ISSN (Print) : 0974-6846
Indian Journal of Science and Technology, *Vol 8(25), DOI: 10.17485/ijst/2015/v8i25/80496, October 2015* ISSN (Online) : 0974-5645

Comparison of Physiological Activity of Solvent Extracts from *Hericium erinaceus*

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Abstract

The present study investigated antioxidant and antimicrobial effects of *Hericium erinaceus* extracts obtained with acetone, ethyl acetate, and methanol. These extracts were evaluated for antioxidant activities by total polyphenol and flavonoid contents, DPPH radical scavenging activity, and ABTS radical scavenging activity. ABTS radical scavenging activity of the methanol extract showed the highest value similar to that of standard antioxidant, ascorbic acid. The acetone extract and ethyl acetate extract had relatively high total flavonoid contents and DPPH radical scavenging activities. Antimicrobial activities were determined by using disc diffusion method with four strains of Gram-negative bacteria and eleven strains of Gram-positive bacteria including nine oral bacteria. The ethyl acetate extract had antimicrobial activity against Gram-positive bacteria, whereas the methanol extract showed inhibitory effect against Gram-negative bacteria. For the microbial activity of oral bacteria, the most of *H. erinaceus* extracts were effective to inhibit the growth of *Streptococcus mutans*.

Keywords: Antioxidant Activity, Antimicrobial Activity, *Hericium erinaceus*

1. Introduction

Recently, owing to aging and increase of living standards, interests in health promotion and aging control have increased. So, studies on the development of natural antioxidants substituting the synthetic antioxidants from various side effects according to consecutive use of the synthetic antioxidants currently used and the development of functional foods using the natural antioxidants are being realized briskly[1–3]. Also, to prevent accidents caused from microbe contamination in the fields of distribution and storage of food, synthetic preservatives are used as additives. As the problem of stability comes to the fore, effort to find natural substances to replace them is being continued[4,5]. Especially, studies on bioactive effects and substances of mushrooms which have been used as food and medicines for a long time are being processed lively[6]. Generally, it is reported that there are antioxidant capacity, anticancer effect, and immune reinforcement effect in β-glucan of mushrooms[7,8]. Polysaccharides in some *Phellinus linteus*, shiitake mushrooms and *Stereum hirsutum* were confirmed to be anticancer substances[9–11]. From manna lichen, matters with effect of reducing blood sugar were checked[12]. Also, various studies on antibacterial matters of mushrooms have been processed. Various antibacterial activity substances such as Coriolin[13] from culture media of *Coriolus consors*, striatins A/B/C[14] from *Cyathus striatus* mycelia, melleolide[15] from *Armillaria mellea*, clavicoronic acid[16] from *Clavicorona pyxidata*, and chlorinated orcinol derivatives[17] from *Hericium erinaceus* have been divided until now.

H. erinaceus belonging to *Aphyllophorales* of *Hymenomycetidae* reseeds at old trees or green trees of broadleaf trees in autumn and distributes evenly in Korea, Japan, China, some of Southeast Asia, Europe and North America and areas except some tropical and Polar Regions. In China, it is called monkey head mushrooms. In Japan, it is called *Yamabushitake*. *H. erinaceus* includes various bioactive substances like polysaccharides, lectins, terpenoids, and erinacine[18,19] and effects of surpassing

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the proliferation of cancer cells and anti-mutation were reported[20]. Plus, from dividing erinacines as a substance to accelerate NGF synthesis which is a matter available for drugs of dementia, the structure was checked[21–23].

In this paper, by extracting *H. erinaceus* with diverse efficacy by various organic solvents, antibacterial activity of extracts of *H. erinaceus* against Gram-positive bacteria, Gram-negative bacteria, and oral bacteria are measured. Besides, antioxidant activities such as total content of polyphenol, content of flavonoid, DPPH radical elimination ability, and ABTS radical elimination ability are to be checked.

2. Materials and Methods

2.1 Mushrooms and Chemicals

H. erinaceus in this experiment was used from being dried naturally and pulverized after purchasing mushrooms which can be obtained at a regular retail market. Then, while kept in a refrigerator at 4℃, it was used after the extraction. The extractant solvents were purchased at Daejung Inc. Reagents except extractants were purchased at Sigma-Aldrich (St, Louis, MO, USA).

