Antioxidant and antimicrobial activities of various extracts from *Engleromyces sinensis* fruiting body

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Abstract: *Engleromyces sinensis*, as rare macro-ascomycetes and traditional ethnomedicine in the southeast part of China, have been applied in anti-infection, anti-inflammatory and anti-tumor for a long time. In this study, the antioxidant activities of ethyl acetate crude extract (EACE), acetone crude extract (ACE), 95% ethanol crude extract (ECE), methanol crude extract (MCE) and water crude extract (WCE) from *E. sinensis* fruiting body were investigated using conventional antioxidant assays *in vitro* for the first time. As results, it was noteworthy that WCE showed the greatest 2,2-diphenyl-1-picrylhydrazil (DPPH) radicals-scavenging activity and reducing power, with EC_{50} values of 3.56 and 19.28mg/mL. MCE and EACE exhibited higher hydroxyl radicals-scavenging activity and ferrous ion-chelating activity significantly, with EC_{50} values of 2.16 and 0.47mg/mL. The total phenolics and total polysaccharides content results revealed that WCE had the highest phenolics and polysaccharides contents with 1.19 mg GAEs/g extracts and 40.07 mg D-glucose/g extracts. The antimicrobial activity of the WCE, ECE, ACE, EACE was assessed in final and two of them, ACE and EACE showed a strong ability to inhibit the microbial growth. The research work demonstrated that *E. sinensis* fruiting body can present a promising source of antioxidant and antimicrobial agents.

Keywords: Antimicrobial activity, antioxidant activity, *Engleromyces sinensis*, the minimal inhibitory concentration.

INTRODUCTION

In the past 20 years, reactive oxygen species (ROS) and free radicals have attracted more and more attentions due to their close relationship with cellular damage and the ageing process (Lee *et al*., 2004). ROS, such as hydroxyl radicals, hydrogen peroxide can cause oxidative damage of nucleic acid, proteins, lipid as well as small cellular molecules, and then result in many human diseases (Lemberkovics *et al*., 2002; Shon *et al*., 2003). It is well-known that the antioxidants play important roles to protect against disorders for oxidant damage and inhibit oxidative chain reaction's initiation or propagation in order to prevent or repair damage to human cells by oxygen (Velioglu *et al*., 1998). Therefore, antioxidant additives or antioxidant-containing foods which may reduce oxidative damage and help the human body keep heathly have been increasingly favored by people.

Engleromyces sinensis (fig. 1), also called "Zhu Jun" in native and identified as a fungus belonging to Xylariaceae in Ascomycota (Whalley *et al*., 2010). It is one of rare medicinal fungi from the bamboo of high mountains around Tibet, Yunnan and Sichuan provinces of China (Whalley *et al*., 2010), where it is boiled in water as a folklore medicine for the treatment of inflammatory diseases, gastric ulcer and cancer. In the recent 40 years, *E. sinensis* has been attracted many researcher's interests for the isolation of active compounds (Pedersen *et al*., 1980; Liu *et al*., 2002; Zhan *et al*., 2003). However, to our best knowledge, there are no reports that screened the antioxidant and antimicrobial activities of this fungus comprehensively, especially for different extractions by various agents. Hence, the research aims to examine the efficiency of commonly used extraction methods for extracting bioactive compounds from *E. sinensis* fruiting body, and determine the contents of total phenol and total polysaccharides of different extracts to reveal their correlation with antioxidant and antimicrobial activities.

MATERIALS AND METHODS

Samples

Fresh fruiting bodies of *E. sinensis* were collected from Yulongcounty, Lijiang City, Yunnan Province, China. All of the samples at the mature stage were selected with no apparent physical or microbial damage.

Preparation of extracts

Fresh fruiting bodies of *E. sinensis* were freeze-dried at -45^{o}C and ground in a mill before analysis. For the extraction process, 10g of *E. sinensis* powdered sample was extracted with 200mL of test solvents (ethyl acetate, acetone, 95% ethanol, methanol and water). Each mixture was boiled for 4 hours under the water-bath reflux and the residue was re-extracted at twice. After extraction, the combined extracts were filtered and then evaporated under rotary evaporator at 40^{o}C , 5-15 kPa, to produce ethyl acetate crude extract (EACE), acetone crude extract (ACE), 95% ethanol crude extract (ECE), methanol crude extract (MCE) and water crude extract (WCE), then the extracts obtained were re-dissolved in water (water extraction) and DMSO (different polarities solvents extraction). All the extracts were stored in dark at 4^{o}C until use.

