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Some Biological Activities of *Lactarius vellereus* (Fr.) Fr. In Turkey

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Abstract: *Lactarius vellereus* is a well known macro fungus used for food in Turkey. Although it has been consumed as food, there is no information on the biological properties. Therefore we aimed to reveal phenolic substances, antioxidant capacity and antimicrobial effect of *L. vellereus.* Seven phenolics were analysed by High Performance Liquid Chromatography (HPLC) in *L. vellereus* and four of them were determined to be catechin, ferulic acid, *p*-coumaric acid and cinnamic acid. The scavenging effect of *L. vellereus* on 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH) was measured as 86% at 1 mg mL^{-1} concentration and its reducing power was 0.205 at 0.4 mg mL^{-1} concentration. The IC_{50} values were determined as 0.68 mg mL^{-1} of *L. vellereus*, 0.43 mg mL^{-1} of BHA and 0.72 mg mL^{-1} of BHT. Chloroform, acetone and methanol extracts of *L. vellereus* were tested for its antimicrobial activities using a micro dilution method against four gram-positive and five gram-negative bacteria. The Minimum Inhibitory Concentration (MIC) was observed with the acetone extract (MIC, 19 µg mL^{-1}) against *K. pneumoniae*. All extracts of the fungus was usually found to be antimicrobial activity against the test microorganisms. The results showed that *L. vellereus* can be recommended as a source of natural antioxidants and antimicrobials.

Key words: Phenolics, antioxidant capacity, antimicrobial effect

INTRODUCTION

Some fungal species have been used as tea or nutritious food source for many years in Eastern cultures because of their unique scents and soft structures.

Some species of edible, inedible and poisonous mushrooms are known to have important medicinal properties. Some studies have demonstrated that fungi have high amounts of antioxidants and antimicrobial effects (Mau *et al*., 2002; Yang *et al*., 2002; Cui *et al*., 2005; Barros *et al*., 2007a; Nakajima *et al*., 2007; Jua *et al*., 2010). Antioxidants, or the molecules that have a scavenging effect on DPPH radicals, are known as potentially protective substances (Ramirez-Anguiano *et al*., 2007). This protective effect is mainly attributed to well-known antioxidants such as ascorbic acid, tocopherols and β carotenes, but plant phenols also play an important role. According to Barros *et al*. (2007b), mushrooms can be regarded as rich sources for molecules with antioxidant, antitumor and antimicrobial properties.

L. vellereus is used as a food in Turkey. Local people consume this mushroom and also sell it at the open markets. It grows in coniferous or mixed coniferous and deciduous forests. Although, *L. vellereus* is an important food source, there are no conclusive reports concerning its phenolics, antioxidant activities or antimicrobial effects. The main objectives of the current study are as follows: (1) To reveal the phenolics, (2) To study the antioxidant activity of *L. vellereus* methanolic extract and (3) To find the antimicrobial effect of *L. vellereus*.

MATERIALS AND METHODS

Collection of *L. vellereus* samples: The samples were collected in the mixed *Pinus sylvestris* and *Quercus* sp. forest from Ordu in 2007. The fungarium number of the species is HD3958. The species identification was performed as described in the literature (Basso, 1999). A stock sample of the species was also deposited at the Fungarium of the Mushroom Application and Research Centre, Selçuk University, Konya, Turkey.

Antioxidant activities

Determination of the phenolics: The total phenolic content of the methanol crude extract was determined by the Folin-Ciocalteu method with some modifications, according to Singleton and Rossi (1965).

A Shimadzu 1100 series HPLC equipped with a SIL-10AD vp autosampler and LC-10Advp pump system, Diode Array Detector (DAD) and an Inertsil Agilent Eclipse XDB column (240×4.60 mm, 5 µm particle size) was used to analyse phenolic compounds. The mobile phase consisted of (A) 100% methanol and (B) 3% (v/v)

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aqueous acetic acid. HPLC separation was performed as described by Maltas and Yildiz (2011). Gallic acid, catechin, caffeic acid, *p*-coumaric acid, ferulic acid, cinnamic acid and quercetin (Sigma-Aldrich) were analysed as standard phenolic structures. The samples were run in triplicate.