2.2 Bacterial Strains and Culture Conditions

Antibacterial activity of *H. erinaceus* extract was investigated targeting three kinds of Gram-positive bacteria like *Bacillus subtillis*, *Staphlyococcus aureus*, and *Micrococcus luteus* and three kinds of Gram-negative bacteria like *Escherichia coli, Pseudomonas aeruginosa*, and *Enterobacter cloacae*. Strains used in the antibacterial activity investigation against oral bacteria of *H. erinaceus* were *Streptococcus sobrinus* (KCTC3308), *Staphylococcus aureus* (KCTC1927) *Streptococcus mutans* (KCTC3065), *Streptococcus ratti* (KCTC3655), *Streptococcus sanguinis* (KCTC3284), *Actinomyces viscosus* (KCTC5531), *Actinomyces naeslundii* (KCTC5525), *Streptococcus anginosus* (KCTC3983) and *Aggregatibacter actinomycetemcomitans* (KCTC3698). Strains for the test were used from being distributed by Korean Collection for Type Cultures (KCTC, Daejeon, Korea). The used medium and cultivation conditions are like (Table 1). The rest bacteria except oral bacteria were cultured in the incubator of 37°C and the oral bacteria was cultured in the anaerobic incubator of 37°C for 24 hours. After adjusting the turbidity of bacterial culture media into the 0.5 McFarland standard (about 1.0×10^6 CFU/ml), they were used at the test.

Table 1. List of stains used for antibacterial experiments

	Strains	Media	Temp(°C)
Gram-positive bacteria	*Bacillus subtilis*	LB	37
	Micrococcus luteus	LB	37
	Staphylococcus aureus	LB	37
Gram-negative bacteria	*Enterobacter cloacae*	LB	37
	Escherichia coli	LB	37
	Pseudomonas aeruginosa	LB	37
Oral bacteria	*Staphylococcus aureus*	BHI	37
	Streptococcus mutans	BHI	37
	Streptococcus ratti	BHI	37
	Streptococcus sanguinis	BHI	37
	Streptococcus sobrinus	BHI	37
	Actinomyces naeslundii	TSB	37
	Actinomyces viscosus	TSB	37
	Aggregatibacter actinomycetemcomitans	TSB	37
	Streptococcus anginosus	TSB	37

BHI, Brain-Heart Infusion; TSB, Trypticase Soy Broth.

2.3 Extraction of *Hericium erinaseus*

To the 50 g powder of pulverized *H. erinaceus*, each 400 ml of acetone, Ethyl acetate (EtAc) and methanol (MeOH) as solvents is put. Then, it is moved to a separatory funnel and shaken for 2 hours after stirring it for 72 hours. After filtering only the supernatant of each solution, vacuum filtration was conducted. So, it was concentrated with a rotary evaporator (EYELA A-1000S, Tokyo Rikakikai Co, Tokyo, Japan). Each extract was used by melting it in 5 ml Dimethyl Sulfoxide (DMSO). From deciding the concentration of each extract, the test was processed fitting to minimal concentration (192 mg/ml). Antibacterial tests all against oral bacteria were processed from being diluted into 100 mg/ml. Each extract was used by keeping it at 4 -> 4 °C.

2.4 Antimicrobial Activity

The antimicrobial effect of extracts of *H. erinaceus* against multi-resistant bacteria was measured by the disc diffusion method from transforming the Bauer method[24]. After the cultivated strains were smeared on the agar plate which was prepared already with cotton swabs, placing paper discs (ϕ6 mm, Whatman AA discs, Whatman

International) that each extract of 30 µl was absorbed and dried on smeared plates, plates to investigate antimicrobial effect of Gram-positive strains and Gram-negative strains against oral bacteria were cultivated in the 37°C incubator and other plates to investigate antimicrobial effect of oral bacteria were cultivated in the 37°C CO_2 incubator for 24 hours. So, clear zones around discs were measured. As negative control, DMSO was used. Each experiment was repeatedly measured three times.