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Total phenolic content

Total phenolic content of five different extracts was examined by Folin-Ciocalteau's method as previously described (Singleton and Rossi, 1965). The phenols content was calculated by a comparison of the values obtained from the calibration curve of gallic acid standard solutions (GAEs/g extract).

Total polysaccharides content

The content of WCE polysaccharides was measured by phenol-sulfuric acid methods with a slightly modified (Dubois *et al*., 1956). The polysaccharide solution was prepared at a concentration of 0.1mg/mL, 400µl of sample solution was transferred into a 10mL tube and then 1mL of concentrated sulphuric acid, 200µl 6% phenol were added to the tube, successively. The mixture was stirred vigorously and then incubated in boiled water at 100°C for 15 min. After that, all tubes were placed in cold water for 5 min to stop the reaction. Absorbance at 490 nm of the mixture solution was measured, and the total polysaccharides was calculated with D-glucose as standard (D-glucose/g extract).

DPPH free radicals-scavenging assay

The DPPH free radicals-scavenging activity were measured by using a modified method of Guo *et al*. (2010). Different concentrations (1-20mg/mL) of all the five samples were mixed with the same volume (80µg/mL) of a Methanolic solution of DPPH. The absorbance was measured with a spectrophotometer at 517nm against a blank, butylatedhydroxytoluene (BHT) and Ascorbic Acid (Vc) were used as positive controls.

Hydroxyl radicals-scavenging assay

Method of Guo *et al*. (2010) were used for this assay with some modifications. Briefly, 150µL of 20mM sodium salicylate, 500µL of 1.5mM $FeSO_4$, 500µL of various concentrations (0.75-12mg/mL) of sample solution and 350µL of 6mM H_2O_2 were mixed in order, after 1 hour water bath at 37^oC, the absorbance of the mixture was measured at 510 nm against a blank. Vc was used as the positive control.

Ferrous ion-chelating assay

Ferrous ion-chelating assay were evaluated by the method of Guo *et al*. (2010) with slight modification. Briefly, 200 µL of each sample with various concentrations (0.625-30mg/mL) was mixed with 20µL of $FeCl_2 \cdot 4H_2O$ (2.0 mM) and 740µL of deionized water. Then, 40µL of ferrozine (5.0mM) were added to initiate the reaction. Finally, the absorbance of mixture was measured at 560 nm against a blank after 20 min incubation. In this assay, EDTA was used as the positive control.

Reducing power assay

The reducing powers were followed by Munazir *et al*. (2015) and measured at 700 nm against a blank. BHT and Vc were used as the positive controls in this assay.

Microbial strains

All test organisms used in this work were obtained from China General Microbiological Culture Collection Center (CGMCC) and American Type Culture Collection (ATCC), the different polarities crude extracts were tested against three species of gram-positive bacteria: *Bacillus subtilis* (CGMCC 1.769), *Listeria monocytohenes* (ATCC 27708), *Staphyloccocus aureus* (ATCC 12600) and three species of gram-negative bacteria: *Escherichia coli* (CGMCC 1.1103), *Proteus vilgaris* (ATCC 33420) *Salmonellae enteritis* (ATCC 14208) and two species of fungi: *Saccharomyces cerevisiae* (CGMCC 2.1793), *Canidia albicans* (CGMCC 2.2086).

Agar diffusion method

Antimicrobial activity was followed by the method of Hajji *et al* (2010). 100µL of the tested microorganisms (10^6 CFU/mL) were spread on Muller-Hinton agar (MHA), then 40µL of each extract (100mg/mL) was loaded to appropriate bore (3mm depth, 5 mm diameter). Tetracycline and Streptomycin (100µg/mL) were used as positive references for test, whereas DMSO (without extract) was the negative control. Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones (including well diameter).

Determination of the minimal inhibitory concentration

The minimal inhibitory concentration (MIC) values, which represent the lowest extracts concentration that inhibits the growth of tested bacteria and fungi, were determined by a micro-well dilution method as described by Basri *et al*. (2012) with some modifications. 100 mg/mL different extracts were prepared with DMSO/water, the final concentrations range from 50mg/mL to 0.05mg/mL. Different concentrations of tetracycline and streptomycin ranging from 50 to 0.05µg/mL was used as positive control, DMSO was used as negative control. Finally, MTT were used to detect each well's biological activities.

