Indian Journal of Experimental Biology
Vol. 57, January 2019, pp. 15-20

Anticancer, antibacterial and antioxidant activities of *Cordyceps militaris*

Madhavi Joshi[1*], Anand Sagar[1], Shamsher Singh Kanwar[2] & Sukhdev Singh[2]

[1]Department of Biosciences; [2]Department of Biotechnology, Himachal Pradesh University, Shimla-171 005, India

Received 14 July 2016; *revised 26 September 2017*

Cordyceps militaris (L.) Link has become popular as a medicinal mushroom. Here, we explored the mushroom *C. militaris* for possible bioactive molecules by studying its biological activities. Antioxidant activity of the methanolic extracts was tested using DPPH. Antibacterial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli*. Minimum inhibitory concentrations of the extracts were estimated using broth microdilution method. Observed IC_{50} value was 0.72 mg/mL compared with standard L-ascorbic acid (IC_{50}= 0.062 mg/mL).The MIC's of the extracts ranged from 1.25 to 10 mg/mL against *S. aureus* and 0.625 to 5 mg/mL against *E. coli.* Additionally, cytotoxicity was also investigated towards cancer cell line HEp-2 using MTT. *C. militaris* extracts reduced HEp-2 cells viability with IC_{50} value 20 μg/mL. Bioactive components (phenols, flavonoids, ascorbic acid, β-carotene, and lycopene) were estimated spectrophotometrically. Present study revealed that *C. militaris* has significant antimicrobial, antioxidant and anticancer effects which may be the scientific basis of its medicinal use by herbal practitioners.

Keywords: Antimicrobial, Ascorbic acid, β-Carotene, Cytotoxic, DPPH, Flavonoids, HEp-2 cell line, Herbal, Lycopene, Phenols

A medicinal herb of extensive and remarkable history, *Cordyceps* is a macrofungus of family Clavicipitaceae[1], parasitic on insects, is used as a source of functional food and medicines. *C. militaris*, contains many kinds of active compounds is now used for an assortment of medicinal purposes[2,3], including beneficial effects on the human circulatory, immune, respiratory, and glandular systems. It is commonly used in the treatment of hyperglycemia, hyperlipidemia renal dysfunction and liver diseases[4]. Recently, several studies confirmed that extracts of *C. militaris* have manifold pharmacological actions which integrated anti-inflammatory activity[5], improvement of insulin resistance, insulin secretion, and tumor suppression activity[4]. Traditionally in Asia, mushrooms have been used for medicines and foods[6]. Various polyphenolic compounds present in mushrooms are recognized as an outstanding antioxidant because of their capability to scavenge free radicals by single electron transfer[7]. Some commonly consumed mushrooms in Asian culture have now been found to possess large quantity of phenolic compounds, which is well interrelated with their antioxidant activity[8].

*Correspondence:
E-mail: madhavi.joshi1986@gmail.com

Northwestern Himalayan region of India has been known as a belt containing rich varieties of wild mushrooms and *C. militaris* is a new species which has been collected, worked out and identified by earlier workers[9]. The therapeutic significance of mushrooms lies in some chemical substances that produce a distinct physiological effect on the human body. Majority of these bioactive constituents are phenolic compounds and flavonoids[10]. Hence, in the present work we determined for the first time, the antibacterial activity, cytotoxic activities and antioxidant activities of the extracts of the fruiting body. The contents of potential bioactive component, such as phenols, flavonoids, ascorbic acid, β carotene, and lycopene have also been estimated.

Materials and Methods

Standards and reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl), L-ascorbic acid, tannic acid, (+) catechin, FCR were obtained from Sigma (St.Louis,Mo,USA). Foetal calf serum obtained from PAA Laboratories. The culture media nutrient broth was obtained from Himedia (Himedia labs, Mumbai, India). Water was treated in a Milli-Q water purification system (TGI Pure water Systems, Greenville, SC, USA) before use. All other chemicals and solvents were of analytical grade.