2.5 Total Polyphenol Content

To check the polyphenol content of *H. erinaceus* extracts, it was measured from transforming some of the Folin-Denis method[25]. From adopting the mixture of 45 µl *H. erinaceus* extract, mixing and 45 µl of 1 N Folin-Ciocalteu (Folin-Ciocalteu:extract, 1:1, v/v), after fixing it at room temperature for three minutes, 910 µl of 2% Na_2CO_3 was added. From letting the mixed liquid responded at room temperature for 30 minutes, the absorbance was measured at 760 nm (MECASYS, Daejeon, Korea). By writing down standard curves with gallic acid, total phenolic content was expressed by mg GAE/g extract. Experiments of each extract were implemented 3 times.

2.6 Total Flavonoid Content

To investigate flavonoid content of *H. erinaceus* extracts, it was measured from changing some of the method of Lee et al[26]. By mixing 500 µl mushroom extract and 500 µl of 2% $AlCl_3$, after responding it at room temperature for one hour, the absorbance was measured at 420 nm. From the standard curve written by using quercetin as a standard substance, the total flavonoid content was calculated. It was expressed by mg (QE)/g extract. Tests for each extract were conducted 3 times.

2.7 DPPH Radical Scavenging Activity

By using the Blois method, antioxidant activity of *H. erinaceus* was investigated from being measured by DPPH radical scavenging ability[27]. By mixing 30 µl extract with regular concentration and 970 µl of 0.1 mM DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich, St. Louis, MO, USA) solution, after reacting them in the dark for 30 minutes, by measuring the absorbance at 517 nm, the absorbance decrease by the reduction of DPPH was investigated. As a comparison group, 1 mM ascorbic acid was used. The average value was found from implementing 3 time tests.

Radical scavenging activity (%) = {1 – Abs (experiment)/Abs(control)} × 100

2.8 Measurement of ABTS Radical Scavenging Activity

ABTS radical scavenging ability was measured according to the method of Re et al[28]. 7.4 mM ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, Sigma-Aldrich, St. Louis, MO, USA) and 2.6 mM potassium persulphate (Sigma-Aldrich) were left in the dark for 24 hours. ABTS solution is prepared. By mixing 970 µl ABTS solution and 30 µl extract, the absorbance was measured at 734 nm (MECASYS, Daejeon, Korea). As a comparison group, 1 mM ascorbic acid was used. The average value was found from implementing tests three times.

Radical scavenging activity
(%) = {1 – Abs(experiment)/Abs(control)} × 100

2.9 Statistical Analysis

All tests were implemented three times repeatedly and the results were represented by average ± standard deviation. For the statistical analysis of data, PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA) was used. After the variance analysis with one way ANOVA, it was verified by Tukey. All statistical significance was verified in the level of $P<0.05$.

3. Results and Discussion

3.1 Antimicrobial Activity

By using the paper disc diffusion assay method, the result of measuring antibacterial effect of extracts of *H. erinaceus* which was extracted by solvents of acetone, EtAc, and MeOH is like (Table 2). Among three solvent extracts, antibacterial activity of acetone extract did not appear. EtAc and MeOH extracts showed inhibitory activity against eight bacteria all though strong activity was not. EtAc extract represented weak activity against *S. aureus* and *B. subtilis*. It showed relatively strong activity against *M. luteus*. And, about *E. coli*, *P. aeruginosa*, and *E. cloacae* as Gram-negative bacteria, all showed clear zones. Plus, MeOH extract showed weak activity about *B. subtilis* as Gram-positive bacteria and strong activity against *S. aureus* and *M. leteus*. It presented weak activity about *E. coli* and *P. aeruginosa* as Gram-negative bacteria and somewhat good activity about *E. cloacae*. EtAc and MeOH

extracts showed somewhat high activity relatively with *M. luteus* (9.3 mm) and *E. cloacae* (8.3 mm) compared with other bacteria. It was checked that MeOH extract showed somewhat bigger clear zones in other bacteria except *M. luteus* and *B. subtilis* than EtAc extract. Wong et al.[29] reported that MeOH extract of *H. erinaceus* showed 8-12 mm clear zones compared with various strains like *B. cereus* and *B. subtilis*. But, in this study, weaker activity was checked than that.