STATISTICAL ANALYSIS

All the experiments were carried out in triplicates and the results were presented as mean values ±SD (standard deviations). The significant differences among the results for extraction yield, total phenols content, total polysaccharides content, EC_{50} value and inhibition zone was analyzed. EC_{50} value represents the effective concentration at which free radicals were scavenged by 50%. In this report, the one-way analysis of variance (ANOVA) was used. Differences at $p<0.05$ were considered statistically significant using SPSS 19.0 software.

RESULTS

Extraction yield, total phenolic and polysaccharides contents

The yield of the different solvent extracts, the phenolic and polysaccharides contents are all presented in table 1. As shown from this table, it is noticeable that MCE has the highest amount of total extractable compounds, and the lowest yields were from ethyl acetate extracts and acetone extracts. The total phenol content of different solvents followed the order: water >methanol >95% ethanol > acetone and ethyl acetate, and water extract contain the highest levels of total phenolic content with 1.19mg GAEs/g extract of *E. sinensis*. The polysaccharides content of WCE was determined, with 40.07 mg D-glucose/g extract (4.007%) of *E. sinensis*.

Fig. 1: Image of fruiting body of *E. sinensis* on *Sinarundinaria* sp.

Antioxidant activities

Fig. 2(A) represents the DPPH radicals-scavenging activity of various solvent extracts from *E. sinensis*. The result indicated that WCE showed the greatest antioxidant ability with 84.35% at 10mg/mL. At the same concentration, other crude extraction was significant lower than WCE (48.94%-23.55%). With regard to EC_{50}, the WCE exhibited the highest DPPH radicals-scavenging activity with the lowest EC_{50} (3.56mg/mL) amongst all the extracts examined.

Fig 2(B) illustrates regression analysis results in case of hydroxyl radicals-scavenging assay for various solvent extracts of *E. sinensis*. At 6mg/mL, the scavenging activity of MCE was 98.98%, followed by WCE (95.99%), while ECE, ACE and EACE, being in the range of 61.86%-66.40%, were lower than those of MCE and WCE. The EC_{50} values of hydroxyl radicals-scavenging activities were 5.23mg/mL, 4.04mg/mL, 3.46mg/mL, 2.47mg/mL, 2.16mg/mL for ECE, ACE, EACE, WCE and MCE, respectively.

Fig 2(C) shows the Fe^{2+} chelating activities of *E. sinensis* different solvent extracts. At 2.5mg/mL, Fe^{2+} chelating activities were ranked in the order: EACE (96.75%) >MCE (95.30%) >ACE (88.56%) >WCE (63.57%) >ECE (20.89%). At 10mg/mL, the order is: ACE (99.80%) >EACE (99.30%) >MCE (98.45%) >WCE (95.05%) >ECE (78.16%). EC_{50} of the various extracts in Fe^{2+}chelating ability varied from 0.47 to 5.36mg/mL depending on solvents used.