Preparation of the methanol extracts for testing antioxidant activities: Briefly, 100 g of the dried and powdered sample was extracted with stirring in methanol at 60°C for 6 h in a Soxhlet apparatus. The extract was then filtered through Whatman No. 4 filter paper and concentrated under a vacuum at 45°C using a rotary evaporator. The extracts were then lyophilised and stored in the dark at 4°C.

Scavenging effect on DPPH radicals: A 5 mL of the methanol extract in a range of concentrations (0.5-5 mg mL^{-1}) was added to 1 mL of a DPPH radical solution in methanol (the final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank using the Hitachi U-2001 spectrophotometer (Shimada *et al*., 1992). BHT and BHA were used as standard controls. The inhibition of DPPH free radicals was calculated in percent (I%) using the following equation:

$$I\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The extract concentration providing 50% inhibition (IC_{50}) was calculated from a graph plotting inhibition percentage against extract concentration. Tests were performed in triplicate.

β-carotene-linoleic acid assay: One milliliter of β-carotene solution in chloroform (3.34 mg mL^{-1}) was pipetted into a flask containing 40 mg linoleic acid and 400 mg Tween 20. The chloroform was then removed using a rotary evaporator at 40°C for 5 min. To the resulting residue, 100 mL of oxygen passed through distilled water was added slowly with vigorous agitation to form an emulsion. A 5 mL aliquot of this emulsion was added to a tube containing 0.2 mL of the 200 mg mL^{-1} antioxidant solution and the absorbance was measured immediately at 470 nm against a blank, which consisted of the emulsion without β-carotene. The tubes were then placed in a water bath at 40°C and the absorbance was measured again at 15 min intervals.

Reducing power: Each extract (0.04-0.4 mg mL^{-1}) in methanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20 min. Next, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture was centrifuged at 200 g for 10 min. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank in a Hitachi U-2001 spectrophotometer.

Determination to antimicrobial effects

Preparation of the extracts: Powdered fungus sample (30 g) was extracted with 250 mL of chloroform in a glass beaker for 8 h at 55°C. The resultant extract was concentrated using a rotary evaporator at 40°C and low pressure and the desired phase was separated from the crude extract with chloroform. Later, the residue was extracted with acetone and methanol, respectively. After extraction, all the semi-solid extracts were dried by a freeze-dryer to yield powders. The powdered extracts were dissolved in DMSO:PBS (1:1) at a 100.000 μg mL^{-1} concentration. These extracts were filtered through a sterile filter (0.45 μm) and stored at 4°C.

Test microorganisms: All the microorganisms were obtained from the Department of Biology, Faculty of Science, Selçuk University. Four Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6633, *Listeria monocytogenes* type 2 NCTC 5348 and *Streptococcus pyogenes* ATCC 19615) and five gram-negative bacteria (*Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 15442, *Proteus vulgaris* ATCC 7829 and *Salmonella enteritidis* RSHMB) were chosen as the test bacteria. *Candida albicans* ATCC 1023 was chosen as the test yeast.

Antimicrobial assay: Brain Heart Infusion Broth (BHIB, Oxoid) was used to cultivate the bacteria and Malt Extract Broth (MEB, Difco) was used for the yeast. Each bacterial species obtained from stock cultures were added into 4-5 mL BHIB and incubated at 35°C for 24 h. The bacterial cultures were prepared in the same medium at a density adjusted to 0.5 McFarland turbidity standards (10^8 CFU mL^{-1}) and the final concentration of each bacterial culture was adjusted to 10^5 CFU mL^{-1}. The yeast strain obtained from the stock culture was added into 4-5 mL MEB and incubated at 25°C for 48 h. After incubation, the final concentration of the yeast was adjusted to 10^4 CFU mL^{-1}.

Determination of antimicrobial activity by the micro dilution method: *L. vellereus* extract in the stock solutions was prepared at 20000 μg mL^{-1} concentration in PBS: DMSO (1:1). MHB (100 μL) was dispensed into each well of a flat-bottom, 96-well microtiter plate. To prepare serial dilutions, 100 μL of *L. vellereus* chloroform, acetone or methanol extract was separately dispensed into MHB-containing microplate wells (A1, A2, A3, etc.) and mixed well. Next, 100 μL of this mixture was pipetted from the first well into a second well, generating a ½ dilution. Finally, this process was repeated to generate a dilution series of each extract from 20000 to 0.305 μg mL^{-1}. After the dilution series of each extract was prepared, 100 μL of each bacterial suspension were separately added into each well containing the MHB and the mushroom extract mixture. This procedure was also repeated for the yeast in different plate wells.