Table 2. Antimicrobial activities of various solvent against Gram-positive bacteria and Gram-negative bacteria from *Hericium erinaceus*

Strains	Diameter of inhibition zone (mm)		
	Acetone	EtAc	MeOH
B. subtilis	-	7.7	6.3
E. cloacae	-	7.3	8.3
E. coli	-	6.7	7.3
M. luteus	-	9.3	8.0
P. aeruginosa	-	7.0	7.3
S. aureus	-	7.0	8.0

EtAc, ethyl acetate; MeOH, methanol.

Antibacterial activity result of extracts of *H. erinaceus* is like (Table 3). In resistant bacteria clear monovalence was shown though it was weak. But, antibacterial activity against oral bacteria did not almost appear. Among nine kinds of oral bacteria, clear zones could be checked in only three kinds. In EtAc extract which showed antibacterial activity in all resistant bacteria, antibacterial activity could not be checked. On the other hand, in acetone extract which did not show antibacterial activity, clear zones against *S. aureus*, *S. mutans*, and *S. sanguinis* could be checked though weak. Plus, in MeOH extract, weak antibacterial activity against *S. mutans* was shown. Conclusively, it was checked that *H. erinaceus* showed weak antibacterial activity against *S. mutans* and *S. sanguinis* which are concerned with the induction of dental caries. Additionally, it was checked that *H. erinaceus* has better antibacterial effect on resistant bacteria than oral bacteria. There is some report[30,31] that as the content of phenolic compounds is higher, antibacterial activity is higher, however, in this study, clear zones were not shown in acetone extract which has relatively higher polyphenol content than other solvent extracts. So, it is thought that other bioactive substances like alkaloid and terpenoid showing antibacterial activity as well as phenolic compounds act on antibacterial activity.

Table 3. Antimicrobial activities of various solvent against oral bacteria from *Hericium erinaceus*

	Acetone	EtAc	MeOH
Actinomyces naeslundii	-	-	-
Actinomyces viscosus	-	-	-
Aggregatibacter actinomycetemcomitans	-	-	-
Staphylococcus aureus	+	-	-
Streptococcus anginosus	-	-	-
Streptococcus mutans	+	-	+
Streptococcus ratti	-	-	-
Streptococcus sanguinis	+	-	-
Streptococcus sobrinus	-	-	-

-, no inhibition (<6 mm); +, slight inhibition (6-8 mm); ++, moderate inhibition (~10 mm); +++, heavy inhibition (>10 mm). EtAc, ethyl acetate; MeOH, methanol

3.2 Total Content of Polyphenol and Flavonoid

To investigate the total content of polyphenol and flavonoid of *H. erinaceus*, by using three kinds of solvents such as acetone, EtAc, and MeOH, extracts were made. Contents of phenolic compounds and flavonoids differed according to extract solvents. The result of total contents of polyphenols and flavonoids is like (Figure 1 and Figure 2). The total polyphenol content was the highest in acetone extract with 8.35 ± 0.03 mg GAE/g extract. It was investigated that EtAc and MeOH extracts were 5.79 ± 0.18 mg GAE/g extract and 2.53 ± 0.08 mg GAE/g extract. The study result did not coincide with the report[32,33] that the more increasing is polarity of solvent; the more increasing is the concentration of phenolic compounds. Also, overall, similar to polyphenol content, the result about total content of flavonoids checked that acetone extract was the highest with 1.02 mg QE/g extract and then EtAc and MeOH extracts were in order. Most phenolic compounds originated from plants exist in diverse types such as flavonol and anthocyanidin as flavonoid components and also phenolic acid and coumarin as non-flavonoid compounds[34,35]. The fact that polyphenol content was higher than flavonoid content is thought to be that non-flavonoid phenolic compounds except flavonoids belonging to phenols were extracted more.