Sample collection

Fruiting bodies of *C. militaris*, were collected from Glen forest of North-Western Himalayas, India and identified at Department of Biosciences, Himachal Pradesh University, Shimla. Fresh fruiting bodies of mushrooms were cut into small pieces and sun-dried. A coarse powder was obtained using a electronic mill.

Extract preparation for antioxidant activity

For preparation of the extract, 10 g sample was extracted by stirring at 100 rpm with 100 mL methanol at 30°C for 24 h and filtered through Whatman No. 1 filter paper. The residue was then extracted with two additional 100 mL portions of methanol, in a similar manner. The combined methanolic extracts were evaporated by rotary evaporator at 40°C to dryness, re-dissolved in methanol to a concentration of 20 mg/mL and stored in dark at 4°C for further use[11].

Scavenging ability on 2, 2-diphenyl-1-picrylhydrazyl radicals

The hydrogen atoms or electron donation ability of the extracts was measured from the bleaching of the purple coloured DPPH methanolic solution with little modification. About three mL of different concentrations of the extracts was added to one mL of methanolic DPPH (final concentration of DPPH was 200 μM). The mixture was shaken briskly and allowed to stand for 30 min at room temperature (25°C) and absorbance of the resulting solution was measured at 517 nm using spectrophotometer (Merck SpectroquantPharo 100)[12]. Inhibition of the DPPH free radicals in (%) was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{absorbance control - absorbance sample}}{\text{absorbance control}} \times 100$$

Whereas, control containing all reagents except the test compound. L-ascorbic acid was used as standard.

Preparation of extracts for antibacterial activity

Aqueous, ethanol, chloroform, acetone and methanolic extracts of experimental fruiting bodies were prepared following Indian Pharmacopoeia[13]. From the dried extracts, stock solution of 40 mg/mL concentration was prepared in the distilled water. Streptomycin was used as reference and its solution was prepared at a concentration of 1.0 mg/mL in sterile distilled water.

Test Microorganisms

The microorganisms used were clinical isolates from patients hospitalized in various department Hospital center of Indira Gandhi Medical College, Shimla, India. Gram-positive bacteria *Staphylococcus aureus* isolated from wound exudates and Gram-negative bacteria *Escherichia coli* isolated from urine.

Test assays for antibacterial activity

Antibacterial activity of different extracts was tested using agar well diffusion method[14]. The well in each plate was loaded with 10-40 mg/mL concentration of prepared extracts of *C. militaris*. The plates were incubated at 37±2°C for 24 h in incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control diameter from the values of inhibition diameter using control as standard[15].

$$\text{Percentage of growth inhibition} = \frac{Control - Test}{Control} \times 100$$

Control = average diameter of bacterial colony in control. Test = average diameter of bacterial colony in treatment sets.

Determination of MIC

To screen for MIC's, 96-well plates were prepared[16]. The test solution was prepared by mixing 20 mg of the solidified extract into 1.0 mL of sterile distilled water. About 98 μL of the prepared extract were added to initiate the serial dilutions and the plates were then incubated at 37°C for 24 h. MIC was defined as the lowest concentration of the extract that inhibited bacterial growth. Bacterial growth was observed using an indicator solution of resazurin, which turns from blue to pink in the presence of growing bacteria.

Cytotoxicity of aqueous extract of *C. militaris* on HEp-2 cell line

Established HEp-2 cell line (procured from Central Research Institute, Kasauli, India) was used to evaluate the cytotoxic effects of *C. militaris.* HEp-2 cells were grown in DMEM (Himedia) supplemented with 10% heat inactivated foetal calf serum and incubated in a humidified atmosphere of 4% CO_2 at 37°C. The cytotoxicity was measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The assay detects the reduction of MTT by mitochondrial dehydrogenase to a blue formazan product which reflects the normal function of mitochondria and cell viability. For testing, cells