The absorbance of each well was measured using an ELISA reader at 630 nm (EL x 800). After the first reading was finished, all the plates were covered and incubated at 37°C for 24 h. Afterwards, the absorbance was measured again. The first absorbance was subtracted from the second absorbance and the difference was used to calculate the MIC values. The lowest concentration that produced an inhibitory effect was recorded as the Minimum Inhibitory Concentration (MIC) for each extract (as described by Devienne and Raddi (2002), with some modifications). Ampicillin (100 μg mL^{-1} concentration) for bacteria and Amphotericin B (50 μg mL^{-1} concentration) for yeast was used as positive controls. Each experiment was conducted in triplicate. Due to a lack of correlation and statistical significance between each bacterial species, statistical analysis was not performed.

RESULTS AND DISCUSSION

Phenolic contents of *L. vellereus*: The amount of phenolic compounds in the methanol extract of *L. vellereus* was determined to be 0.17 mg mL^{-1} gallic acid equivalents. The phenolic composition of *L. vellereus* was reported here for the first time (Table 1). The amount of total phenolics in wild mushrooms varies in different ranges in the literature. The phenolics were found to be 23.28 mg g^{-1} of *Coriolus versicolor* (L.) Quél., 47.25 mg g^{-1} of *Ganoderma lucidum* (Curtis) P. Karst. and 51.28 mg g^{-1} of *G. tsugae* Murril (Mau *et al*., 2002). Wong and Chye (2009) observed the total phenolics of *Pleurotus porrigens* (Pers.) P. Kumm., *Xerula furfuracea* (Peck) Redhead, Ginns and Shoemaker, *Schizophyllum commune* Fr., *Polyporus tenuiculus* (P. Beauv.) Fr., *Hygrocybe conica* (Schaeff.) P. Kumm. and *Pleurotus florida* singer with petroleum ether and methanolic extract. According to their results, the amount of the total phenolics in wild mushrooms varied from 0.98 to 28.23 mg GAE g^{-1} of PE extract. Among the PE and methanolic extracts, *H. conica* had the highest phenolic content of 28.23 and 42.21 mg GAE g^{-1}, respectively while other species varied between 0.83-27.15 mg GAE g^{-1} for both PE and methanolic extract. Barros *et al.* (2007b) studied the phenolics of three mushrooms and they found that *Leucopaxillus giganteus* (Quél.) Singer extracts showed the highest phenolic content (6.29 mg g^{-1}) whereas *Agaricus arvensis* Schaeff. extracts (2.83) was slightly lower than in *Sarcodon imbricatus* (L.) P. Karst. (3.76 mg g^{-1}). The total phenolic content of *Pleurotus ostreatus* (Jacq.) P. Kumm. was found to be 5.49 g/100 g (Jayakumar *et al*., 2009). Compared to relevant literature, the total phenolic of *L. vellereus* is relatively lower. The yield of extracts do not associated with the total phenolics content of the fungal species but might be influenced by the types of phenolic compounds present along with the extractability of the solvent used in the preparation. These results indicate that phenolic quantities in fungal species can vary dependent on the methods applied or the solvents.

Seven phenolics from the methanolic extract of *L. vellereus* were analysed and four of them were identified as catechin, ferulic acid, *p*-coumaric acid and cinnamic acid as illustrated in Table 1, there are significant qualitative and quantitative differences in the components of the extracts. Catechin is the predominant phenolic acid with a value of 20 mg g^{-1}, followed by ferulic acid at 18 mg g^{-1}, *p*-coumaric acid at 12 mg g^{-1} and cinnamic acid at 8 mg g^{-1}. However, the extract did not contain any flavonoids, such as quercetin, gallic acid and caffeic acid. Antioxidant, antimicrobial, antiallergy and anticancer effects of catechin were reported in some previous studies (Kondo *et al*., 2000; Shimamura *et al*., 2007). Catechin was found in *R. delica* at 5.33 mg g^{-1} by Yaltirak *et al.* (2009). In the present study, it was found at 20 mg g^{-1} and this ratio is quite high. Antimicrobial activities were already appearing in high-impact in the present study and this can be related with high catechin level.