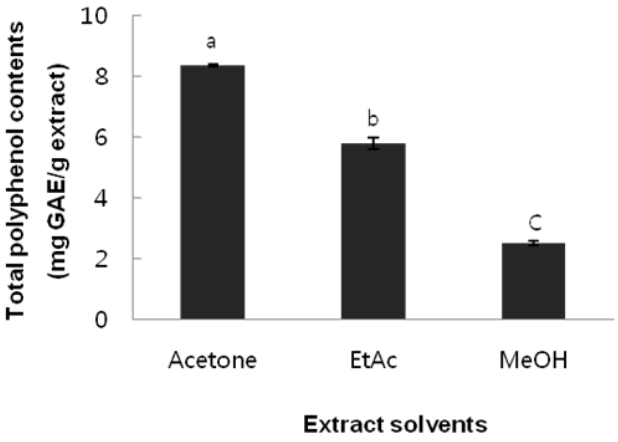

Figure 1. Total polyphenol contents of extracts from *Hericium erinaceus.*

The results represent the mean±SD of values obtained from three independent experiments. MeOH, methanol; EtAc, ethyl acetate. Values are expressed as mg gallic acid equivalent (GAE) per g of extract. a,b,c Mean with different letter on the bars are significantly different by Tukey (P<0.05).

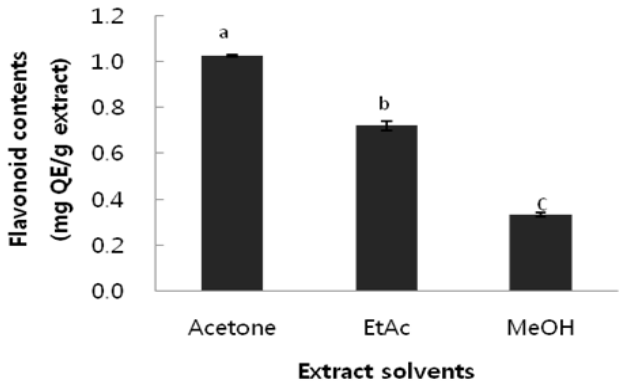

Figure 2. Total flavonoid contents of extracts from *Hericium erinaceus.*

The results represent the mean±SD of values obtained from three independent experiments. MeOH, methanol; EtAc, ethyl acetate. Values are expressed as mg quercetin equivalent (QE) per g of extract. a,b,c Mean with different letter on the bars are significantly different by Tukey (P<0.05).

3.3 Measurement of DPPH Radical Scavenging Ability

DPPH radical scavenging ability is a measuring method of antioxidant activity using the principle that DPPH free radicals are reduced by antioxidant substances and bleached into deep purple[36]. The result to measure the DPPH radical scavenging ability of *H. erinaceus* extracts is like the (Figure 3). The DPPH radical scavenging ability of *H. erinaceus* extracts was investigated to be 46.1 ± 0.23%, 46.0 ± 0.18%, and 18.6 ± 0.82% in EtAc, acetone, and MeOH respectively. Contents of polyphenols and flavonoids and the DPPH radical scavenging ability showed positive correlation (r = 0.90). It was known that according to the increase of polyphenol and flavonoid contents, the DPPH radical elimination ability increased. It is thought to be that phenolic compounds act greatly on the DPPH radical elimination ability of *H. erinaceus*. Kim et al.[37] reported that the DPPH radical elimination ability of *H. erinaceus* extracts was 87.78%. There was big difference with the DPPH radical elimination ability of organic solvent extracts. Therefore, like the report[38] that solubility of polyphenols in the solvent of mixing water and organic solvents is increased, since increase of polyphenol content can heighten antioxidant activity, it is considered that additional activity researches using mixed solvents should be realized in the future.

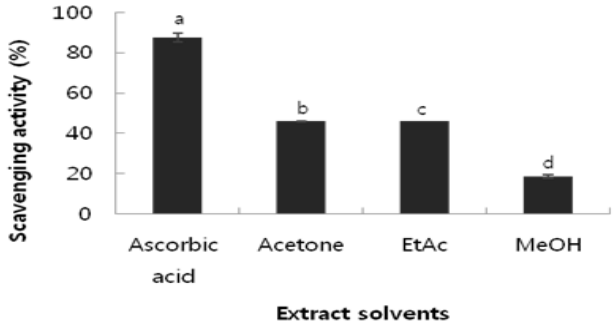

Figure 3. DPPH free radical scavenging activity of extracts from *Hericium erinaceus.*

The results represent the mean±SD of values obtained from three independent experiments. Ascorbic acid was used as positive control. MeOH, methanol; EtAc, ethyl acetate. a,b,c,d Means with different letter on the bars are significantly different by Tukey (P<0.05).