were washed in DMEM, harvested by trypsinization and seeded in a 96-well microplate (CELLSTAR, Greiner Bio-one). After 48 h incubation when monolayer was observed, cells were treated with different concentrations of aqueous extract of *C. militaris* and re-incubated for 24 h. Then 20 µL of MTT solution (5 mg/mL) was added to each well. After 1 h incubation with MTT, medium were aspirated from each well and 100 µL DMSO was added to each well and gently shaken. Reduced MTT was assayed at 570 nm using a microplate reader (Thermo Electron Corporation). Culture medium containing water was used as positive control and untreated cells were used as negative control. The % of cell inhibition was calculated using following formula:

$$\text{Cell Inhibition (\%)} = \frac{\text{absorbance control - absorbance sample}}{\text{absorbance control}} \times 100$$

Determination of bioactive components

Total phenolic content of the extract was determined following Makkar *et al.*[17]. The reaction mix was prepared by amalgamation of the extract (0.1 mL) and double distilled water (0.9 mL). Subsequently; 2.5 mL of sodium carbonate solution (20%) was added to it, followed by 0.5 mL of FCR (1N). Absorbance was read at 725 nm after 40 min incubation at room temperature. Tannic acid (0.5 mg/mL) was used to prepare standard curve. The results were expressed as mg of TAEs per g of the extracts.

The quantity of total flavonoids (TFC) was estimated colorimetrically[18]. A suitable aliquot 250 µL taken for estimation was mixed with 1.0 mL of water in a test tube. About 75 µL of aqueous $NaNO_2$ (5%) was added to the test tube, then after 5 min, 150 µL of 10% $AlCl_3$ and after 6 min, 500 µL of 1.0 M NaOH were added sequentially. Finally, 275 µL distilled water was added. The reaction mixture was mixed thoroughly. The absorbance was noted at 510 nm using a spectrophotometer. TFC, calculated using a standard calibration curve, were reported as (+) catechin equivalents (mg CE/g of the extract).

β-carotene and lycopene were determined according to the method of Nagata and Yamashita[19]. The dried extract (100 mg) was vigorously shaken with 10 mL of acetone-hexane mixture (4:6) for 1 min and filtered through Whatman No.1 filter paper. The absorbance of the filtrate was measured at 453, 505, and 663 nm. The contents of β-carotene and lycopene were calculated according to the following equations:

$$\text{Lycopene (mg/100 mL)} = -0.0458A_{663} + 0.372A_{505} + 0.0806A_{453}$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

Ascorbic acid content was determined from dried methanolic extract. A 100 mg of the extract was mixed with 1% metaphosphoric acid (10 mL) and incubated at room temperature for 45 min and filtered. 1.0 mL of filtrate was mixed with 9 mL of 2, 6-dichloroindophenol and absorbance was recorded at 515 nm in 30 min against a blank. The ascorbic acid content was calculated using calibration curve of L-ascorbic acid. The results were expressed in terms of mg of ascorbic acid equivalents per g of extract (AAEs).

Results

DPPH radical scavenging activity

The results depicted in Fig. 1 indicate the increase in % inhibition of DPPH free radical on increasing concentration of the methanolic extract. The concentration of antioxidant needed to reduce the initial DPPH concentration by 50% (IC_{50}) is a parameter generally used to measure the antioxidant activity[7]. IC_{50} value of the methanolic mushroom extract was 0.72 mg/mL compared with standard L-ascorbic acid IC_{50}=0.062 mg/mL. This result showed that the methanol extract has good scavenging ability on DPPH radicals.