Ferulic acid, likes many phenols, is an antioxidant '*in vitro*' in the sense that it is reactive towards free radicals, such as Reactive Oxygen Species (ROS). ROS

Table 1: Percentage values of Identified Phenolic substances of *L. vellereus*

Phenolics	Composition (mg g^{-1})
Caffeic acid	ND
Catechin	20
Cinnamic acid	8
Ferulic acid	18
Gallic acid	ND
p-coumaric acid	12
Quercetin	ND

ND: Not deleted

and free radicals are implicated in DNA damage, cancer and accelerated cell aging. Ferulic acid may be effective at preventing cancer induced by exposure to carcinogenic compounds (Kampa *et al*., 2004).

Coumarin derivatives are important substances for human health. They have anti-thrombotic, anti-inflammatory and vasodilatory effects as well as antiviral and antimicrobial activities (Cowan, 1999).

Cinnamic acids are common representatives of a wide group of phenylpropane-derived compounds that are in the highest oxidation state. Cinnamic acids are effective against viruses, bacteria and fungi (Cowan, 1999).

As a result, high and varied phenolic compounds in *L. vellereus* increase its medical importance.

Antioxidant activity results

The scavenging effects on DPPH radicals: The DPPH radical scavenging effects of *L. vellereus*, as well as those of BHA and BHT controls increased with the increasing concentration from 0.5 to 5 mg mL^{-1} (Fig. 1). The scavenging values at 0.5 mg mL^{-1} concentration was 0.046 mg mL^{-1} for *L. vellereus*, 0.047 mg mL^{-1} for BHT and 0.038 mg mL^{-1} for BHA, while the scavenging values at 5 mg mL^{-1} was 0.035 mg mL^{-1} for *L. vellereus*, 0.035 mg mL^{-1} for BHT and 0.028 mg mL^{-1} for BHA. As illustrated in Fig. 1, the scavenging effect of *L. vellereus* extract on DPPH radicals rose with the increasing concentrations.

The IC_{50} value of *L. vellereus* extract was higher than BHA and lower than BHT. The IC_{50} values was 0.68 mg mL^{-1} for *L. vellereus*, 0.43 mg mL^{-1} for BHA and 0.72 mg mL^{-1} for BHT (Fig. 2). A lower IC_{50} value indicates higher antioxidant activity (Pourmorad *et al*., 2006). The IC_{50} of *L. vellereus* was lower than the standard of BHT and it has an effective antioxidant activity. The inhibition values of *L. vellereus* extract and the standards on the DPPH radicals at 5 mg mL^{-1} were 88% for *L. vellereus*, 90% for BHA and 88% for BHT (Fig. 3).

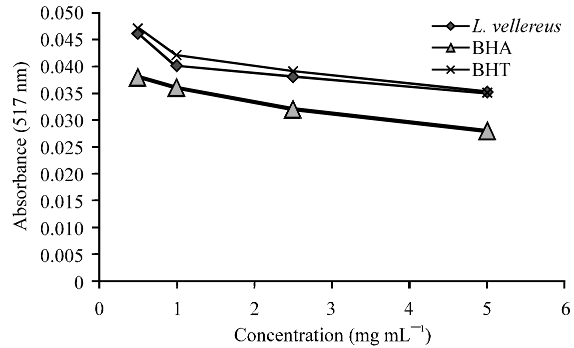

Fig. 1: The DPPH radical scavenging effects of *L. vellereus*, BHA and BHT

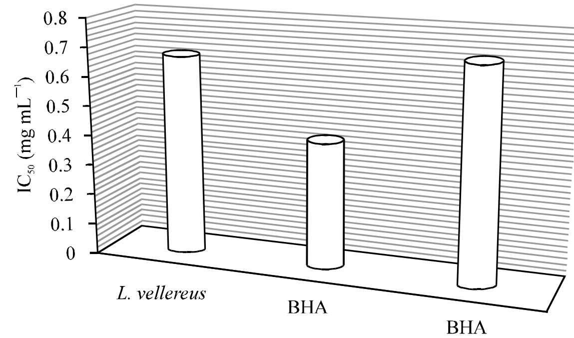

Fig. 2: IC_{50} values of *L. vellereus*, BHA and BHT

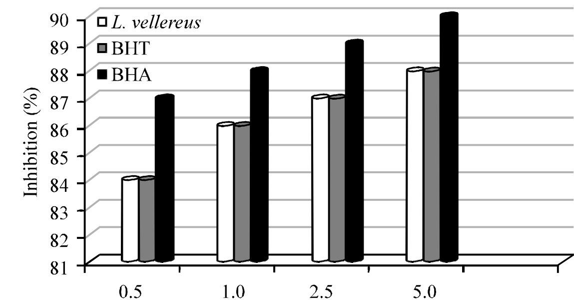

Fig. 3: Percentage inhibition values of *L. vellereus* and standards on the DPPH radicals