3.4 Measurement of ABTS Radical Scavenging Ability

ABTS radical scavenging ability is a measuring method of antioxidant capacity using the principle that blue-green ABTS radicals which were created from responding with potassium persulfate are bleached from acting with antioxidant substances among specimens[3]. The result of measuring the ABTS radical scavenging ability of *H. erinaceus* is like (Figure 4). In the ABTS radical scavenging ability according to extract solvents, MeOH extract was higher with 92.8 ± 2.13% than ascorbic acid as a comparison group. In acetone and EtAc extracts, high activity was shown with 89.4 ± 0.27% and 88.5 ± 0.71%, respectively. Generally, like the report[39] that pigment components such as anthocyanine and carotenoid impede

the measuring DPPH radical elimination ability and low antioxidant values are shown, in this report, it was checked that ABTS radical scavenging ability had much higher activity compared with DPPH radical scavenging ability. Plus, as ABTS radical scavenging ability and polyphenol content showed negative correlation (r = -0.79), it is considered that not phenolic compounds but other biological activity substances act in the ABTS radical elimination test.

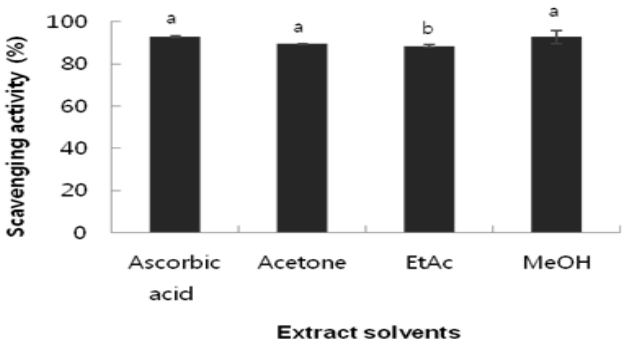

Figure 4. ABTS activity of extracts from *Hericium erinaceus*.

The results represent the mean±SD of values obtained from three independent experiments. Ascorbic acid was used as positive control. MeOH, methanol; EtAc, ethyl acetate. a,b Means with different letter on the bars are significantly different by Tukey (P<0.05).

4. Conclusions

In this study, after *H. erinaceus* was extracted from using organic solvents like acetone, EtAc, and MeOH, antioxidant activities such as contents of polyphenols and flavonoids, DPPH radical scavenging ability, ABTS radical scavenging ability as well as antibacterial activity against resistant bacteria and oral bacteria were investigated. At the result of measuring antibacterial activity against resistant bacteria, clear zones against eight kinds of bacteria used in the extracts of EtAc and MeOH except acetone extract could be checked. Although these extracts did not show strong activity, regardless of kinds of Gram-positive bacteria and Gram-negative bacteria, it was checked that all bacteria used in the experiment had activity. At the result of antibacterial activity against nine kinds of oral bacteria, weak inhibition effect against *S. aureus*, *S. mutans*, and *S. sanguinis* was shown in acetone extract. In MeOH extract, only antibacterial activity against *S. mutans* was represented.

The content of total polyphenols of *H. erinaceus* was high in order of acetone, EtAc, and MeOH extracts. The flavonoid content showed the same result. DPPH radical elimination ability was measured about 46.0% in EtAc and acetone extracts and about 19% in MeOH extract. Through the correlation of the contents of polyphenols and flavonoids and the quantity of DPPH radical scavenging ability, it is thought that phenolic compounds in DPPH radical scavenging ability of *H. erinaceus* act greatly. Plus, it was presented that ABTS radical scavenging ability was the most excellent in MeOH extract. As seen in the investigation result that 88.5 to 92.8% were in order of acetone extract and EtAc extract, ABTS radical scavenging ability was higher significantly than DPPH radical elimination ability. Based on the study results, it is considered that *H. erinaceus* will be available as a valuable material as a natural preservative and natural functional food.

