens AND *Cantharellus cibarius*

Fatty acid	*R. virescens*	*C. cibarius*
C16:0 Palmitic acid	15.81	19.34
C16:1 Palmitolic acid	1.32	5.40
C16:3 Hiagonic acid	1.69	0.25
C18:l Oleic acid	4.96	8.33
C18:2 Linoleic acid	74.31	63.99
C18:3 Linolenic acid	1.02	2.41
C20:4Arachidonic acid	0.89	0.28
Total saturates	15.81	19.34
Total unsaturates	84.19	80.66

Values are expressed as percentage of total fatty acids.

acids common to all species included the C18:2 and C18:3. These two fatty acids constituted 75.33 and 66.40 % for *Russula virescens* and *Cantharellus cibarius*, of the total fatty acids. Other fatty acids, for example C16:1, C16:3 and C18:3, C20:4 were found only in minor amounts.

Both samples presented in Table-3 are characterized by a high concentration of unsaturated fatty acids and more than 80 % of total fatty acid content. This is consistent with the observations that, in mushrooms, unsaturated fatty acids predominate over the saturated fatty acids[1,2,22]. The high concentration of unsaturated fatty acids, particularly linoleic acid, in the wild edible mushrooms is very significant from a nutritional standpoint[7].

Amino acid composition: The amino acid compositions of *Russula virescens* and *Cantharellus cibarius* are shown in Table-4. The results showed that the two wild species contained 17 known amino acids. The percentage of the essential amino acids (EAAs) in total amino acids was 44.4 % in *Russula virescens* and 53.8 % in *Cantharellus cibarius* and the ratio of EAAs to non-EAAs was 0.8 in *Russula virescens* and 1.2 in *Cantharellus cibarius*, which meets well the reference values of 40 % and 0.6 recommended by FAO/WHO[24]. The levels of the essential amino acids histidine and valine are present in relatively large quantities exceeding the FAO/WHO[24] reference protein requirements. The phenylalanine, tyrosine, methionine and isoleucine were slightly deficient in the two mushrooms.

TABLE-4
AMINO ACID COMPOSITION OF *Russula virescens* AND *Cantharellus cibarius* FRUITING BODY PROTEINS (mg/g PROTEIN)*

Amino acid	*Russula virescens*	*Cantharellus cibarius*	Whole hen's egg
Glutamate	168.3	114.0	127.4
Proline	131.9	71.9	41.6
Alanine	88.4	50.9	59.2
Glycine	48.6	38.4	33.1
Arginine	52.0	46.2	61.0
Serine	47.3	44.8	–
Histidine	27.3	25.6	24.3
Lysine	55.6	57.3	69.8
Cysteine	25.9	25.5	24.3
Isoleucine	42.7	34.8	62.9
Threonine	52.9	47.5	51.2
Leucine	74.3	61.3	88.2
Aspartate	87.5	78.4	–
Tyrosine	25.3	30.0	41.6
Valine	145.6	181.9	68.5
Methionine	6.4	13.2	34.6
Phenylalanine	42.9	40.1	56.3

*Average values of three independent determinations.

Bioactive compounds: Table-5 presents phenol, flavonoid, ascorbic acid and carotenoid concentrations obtained in the two mushroom extracts. Phenolics

exhibit a wide range of biological effects including antibacterial, antiinflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions. Phenols were the major antioxidant components found in the extracts, followed by flavonoids (about 4.5 mg/g). β-carotene was found in only vestigial amounts (about 0.03 mg/g), ascorbic acid and lycopene was not determined.

TABLE-5
TOTAL BIOACTIVE COMPOUNDS OF TWO WILD EDIBLE MUSHROOMS

	R. virescens	*C. cibarius*
Flavonoids (mg/g)	4.27 ± 0.21	4.71 ± 0.20
Ascorbic acid (mg/g)	ND*	ND*
Total phenols (mg/g)	56.10 ± 11.86	26.55 ± 10.79
β-carotene (μg/g)	28.37 ± 2.41	30.62 ± 4.58
Lycopene (μg/g)	ND*	ND*

*ND: Not determined.

Antioxidant activity: The antioxidant properties were evaluated using the methanolic extracts of the mushrooms, 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 colour reduction in the Fe^{2+}-ferrozine complex) and scavenging effect on hydroxyl free radicals (measured by the colour intensity of Fenton reaction system).