In previous studies, the scavenging effects of mushroom extracts ranged from 36-96% at the concentrations of 5 to 10 mg mL^{-1}. Because different methods and concentrations were used in the literature, there are no standard levels for measuring scavenging effects. Some studies can be summarised as follows. The methanolic extract of *Dictyophora indusiata* (Vent.) Desv. was 92% at 6.4 mg mL^{-1} (Mau *et al*., 2004). Yang *et al*. (2002) investigated mushrooms of some commercial importance (*Flammulina velutipes* (Curtis) Singer, *Lentinula edodes* (Berk.) Pegler, *Pleurotus ostreatus* (Jacq.) P. Kumm. and *P. cystidiosus* O.K. Mill.) and they found that the scavenging effects of these mushrooms varied between 42.9 and 81.8% at 6.4 mg mL^{-1}. The scavenging effect of *Volvariella volvacea* (Bull.) Singer was also found as 57.8% at 9 mg mL^{-1} (Cheung *et al*., 2003). Mau *et al*. (2004) used 10 mg mL^{-1} concentration in their studies, which reported scavenging effects of 78.8%, 79.9% and 94.1% for *Termitomyces albuminosus* (Berk.) R. Heim, *Grifola frondosa* (Dicks.) Gray and *Morchella esculenta* (L.) Pers., respectively. Additionally, Lee *et al*. (2008) studied the fruiting body and mycelia of *Hypsizygus marmoreus* (Peck) H.E. Bigelow with hot water and methanol extraction. According to their results, the

scavenging effects of the fruiting body and mycelia ethanol extracts, at 5 mg mL^{-1} concentration, were both 75.5%, while the scavenging effects of the fruiting body and mycelia hot water extracts were 36.8 and 55.5%, respectively, also at 5 mg mL^{-1}. The scavenging effect of *Russula delica* extract at 10 mg mL^{-1} was found as 26% (Yaltirak *et al*., 2009). Compared with the inhibition values reported in those studies, *L. vellereus* is effective at lower concentration. In the present study, inhibition levels of *L. vellereus* reached 88% at 5 mg mL^{-1}.

β-carotene-linoleic acid assay: The rate of absorbance change was calculated from T_0 to 120th min and used to calculate the coefficient of oxidation prevention as a percent (%). The bottom absorbance curve in Fig. 4 is the control sample. The absorbance at the 120th min was measured as 1.643 mg mL^{-1} for *L. vellereus*, 0.73 mg mL^{-1} for control, 1.74 mg mL^{-1} for BHT and 1.89 mg mL^{-1} for BHA. Antioxidant activity was assayed as the ability to inhibit the peroxidation of linoleic acid. The inhibition values of *L. vellereus* were determined to be higher than Trolox, but lower than BHT and BHA. *L. vellereus*, BHA, BHT and Trolox exhibited 64.6, 80.8, 73.7 and 50.51% inhibition, respectively (Fig. 5).

According to literature, the inhibition values of mushroom extracts vary from 50 to 96% at the different concentrations. The antioxidant activity of methanolic extracts of young and mature *Agaricus brasiliensis* Fr. specimens were evaluated with β-carotene and were found to inhibit oxidation 92% at 0.2 mg mL^{-1} (Soares *et al*., 2009). Gursoy *et al*. (2009) studied six Morel species (*Morchella rotunda* (Pers.) Boud., *M. esculenta* (L.) Pers. var. *umbrina* (Boud.) S. Imai, *M. deliciosa* Fr., *M. elata* Fr., *M. conica* Krombh. and *M. angusticeps* Peck) and found *M. esculenta* var. *umbrina* and *M. angusticeps* to be the most active species (96.89 and 96.88%, respectively, at 4.5 mg mL^{-1} concentration). Sarikurkcu *et al*. (2010) investigated *Amanita caesarea* (Scop.) Pers., *Clitocybe geotropa* (Bull.) Quél. and *Leucoagaricus pudicus* (Bull.) Bon and they discovered that *L. pudicus* possessed the highest oxidation level (81.8% at 25.5 mg mL^{-1}), followed by *A. caesarea* (70.1%) and *C. geotropa* (61.3%). In comparison to previous studies, *L. vellereus* has a high oxidation level even at a low concentration (64.6% at 2.284 mg mL^{-1} concentration).