5. References

1. Han JH, Moon HK, Chung SK, Kang WW. Comparison of antioxidant activities of radish bud (*Raphanus sativus L.*) according to extraction solvents and sprouting period. J Korean Soc Food Sci Nutr. 2013; 42:1767–75.
2. Lee HJ, Do JR, Jung SK, Kim HK. Physiological properties of Sarcodon aspratus extracts by ethanol concentration. J Korean Soc Food Sci Nutr. 2014; 43:656–60.
3. Kang HW. Antioxidant and anti-inflammatory effect of extracts from *Flammulina velutipes (Curtis)* singer. J Korean Soc Food Sci Nutr. 2012; 41:1072–8.
4. Choe SB, Kang ST. Investigation of antimicrobial activity and stability of *Orixa Japonica Thunb*. leaf extract. Korean J Food Sci Technol. 2014; 46:39–43.
5. Youm TH, Lim HB. Antimicrobial activities of organic extracts from fruit of *Thuja orientalis L*. Korean J Medicinal Crop Sci. 2010; 18:315–22.
6. Kim JH. Biological activities of water extract and solvent fractions of an edible mushroom, *Hericium erinaceus*. Kor J Mycol. 2012; 40:159–63.
7. Nakajima A, Ishida T, Koga M, Takeuchi T, Mazda O, Takeuchi M. Effect of hot water extract from *Agaricus blazei murill* on antibody-producing cells in mice. Int Immunopharmacol. 2002; 2:1205–11.
8. Mizuno M, Morimoto M, Minato K, Tsuchida H. Polysaccharides from *Agaricus blazei* stimulate lymphocyte t-cell subsets in mice. Biosci Biotechnol BioChem. 1998; 62:434–7.
9. Choi J, Ha TM, Kim YH, Rho YD. Studies on the main factors affecting the mycelial growth of *Phellinus iitenus*. Kor J Mycol. 1996; 24:214–22.
10. Chihara G, Hamuro J, Maeda Y, Arai Y, Fukuoka F. Fractionation and purification of the polysaccharide with marked antitumor activity, especially Lentinan from *Lentinus edodes (Berk.) Sing*. (an edible mushroom). Cancer Res. 1970; 30:2776–81.