Antibacterial assay

In the range of tested concentrations of the different extracts (10-40 mg/mL) there was significant

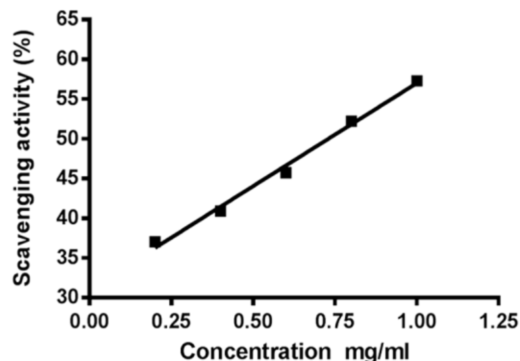

Fig. 1 — Antioxidant activity of *Cordyceps militaris* extract using DPPH test. [Slope = 25.91±1.288, Y- Intercept = 31.09±0.8545, r^2= 0.9926]

inhibition of both the pathogens (Fig. 2). Control did not inhibit the growth of the bacteria. However, the reference antibacterial agent streptomycin was effective against both the bacteria and showed inhibition zone of 23.1±0.01 mm and 22.6±0.12 mm against *S. aureus* and *E. coli*, respectively. Various mushroom extracts used in the study were found to display various degrees of antimicrobial effects against the tested microorganisms. The ethanolic and acetone extracts were maximum active against the inhibition of growth of *E. coli* and resulted in 30.71% and 33.97% inhibition at 100% (40 mg/mL) concentration of the extract. Aqueous extracts were poor in inhibiting the growth of tested bacteria. Whereas, acetone extracts significantly inhibited the growth of *S. aureus* in all the concentration range studied and displayed 41.04% inhibition at 100% (40 mg/mL) concentration of the extract.

Minimum inhibitory concentration (MIC)

With the initial concentration of 20 mg/mL, all the extracts demonstrated signs of antibacterial effects against *S. aureus* and *E. coli* (Table 1). MIC of acetone extract being lowest 0.62 mg/mL for *E. coli* and >1.0 mg/mL for all other extracts. Against *S. aureus* MIC's were more than 1 mg/mL for all the extracts tested and aqueous extracts displayed minimum inhibition at 10 mg/mL concentration. Antibiotic streptomycin exhibited MIC of 0.03 mg/mL and 0.06 mg/mL against *S. aureus* and *E. coli*, respectively.

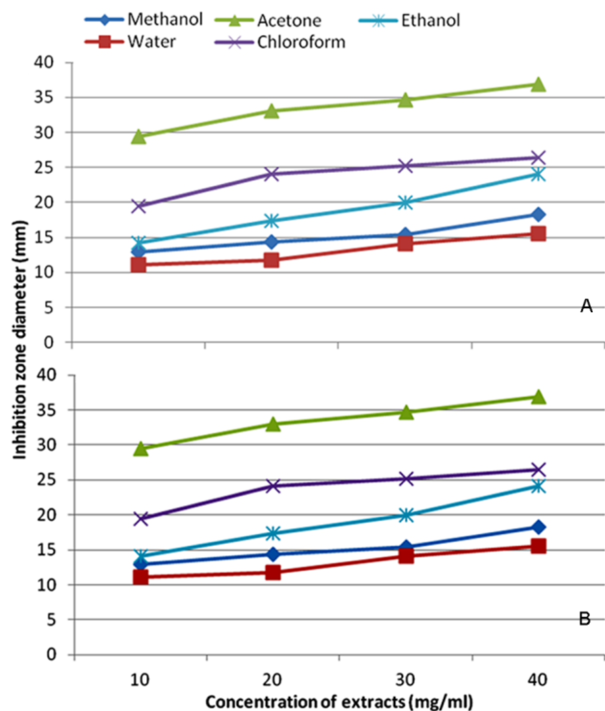

Fig. 2 — Antibacterial activity of *Cordyceps militaris* exracts against (A) *E. coli*; and (B) *S. aureus*

Cytotoxic effects of aqueous extract of C. militaris

To address the anticancer potential of *C. militaris*, HEp-2 cells (human epithelial carcinoma) were treated with different concentrations of aqueous extract of *C. militaris* and assayed for viability using MTT. *C. militaris* reduced approximately 43% viability with IC_{50} (concentration at which 50% cell viability remains) 20 µg/mL when examined upto 30 µg/mL (Table 2).