Reducing power: The reducing power of *L. vellereus* showed a parallelism with the increasing concentration (Fig. 6). The highest reducing power, 0.04 to 0.4 mg mL^{-1}, was observed with BHA (1.895 mg mL^{-1}) followed by BHT (1.654 mg mL^{-1}) and *L. vellereus* (0.205 mg mL^{-1}).

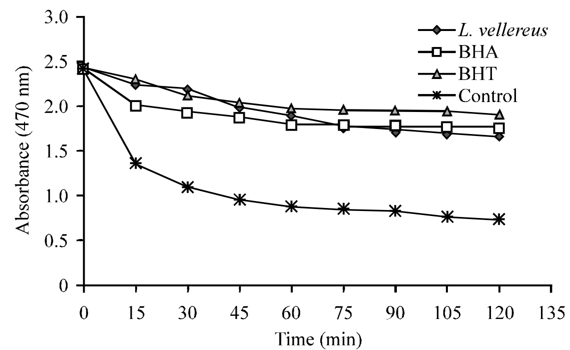

Fig. 4: Oxidation of linoleic acid depented on the time

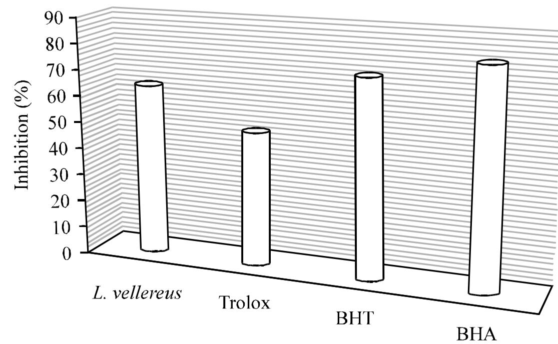

Fig. 5: Percentage of inhibition of linoleic acid oxidation

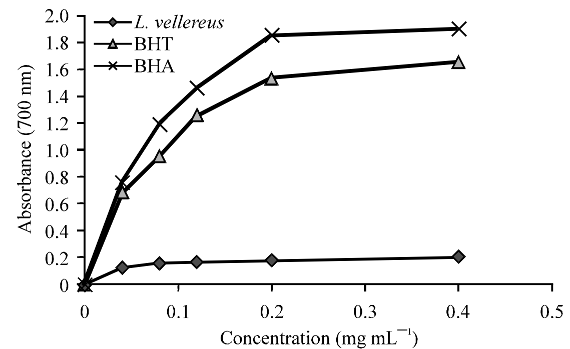

Fig. 6: Reducing powers of *L. vellereus* and synthetic antioxidants

According to Elmastas *et al*. (2007), the reducing power of *R. delica* and *Verpa conica* (O.F. Müll.) Sw extracts were 1.32 and 1.22 μg mL^{-1}, respectively, at 200 μg mL^{-1}. At 20 mg mL^{-1}, the reducing powers of *A. caesarea*, *C. geotropa* and *L. pudicus* were 1.5, 1.2 and 1.3 mg mL^{-1}, respectively (Sarikurkcu *et al*., 2010). In the present study, the reducing power of *L. vellereus* was 0.205 at 0.4 mg mL^{-1} concentration. In comparison to the relevant studies, *L. vellereus*

possesses a high reducing power at a low concentration. Antimicrobial results: According to Craig (1998), to evaluate antimicrobial activity, MIC values should be measured from the 4th through the 16th dilutions. The antimicrobial effects of *L. vellereus* against bacteria and yeast were measured in accordance with the following ranges (Gulay, 2002; Morales *et al*., 2008):