11. Tsukagoshi S, Ohashi F. Protein-bound polysaccharide preparation, PS-K, effective against mouse sarcoma-180 and rat ascites hepatoma AH-13 by oral use. Gan. 1974; 65:557–8.
12. Choi HJ, Kim NJ, Kim DH. Hypoglycemic effect of GE974 isolated from *Gyrophora esculenta* in normal and diabetic mice. Kor J Pharmacogn. 2000; 31:268–72.
13. Takeuchi T, Iinuma H, Iwanaga J, Takahashi S, Takita T, Umezawa H. Coriolin, a new basidiomycetes antibiotic. J Antibiot (Tokyo). 1969; 22:215–7.
14. Anke T, Oberwinkler F, Steglish W, Hofle G. The striatins-new antibiotics from the basidiomycete *Cyathus striatus (Huds. ex Pers.) willd.* J Antibiot (Tokyo). 1977; 30:221–5.
15. Midland SL, Izac RR, Wing RM, Zaki AI, Munnecke DE, Sims JJ. Melleolide, a new antibiotic from *Armillaria mella.* Tetrahedron Lett. 1982; 23:2515–8.
16. Erkel G, Anke T, Gimenez A, Steglich W. Antibiotics from basidiomycetes. XLI. Clavicoronic acid, a novel inhibitor of reverse transcriptases from *Clavicorona pyxidata (Pers. ex Fr.) doty.* J Antibiot (Tokyo). 1992; 45:29–37.
17. Shimada A, Shirai R, Sakamoto H, Yoshida S, Ojima F, Ishiguro Y, Kawagishi H. Antimicrobial chlorinated orcinol derivatives from mycelia of *Hericium erinaceum*. Phytochem. 1993; 34:1445–6.
18. Lee EW, Shizuki K, Hosokawa S, Suzuki M, Suganuma H, Inakuma T, Li J, Ohnishi-Kameyama M, Nagata T, Furukawa S, Kawagish H. Two novel diterpenoids, erinacines H and I from the mycelia of *Hericium erinaceum.* Biosci Biotechnol Biochem. 2000; 64:2402–5.
19. Kenmoku H, Shimai T, Toyomasu T, Kato N, Sassa T, Erinacine Q. A new erinacine from *Hericium erinaceum,* and its biosynthetic route to erinacine C in the basidiomycete. Biosci Biotechnol Biochem. 2002; 66:571–5.
20. Kim SR, Kim MR. Physicochemical characteristics and antioxidant activity, antimutagenicity and cytotoxicity of hot-water extract of Hericium erinaceus. Korean J Food Cookery Sci. 2012; 28:569–77.
21. Kawagishi H, Shimada A, Shirai R, Okamoto K, Ojima F, Sakamoto H, Ishiguro Y, Funkawa S. Erinacines A, B and C strong stimulators of Nerve Growth Factor (NGF) - synthesis from the mycelia of *Hericium erinaceus.* Tetrahedron Lett. 1994; 35:1569–72.
22. Kawagishi H, Shimada A, Hosokawa S, Mori H, Sakamoto H, Ishiguro Y, Sakemi S, Bordner J, Kojima N, Furukawa S. Erinacines E, F and G, stimulators of Nerve Growth Factor (NGF) - synthesis from the mycelia of *Hericum erinaceus.* Tetrahedron Lett. 1996; 37:7399–402.
23. Kawagishi H, Shimada A, Shizuki K, Mori H, Okamoto K, Sakamoto H, Furukawa S. Erinacine D, A stimulator of NGF - synthesis from the mycelia of *Hericum erinaceus.* Heterocyclic Communications. 1996; 2:51–4.
24. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493–6.
25. Folin O, Denis W. A colorimetric method for determination of phenols (phenol derivatives) in urine. J Biol Chem. 1915; 22:305–8.
26. Lee GD, Chang HG, Kim HK. Antioxidative and nitrite-scavenging activities of edible mushroom. Korean J Food Sci Technol. 29:432–6.
27. Blois MS. Antioxidant determination by the use of a stable free radical. Nature. 1958; 181:1199–200.
28. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med. 1999; 26:1231–7.
29. Wong KH, Sabaratnam V, Abdullah N, Kuppusamy UR, Naidu M. Effects of cultivation techniques and processing on antimicrobial and antioxidant activities of *H. erinaceus.* Food Technol Biotechnol. 2009; 47:47–55.
30. Clark AM, El-Feraly AS, Li WS. Antimicrobial activity of phenolic constituents of *Mangnolia grandiflora.* J Pharm Sci. 1981; 70:951–2.
31. Kim JY, Lee JA, Yoon WJ, Oh DJ, Jung YH, Lee WJ, Park SY. Antioxidative and antimicrobial activities of *Euphorbis jolkini* extracts. Korean J Food Sci Technol. 2006; 38:699–706.
32. Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 2003; 81:249–55.
33. Moure A, Franco D, Sineiro J, Dominguez H, Nunez MJ, Lema JM. Evaluation of extracts from *Gevuina avellana Hulls* as antioxidants. J Agric Food Chem. 2000; 48:3800–7.
34. Hollman PCH, Hertog MGL, Katan MB. Analysis and health effects of flavonoids. Food Chem. 1996; 57:43–6.
35. Robbins RJ. Phenolic acids in foods: An overview of analytical methodology. J Agric Food Chem. 2003; 51:2866–87.
36. Kim HS, Hong MJ, Kang IY, Jung JY, Kim HK, Shin YS, Jun HJ, Suh JK, Kang YH. Radical scavenging activities and antioxidant constituents of oriental melon extract. J Bio-Environment Control. 2009; 18:442–7.
37. Kim HS, You JH, Jo YC, Lee YJ, Park IB, Park JW, Jung MA, Kim YS, Kim SO. Inhibitory effects of *Phellinus linteus* and rice with *Phellinus linteus* mycelium on obesity and diabetes. J Korean Soc Food Sci Nutr. 2013; 42:1029–35.
38. Lee CY, Kim KM, Son HS. Optimal extraction conditions to produce rosemary extracts with higher phenolic content and antioxidant activity. Korean J Food Sci Technol. 2013; 45:501–7.
39. Arnao MB. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. Trends Food Sci Technol. 2000; 11:419–21.