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

The reducing power of the methanolic extract of *Russula virescens* was excellent and was 0.48 at 1 mg/mL, then dramatically increased to 0.95 as concentrations increased to 5 mg/mL and finally reached a plateau of 0.95-1.11 at 5-15 mg/mL (Fig. 1). Reducing power of methanolic extracts from *Cantharellus cibarius* increased slowly along with the increased concentrations and was 0.26, 0.63 and 0.79 at 1, 5 and 15 mg/mL, respectively. However, 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[25] reported that the methanolic extract of 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.

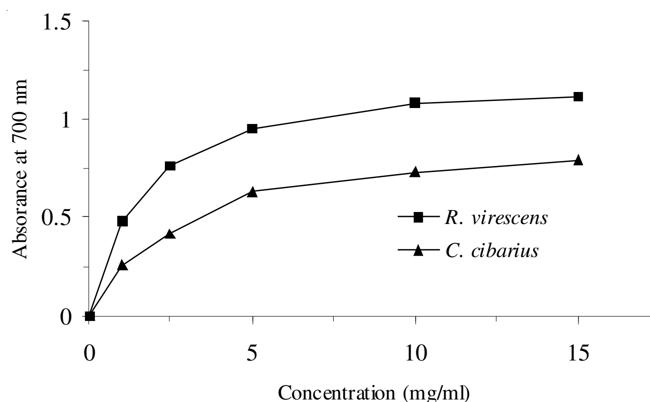

Fig. 1. Reducing power of methanolic extracts of two wild mushrooms

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 mushroom methanolic extracts on DPPH radical increased slowly with the increased concentrations (Fig. 2). At 40 mg/mL, scavenging effect was 84.45 % for *Russula virescens* and 24.94 % for *Cantharellus cibarius*. However, the scavenging effect of BHT at 0.8 mg/mL was 75.86 %. These results revealed that *Russula virescens* was free radical inhibitors or scavengers, acting possibly as primary antioxidants. *Cantharellus cibarius* was not effective in scavenging DPPH radicals.

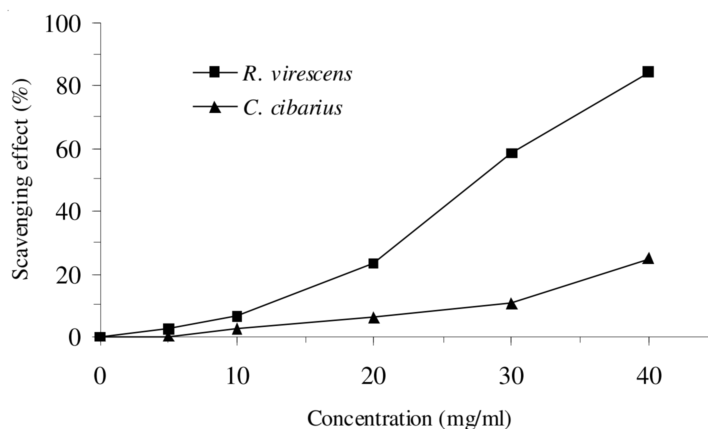

Fig. 2. Scavenging activity (%) on DPPH radicals of methanolic extracts of two wild mushrooms

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 Fig. 3, as concentrations of the methanolic extracts of the

mushroom increased, their $OH^{\bullet}$ of scavenging activity also increased. The scavenging effects of methanolic extracts from two wild mushrooms on hydroxyl free radicals were highest (> 65 %) at 15 mg/mL (Fig. 3). At 10 mg/mL, scavenging effects for *Russula virescens* and *Cantharellus cibarius* were 82.68 and 49.46 %, respectively. In addition, at 1 mg/mL, the scavenging effect of methanolic extracts from *Russula virescens* on hydroxyl radical was 12.16 %, whereas *Cantharellus cibarius* scavenged hydroxyl radical by 15.28 %. However, the scavenging effect of BHT at 1 mg/mL was 6.10 %.