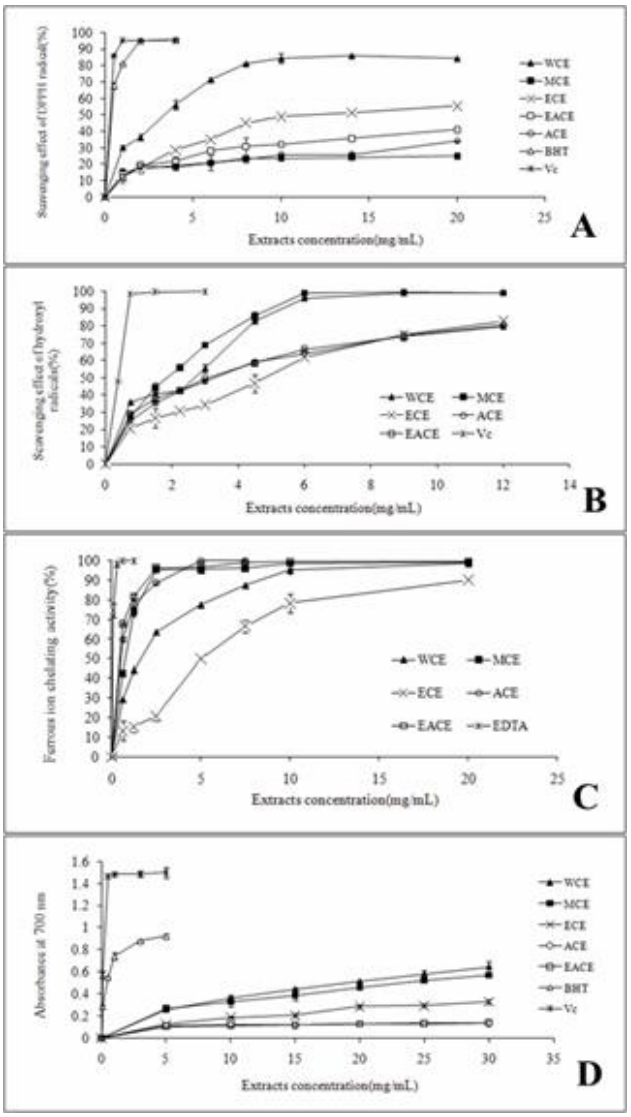

Fig. 2: Antioxidant activities of different solvent extracts tested with four methods: (A) DPPH free radicals-scavenging activity, (B) Hydroxyl radicals-scavenging activity, (C) Ferrous ion-chelating activity, (D) Reducing power. BHT, Vc and EDTA were used as the positive controls. Each value is expressed as a mean ±S.D. (n=3).

As shown in fig 2(D), the reducing power activity of five solvent extracts are much lower than positive control. The highest antioxidant activity amongst the tested samples was obtained for the WCE with 0.645 at 30mg/mL, followed by MCE (0.570), whereas those of others varied range from 0.134 to 0.327. The EC_{50} values of reducing

Table 1: Extraction yield, total phenols and total polysaccharides of different extract of *Engleromyces sinensis* fruiting body

Different extract	Extraction yield (%)	Total phenols content (mg GAEs/g extract)	Total polysaccharides content (mg D-glucose/g extract)
WCE	17.12±0.22[b]	1.19±0.03[a]	40.07±2.10[a]
MCE	19.98±0.73[a]	1.10±0.02[b]	ND
ECE	16.94±2.24[b]	0.92±0.02[c]	ND
ACE	11.72±0.93[c]	0.59±0.00[d]	ND
EACE	11.09±0.17[c]	0.40±0.02[e]	ND

Each value is expressed as a mean± standard deviation (n=3). In each column different letters (a-e) mean significant difference $p<0.05$. ND: No detectable data.

Table 2: EC_{50} value for the antioxidant activity of different extracts from *Engleromyces sinensis* fruiting body.

	EC_{50} value (mg extract/mL)			
	DPPH radicals	Hydroxyl radicals	Ferrous ions	Reducing power
WCE	3.56±0.03[b]	2.47±0.06[d]	2.00±0.03[b]	19.28±0.092[b]
MCE	>20	2.16±0.05[e]	1.04±0.03[c]	23.52±0.082[a]
ECE	13.73±0.03[a]	5.23±0.02[a]	5.36±0.04[a]	>30
ACE	>20	4.04±0.01[b]	0.58±0.03[d]	>30
EACE	>20	3.46±0.03[c]	0.47±0.03[e]	>30
Vc	<0.5	<0.5	ND	<0.5
BHT	<0.5	ND	ND	<0.5
EDTA	ND	ND	<0.1	ND

Each value is expressed as a mean± standard deviation (n=3). Means with different letters within a column are significantly different ($p<0.05$)

Table 3: Inhibition zone (mm) of different solvent extracts against selected microbial strains.