Bioactive Components

The present studies also focus on five different bioactive components, such as phenols, flavanoids, ascorbic acid, β carotene and lycopene (Table 3). Total phenolic content in *C. militaris* were found to be 39.52 mg tannic acid equivalent per g of the extract. Being high in TPC, *C. militaris* visibly can be considered as a superior source of polyphenols.

Table 1 — MIC (mg/mL) of various extracts against microorganisms

Extract	MIC mg/mL	
	S. aureus	*E. coli*
Acetone	1.25	0.62
Chloroform	1.25	1.25
Ethanol	1.25	1.25
Methanol	2.5	1.25
Aqueous	10	5.0
Streptomycin	0.03	0.06

Table 2 — Cytotoxicity of aqueous extract of *C. militaris* against HEp-2 cell line

Concentration (µg/mL)	% Cell Viability	% Cell Death	STDEV ±
0	100	-	-
5	67.65	32.34	14.52
10	61.64	38.35	8.79
15	64.26	35.73	13.53
20	49.36	50.63	14.13
25	48.34	51.65	17.98
30	43.01	56.98	9.18

Table 3 — Bioactive components in *C. militaris* mushroom extract

Total Phenols (mg/g)	39.52±0.040
Total flavanoids (mg/g)	1.56±0.039
Ascorbic acid (mg/100g)	1.81±0.026
β-carotene (mg/g)	0.328±0.045
Lycopene (mg/g)	0.277±0.004

[Contents of total phenols, flavanoids, ascorbic acid, β-carotene and lycopene in the mushroom extract]

Flavonoids are recognized as dietary biochemical agents, which display pH dependent antioxidant behaviour in human body and are also effective for cardiovascular system and work as cardio protective agents[20]. Observed value was found 1.56 mg catechin equivalent per g of the extract.

Ascorbic acid was found in little amounts whereas; β-carotene and lycopene were only found in vestigial amounts.

Discussion

The macrofungi extract showed positive antioxidant activity by fading the violet colour of DPPH solution to yellow and pale violet. Antioxidants react with DPPH, which is a stable free radical and convert it to 1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant[21]. In this method, a preformed stable free radical DPPH reacts with any substance that can reduce DPPH to a stable diamagnetic molecule by donating hydrogen atom. This reduction of the DPPH was followed by the decrease in absorbance at 517 nm[22]. To evaluate antioxidant activities in a relatively short time, DPPH assay is commonly used as compared to other methods[23]. It has the benefit of being unaffected by some side reactions which is common in laboratory-generated hydroxyl and superoxide anion[7]. Compared with the results of other authors in parallel studies it was found that the methanolic extract from *Agaricus blazei* showed a high scavenging ability of 97.1% at 2.5 mg/mL, *C. comatus* 84.5% at 5 mg/mL and *Ramaria botrytis* polysaccharides 82.67% at 1.4 mg/mL[24-26]. These results revealed that *C. militaris* is a free radical scavenger probably acting as primary antioxidant. Their methanolic extracts might react with free radicals (particularly peroxy radicals), which are the chief propagator of the autooxidation series of fat, thereby terminating the chain reaction[27].

Pathania and Sagar[14] reported that methanolic extracts of *C. militaris* were more potent as compared to aqueous extract in inhibiting the growth of *S. aureus* and *E. coli.* In the analogous line fermentation broth of *C. sinensis* was found to show stronger effect on *S. aureus* and *E. coli*[28]. The outcome of the present study are in agreement with the results of the earlier workers[29,30] who have also reported strong antibacterial activity of methanolic extract of *Ganoderma lucidum* against gram negative (*E. coli*) and comparatively less activity against gram-positive (*S. aureus*) bacteria. Due to the emergent antibiotics resistance, diseases that were easily healed are these days becoming a serious problem[31,32]. The association between hospital infections and multi-resistant microbes definitely highlighted the trouble and the pressing necessity for solutions[33]. Cancer is an escalating worldwide health concern and it demands development of safer anticancerous therapeutics. A number of natural extracts have been investigated for anticancer function on tumor cells for the same[34]. The research on mushrooms is extensive and hundreds of species have been confirmed a wide variety of pharmacological activities.