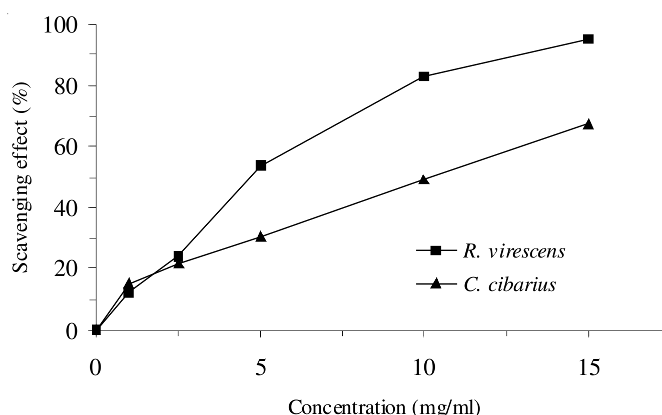

Fig. 3. Scavenging effect of methanolic extracts of two wild mushrooms on hydroxyl free radical

The binding of ferrous ions in methanolic extracts of two wild mushrooms was estimated by Decker and Welch[12]. 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 colour of the complex is decreased. Measurement of colour reduction therefore allows estimation of the chelating activity of the coexisting chelator.

As shown in Fig. 4, the formations of the Fe^{2+}-ferrozine complex were prevented by methanolic extracts of the two mushrooms. The percentage of chelating capacity of methanolic extracts from *Russula virescens* was 20.22 % at 1 mg/mL, then dramatically increased to 56.01 % as concentrations increased to 5 mg/mL and finally reached a plateau of 56.01-68.63 % at 5-20 mg/mL. Chelating effect for *Cantharellus cibarius* was 41.56 % at 1 mg/mL and increased slowly with the increased concentrations (Fig. 4). In case of 20 mg/mL the chelating effect for *Cantharellus cibarius* reached 75.46 %. However, at 2 mg/mL, the chelating effect of BHT and TBHQ showed 5.26 and 1.61 %, respectively. The data obtained from Fig. 4 reveal that the methanolic extracts of the two wild mushrooms in this study demons-trated a marked capacity for iron binding, suggesting that their action as peroxidation protector may be related to its iron binding capacity.

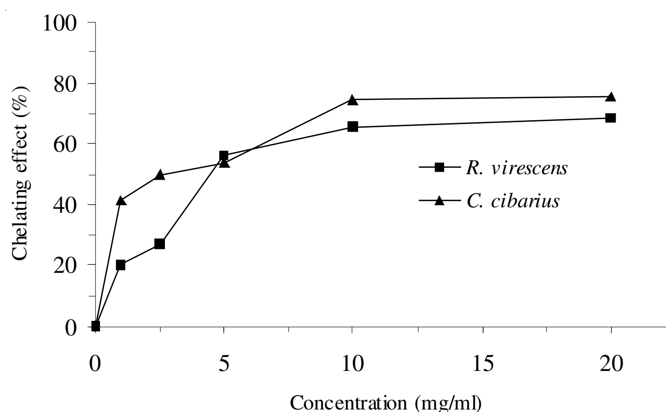

Fig. 4. Chelating effects of methanolic extracts of two wild mushrooms on ferrous ions

Table-6 shows the EC_{50} values for the antioxidant activity assays of the methanolic extracts of two mushrooms.

TABLE-6
EC_{50} VALUES[a,b,c,d] (mg/mL) OF TWO WILD MUSHROOMS EXTRACTS IN THE ANTIOXIDANT ACTIVITY EVALUATION ASSAYS

	R. virescens	*C. cibarius*
Reducing power (EC_{50}[a])	1.45	3.59
DPPH (EC_{50}[b])	29.60	> 40
Hydroxyl free radicals (EC_{50}[c])	4.73	10.27
Ferrous ions (EC_{50}[d])	4.52	2.50

[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.

Finally, *Russula virescens* revealed better antioxidant properties than *Cantharellus cibarius* (lower EC_{50} values), which is in agreement with the higher content of phenols found in the mushroom *Russula virescens*. A relationship between the reducing power, $DPPH^{\bullet}$-scavenging activity and hydroxyl free radicals 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. 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 emphasises the idea that phenolic compounds could make a significant contribution to the antioxidant activity of the mushrooms.

Conclusion

In quantitative and qualitative terms, *Russula virescens* and *Cantharellus cibarius* were found to be an excellent source of protein. Theirs amino acid composition met favourably World Health Organization (WHO) protein standard. The

two wild mushrooms contain considerable amounts of the two fatty acids that are essential in humans (linoleic acid and linolenic acid) and a number of minerals including iron, magnesium and copper. Both samples also contain useful phytochemicals such as phenolics, flavonoids and β-carotene and revealed interesting antioxidant activity. Phenolic compounds 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 two mushrooms make them appropriate for functional food or as nutritional supplements.

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