Test organisms	Diameters of zones of inhibition (mm)					
	MCE	ECE	ACE	EACE	Tetracycline	Streptomycin
Escherichia coli	9.20±0.35[e]	9.21±0.32[e]	10.25±0.09[d]	11.22±0.23[c]	21.28±0.26[b]	24.84±0.36[a]
Proteus vilgaris	8.03±0.05[d]	7.21±0.19[e]	10.27±0.38[c]	10.87±0.48[c]	21.99±0.06[b]	29.56±0.56[a]
Salmonellae enteritis	7.80±1.06[c]	8.07±0.12[c]	10.64±0.66[b]	11.22±0.58[b]	24.20±0.72[a]	24.73±0.06[a]
Bacillus subtilis	7.61±0.62[d]	7.58±0.57[d]	9.58±0.38[c]	10.07±0.12[c]	26.47±0.40[a]	17.64±0.55[b]
Listeria monocytohenes	8.74±0.30[d]	7.61±0.35[e]	10.39±0.65[c]	11.06±0.75[c]	24.23±0.25[b]	26.07±0.40[a]
Staphyloccocusaureus	naa	naa	naa	naa	17.23±0.19[a]	naa
Saccharomyces cerevisiae	0.808±0.01[e]	0.854±0.02[e]	10.76±0.0.9[c]	9.87±0.28[d]	16.22±0.54[b]	21.2±0.31[a]
Canidiaalbicans	9.23±0.21[f]	10.55±0.15[e]	15.45±0.20[c]	13.64±0.07[d]	23.31±0.53[a]	19.88±0.40[b]

Data expressed as mean ±SD of three different observations.
a, b, c, d, e and f: different letters within the same line means significant different at $p<0.05$.
naa means no antimicrobial activity.

Table 4: Mean MIC values of different extracts from *Engleromyces sinensis* fruiting body against test organisms

Test organisms	Mean minimum inhibitory concentration (MIC) value ± 0.00 (SD) (mg/mL)					
	MCE	ECE	ACE	EACE	Tetracycline	Streptomycin
Escherichia coli	50	50	25	25	0.05	0.025
Proteus vilgaris	50	50	25	25	0.1	0.0125
Salmonellae enteritis	50	50	25	25	0.05	0.025
Bacillus subtilis	25	25	12.5	12.5	0.05	0.05
Listeria monocytohenes	50	50	25	25	0.05	0.05
Saccharomyces cerevisiae	50	25	25	25	0.05	0.1
Canidiaalbicans	25	12.5	3.125	12.5	3.125×10^{-3}	0.78×10^{-3}

Lowest MIC value indicates the highest inhibitory effect.

power were found to be 19.28mg/mL and 23.52mg/mL for WCE and MCE, respectively, whilet ECE, ACE, EACE were all higher than 30mg/mL.

Antimicrobial activities

Table 3 presents diameters of inhibition zones exerted by different extracts towards test organisms. Four extracts (MCE, ECE, ACE, EACE) from *E. sinensis* fruiting body showed various degrees of antimicrobial activities against most of microorganism tested. The ACE and EACE have significantly higher antimicrobial activities than other extracts, were in the range of 9.58-15.45 mm. Among all test microorganisms, *C. albicans* was found to be the most susceptible towards all tested extracts, and we observed no distinct inhibition of the *S. aureus*. Quantitative evaluation of antimicrobial activity of MCE, ECE, ACE and EACE were presented in table 4. The results indicated that MIC values ranging from 3.125 to 25mg/mL for the ACE and EACE, and from 12.5 to 50mg/mL for the MCE and ECE. However, ACE and EACE, MCE and ECE were found to exhibit similar MIC values against most microbial species. The lowest MIC value of 3.125mg/mL was obtained by ACE on *C. albicans*.

DISCUSSION

Polyphenols are one of the most abundant products in nature, which play an important role in providing protection against *in vivo* and *in vitro* oxidation (Shahidi and Wanasundara, 2003). Phenolic compounds exist in plants and macro fungus, types include flavonoid, phenolic acids, tannins, stilbenoid and lignanoid (Ignat *et al*., 2010). In this study, WCE have the highest phenol content, with 1.19mg/g, which is higher than other four extracts. The result indicates that high polarity solvent and high temperature may be more suitable for the extraction of polyphenols from *E. sinensis* fruiting body.