- MIC values are lower than 100 μg mL^{-1} = antimicrobial activity is high
- MIC values are between 100 and 500 μg mL^{-1} = antimicrobial activity is moderate
- MIC values are between 500 and 1000 μg mL^{-1} = antimicrobial activity is weak
- MIC values are more than 1000 μg mL^{-1} = no antimicrobial effect

In accordance with these ranges, the antimicrobial results are given in Table 2. The Minimum inhibitory effect on the test microorganisms was observed with acetone extract (MIC, 19 μg mL^{-1}) against *K. pneumoniae*. Overall, all extracts exhibited good antimicrobial effect with values generally lower than 100 μg mL^{-1} (MIC, 39 μg mL^{-1}) placing it in the high activity category, while the MIC of chloroform extract was much lower (MIC, 78 μg mL^{-1}) against *K. pneumoniae*.

These current results are also confirmed by previous studies. Quereshi *et al.* (2010) tested the antimicrobial activity of various solvent extracts of *G. lucidum* (Curtis) P. Karst (40 mg mL^{-1} concentration) against six species of bacteria. Acetone extract exhibited maximum antibacterial activity, while the most susceptible bacterium observed was *K. pneumoniae*. Bala *et al.* (2011) investigated the antimicrobial effect of forty-seven different specimens from Australia and they found that water and ethanol extracts were more effective against *S. aureus* than *E. coli*, whereas a small number of hexane extracts showed better results for their potential antimicrobial effect against *E. coli* at higher concentration. In generally, a number of macrofungi in the genera *Agaricus*, *Amanita*, *Boletus*, *Cantharellus*, *Fomitopsis*, *Hohenbuehelia*, *Lentinus*, *Ramaria* and *Strobilomyces* showed good inhibition rates. Aqueous and methanolic extracts of *Trametes hirsuta* (Wulfen) Lloyd were tested against pathogenic fungi and bacteria.

The maximum antibacterial activity of aqueous extract of *T. hirsuta* was found against *S. aureus* than that of methanol extract. The significant antifungal activity of aqueous extract was found against *Aspergillus flavus* than that of methanol extract (Sivaprakasam *et al*., 2011). The antibacterial effects of three mushrooms extract *G. lucidum*, *Auricularia auricula* (L.) Underw., *Pleurotus floridanus* singer were studied against *S. aureus* and *E. coli*. *A. auricula* showed significant antibacterial activity against *S. aureus*. *P. floridanus* showed some antibacterial activity while *G. lucidum* showed no antibacterial activity. None of the extracts showed any activity against *E. coli* (Iftekhar *et al*., 2011). Antimicrobial activity of *Ganoderma praelongum* Murrill, *G. resinaceum* Boud. and *G. lucidum* were evaluated against thirty strains of clinical isolates of methicillin resistant and methicillin sensitive *S. aureus* (MSSA). Maximum activity of crude extracts was exhibited by ethyl acetate. The MIC of Sesquiterpenoids extract of *G. praelongum* was 0.390-6.25 mg mL^{-1}. Diterpenoids and triterpenoids displayed moderate activity while polysaccharides IIIa and IIIb showed weak activity. All bacterial strains were resistant to polysaccharides I and II (Ameri *et al*., 2011).

Table 2: Minimal inhibition concentration values of *L. vellereus* extracts (μg mL^{-1}) against tested microorganisms

Microorganisms	Chloroform	Acetone	Methanol
B. subtilis	39	39	39
S. aureus	39	39	39
L. monocytogenes	39	39	39
S. pyogenes	39	39	39
C. albicans	39	39	39
E. coli	39	39	39
K. pneumoniae	78	19	39
P. aeruginosa	39	39	39
P. vulgaris	39	39	39
S. enteritidis	39	39	39

*-<100 μg mL^{-1}: High, 100-500 μg mL^{-1}: Moderate, 500-1000 μg mL^{-1}: Low

The current results are similar or more effective than those reported in the literature. To the best of our knowledge, there were previously no reports for the antimicrobial effects of *L. vellereus* and this result was reported here for the first time.

CONCLUSION

The total phenolic, some phenolics, antioxidant activities and antimicrobial effects of *L. vellereus* were measured. Fungal species can be used a source of healthy foods, contributing high levels of antioxidants and antimicrobial activities.

The present results indicate that economically important and edible mushrooms display significant antioxidant and antimicrobial features. Therefore, these studies should be extended to other economically important and edible mushrooms.

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