The polysaccharides content of WCE was determined (as shown in table 1), with 40.07 mg D-glucose/g extract (4.007%) of *E. sinensis*. The fungal polysaccharides have a great help to cure human diseases include tumor, cancer, immunodeficiency and cardiovascular diseases (Ajith and Janardhanan, 2007; Wang *et al*., 1995; Wasser, 2002; Zhang *et al*., 2007). In the present study, the total polysaccharides content from fruiting body of *E. sinensis* were higher than those from fruiting bodies of many medicinal mushrooms sincerely, such as *Pleurotus ostreatus*, 3.32% (Tong *et al*., 2009), *Russula virescens*, 1.94% (Sun *et al*., 2010) and *Ganoderma tsugae* (Ling chih), 1.5%-1.7% (Tseng *et al*., 2008), but lower than those of fruiting body (5.74%) of *Lentinus polychrous* (Thetsrimuang *et al*., 2011) and another Chinese traditional medicine *Cordyceps sinensis* (18.37%) (Dong and Yao, 2008). Miao *et al*. (2011) and Wang *et al*. (2013) have optimized polysaccharides extraction from the fruiting bodies of Chinese truffle and *Gomphidius rutilus* using response surface methodology, the mean extraction yield of polysaccharides was increased 3.2 folds (3.85% to 12.19%) and 1.5 folds (5.49% to 8.02%) under the optimal conditions compare to the lowest yield, respectively. Thus, optimization of extraction process may effectively improve the yields of polysaccharide from *E. sinensis* fruiting body in the further studies.

DPPH scavenging assay is a simple and convenient method to evaluate the antioxidant activity. As for other fungus hot water extracts, *Hypsizygus marmoreus*, *Agaricus bisporus*, *Pleurotus citrinopileatus* fruiting bodies could scavenge DPPH radicals by 20.7%-52.3% at 20 mg/mL (Lee *et al*., 2007; Huang, 2003). At 4-8mg/mL, the scavenging abilities of hot water extracts from *C. sinensis* were more than 80% (Dong and Yao, 2008). For ethanolic extracts, the scavenging activities of *H. marmoreus*, *A. bisporus*, *P. Citrinopileatus* fruiting bodies were 46.6%-68.4% at 5mg/mL (Lee *et al*., 2007; Huang, 2003). At 0.125-2.0mg/mL, the scavenging activities of acetonic, methanolic extracts of *Pleurotus ferulaeon* DPPH radicals ranged from 14.02% to 91.32%, 18.39% to 88.85%, respectively (Alam *et al*., 2010). It is obvious that the scavenging activity of hot water from *E. sinensis* fruiting body was more effective than that of *H. marmoreus*, *A. bisporus*, *P. citrinopileatus* but similar to *C. sinensis*. The better ability of WCE might be due to more polysaccharides and phenolic components extracted by water, which has a better ability of scavenging free radicals and donating hydrogen atom. However, ECE, ACE, MCE have worse scavenging ability than those mentioned above.

The hydroxyl radical is one kind of important active oxygen which may damage protein or DNA bio-molecules in the living body. Therefore, hydroxyl radicals-scavenging is probably one of the most effective methods to protect our human body against various diseases. As for hot water extracts, *P. citrinopileatus* and *H. marmoreus* fruiting bodies scavenged 51.8% and 80.1% of hydroxyl radicals at 20mg/mL, respectively (Lee *et al*., 2007; Huang, 2003). At 5-20mg/mL, scavenging abilities of mature and baby fruiting body of *G. tsugae* were 19.6%-23.2% to 72.4%-73.7% (Mau *et al*., 2005), while *Agrocybe cylindracea*, *A. bisporus*, *P. ostreatus* and *Pleurotus eryngii* were 11.3-33.9% to 38.2-67.9% (Tsai *et al*., 2006; Lo, 2005). However, WCE was 55.30%-95.99% at 3-6mg/mL, therefore, the scavenging ability of WCE can be considered as a good scavenger of hydroxyl radicals. As for methanolic extracts, MCE also showed great hydroxyl radicals-scavenging abilities compared to some commercial mushrooms (29.2%-54.3%, at 40mg/mL) (Yang *et al*., 2002), medicinal mushrooms (38.0%-52.6%, at 16 mg/mL) (Mau *et al*., 2002). These results revealed that the radicals-scavenging abilities of various extracts of *E. sinensis* fruiting body, especially MCE and WCE are more effective than above mushrooms.

The WCE, which was safe, non-toxic and easy to get, showed a great potential of preventing oxidative damage and maintaining health of the human body.

Ferrous ions commonly found in food are also generally considered to be a strong and effective pro-oxidants. With regard to hot water, *H. marmoreus* and *P. citrinopileatus* fruiting bodies chelated 92.6% and 82.1% of ferrous ions at 5mg/mL, respectively (Lee *et al*., 2007; Huang, 2003). As for some precious medicinal fungi, the hot water extracts from mature and baby fruiting body of *G. tsugae* chelated 42.6% and 39.5% ferrous ions at 20mg/mL (Mau *et al*., 2005), while *C. sinensis* hot water extract reached 41.86% at 8mg/mL (Dong and Yao, 2008). At 2-12mg/mL, the Fe^{2+} chelating activities of ethyl acetate, ethanol extracts of *Tuber indicum* ranged from 62.0%–89.9% and 56.9%-77.4%, respectively (Guo *et al*., 2011). It can be concluded that the ferrous ion-chelating activity of WCE, ECE, EACE was more effective than those of *H. marmoreus*, *P. citrinopileatus*, *G. tsugae*, *C. sinensis* and *T. indicum* at lower concentrations. Moreover, the ACE and MCE also exhibited good Fe^{2+} chelating activities. Since ferrous ions are the most effective pro-oxidantsin food field, the high ferrous-ion chelating abilities of the different extracts from the fruiting body of *E. sinensis* would be beneficial to human health for antioxidant protection.

The reducing power assay may serve as a significant index to evaluate extracts potential antioxidant activity. As far as the hot water extracts are concerned, reducing power of *A. cylindracea* were 0.87-0.99 at 5-10mg/mL (Tsai *et al*., 2006). Moreover, mature and baby fruiting body of *G. tsugae* exhibited reducing power of 1.08 and 1.04 at 5mg/mL, respectively (Mau *et al*., 2005). Hot water extracts from *C. sinensis* fruiting body showed reducing powers of 0.6 at 10mg/mL. With regard to methanolic extracts, the reducing power of *Russula delica* and *Verpa conica* exhibited a strong reducing power of 1.32 and 1.22 at 200μg/mL (Elmastas *et al*., 2007). At 5 mg/mL, methanolic extracts of *Pleurotus abalones* (abalone mushroom) and *P. ostreatus* (tree oyster mushroom) showed a reducing power of 0.65 and 0.81, respectively (Yang *et al*., 2002). Therefore, it is obvious that the reducing power of *E. sinensis* fruiting body is not remarkable compared to those from other commercial, medicinal mushrooms.

Unlike the previous studies on the antimicrobial activity of commercial and medicinal plants such as apple skins (Alberto *et al*., 2006), walnut green husk (Fernández-Agulló *et al*., 2013),Tunisian quince pulp and peel (Fattouch *et al*., 2007), the antimicrobial activity of *E. sinensis* fruiting body was not increased by the increasing of total phenol content. For instance, EACE has the lowest total phenol content compared to other crude extracts, however, it shows a great antimicrobial activity and broad spectrum resistance to test organisms (MIC values range from 12.5 to 25mg/mL). Furthermore, the overall results indicated that a low polarity solvent extracts have a better antimicrobial activity than extracts that extracted by a high polarity solvent. Previous studies (Liu *et al*., 2002; Zhan *et al*., 2003) reported that three cytochalasin analogues have been isolated from ethyl acetate soluble part of *E. sinensis* fruiting body. Cytochalasins could produce a variety of cell biological effect (Bossart *et al*., 1975; Godman *et al*., 1975; Tannenbaun *et al*., 1977), this may argued that the less polar components from sporocarp had more anti-microbiological activities. Therefore, a low polarity solvent may be more suitable for the extraction of the antimicrobial substance from *E. sinensis* fruiting body.

CONCLUSION

The results obtained in this study clearly demonstrate that all the tested extracts of *E. sinensis* fruiting body showed antioxidant and radicals-scavenging activities at different magnitudes of potency. The extraction using various solvents and temperatures may have resulted in different active compounds. As a safe solvent, water crude extracts were effective in the overall result of antioxidant activity, especially in the hydroxyl radicals-scavenging activity and ferrous ion chelating activity due to higher phenol and polysaccharides contents. In addition, the interesting antimicrobial activities of the *E. sinensis* fruiting body extracts suggest that a lower polarity solvent extracts have a better antimicrobial activity. Therefore, the results from this study revealed that the extracts of *E. sinensis* fruiting body have potential value for health care, anti-inflammatory drugs, food additives etc. In the further work, we will isolate and identify the active compounds in various extracts that are related to the antioxidant and antimicrobial capability.

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