Phenolic compounds constitute a key class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by free radicals produced inside human body[35]. Several reports emphasised the major role of phenolic compounds as scavengers of free radicals which in turn seems to be associated with antioxidant activity[36]. A number of references are available on insignificant quantities of ascorbic acid, lycopene and β carotene in mushroom fruiting bodies as naturally occurring bioactive components[7,37]. Although, other bioactive components were possibly present in this mushroom extract, the amount of ascorbic acid, lycopene and β carotene found was extremely low, which emphasised the idea that phenolic compounds significantly contribute to the mushrooms antioxidant, antibacterial or cytotoxic activity.

Acknowledgement

The authors express their sincere thanks to the Head and Assistant Scientist Biochemistry, Dr. Nageswar Singh, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur for providing laboratory facilities. Financial support from UGC, (F.17-40/08 SA-1), New Delhi is also gratefully acknowledged.

Conflict of interest

Authors declare that they have no conflict of interest.

References

1 Kaszak BD, *Cordyceps* fungi as natural killers, new hopes for medicine and biological control factors. *Ann Parasitol*, 6 (2014) 151.

2 Seth R & Haider SZ, Mohan M, Pharmacology, phytochemistry and traditional uses of *Cordyceps sinensis* (berk. Sacc): A recent update for future prospects. *Indian J Tradit Knowle*, 13 (2014) 551.

3 Patel KJ & Ingalhalli RS, *Cordyceps militaris* (L.:Fr.) Link – An Important Medicinal mushroom. *J Pharmacogn Phytochem*, 2 (2013) 315.
4 Kim DJ, Kang YH, Kim KK, Kim TW, Park JB & Choe M, Increased glucose metabolism and alpha glucosidase inhibition in *Cordyceps militaris* water extracts treated HepG2 cells. *Nutr Res Pract*, 3 (2017) 180.
5 Liu SC, Chiu CP, Tsai CH, Hung CY, Li TM, Wu YC & Tang CH, Soya cerebrosides, an extract of *Cordyceps militaris*, suppresses monocyte migration and prevents cartilage degradation in inflammatory animal models. *Sci Rep*, (2017) 1.
6 Jayachandran M, Xiao J & Xu B, A critical review on health promoting benefits of edible mushrooms through gut microbiota. *Int J Mol Sci*, 18 (2017) 1934.
7 Joshi M & Sagar A, *In vitro* free radical scavenging activity of a wild edible mushroom, *Sparassis crispa* (Wulf.)Fr., from north western Himalayas, India. *J Mycol*, 2014 (2014) 1.
8 Palacios I, Lozano M, D'Arrigo M, Rostagno MA, Martinez JA, Garcia Lafuente A, Guillamon E & Villares A, Antioxidant properties of phenolic compounds occurring in edible mushroom. *Food Chem*, 128 (2011) 674.
9 Sehgal AK & Sagar A, *In vitro* isolation and influence of nutritional conditions on the mycelia growth of the entomopathogenic and medicinal fungus *Cordyceps militaris*. *Plant Pathol J*, 3 (2006) 315.
10 Lo KM & Cheung PCK, Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita* var. alba. *Food Chem*, 89 (2005) 533.
11 Pal J, Ganguly S, Tahsin KS & Acharya K, *In vitro* free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont.) Singer. *Indian J Exp Biol*, 47 (2010) 1210.
12 Alam N, Yoon KN, Lee KR, Shin PG, Cheong JC, Yoo YB, Shim MJ, Lee MW, Lee UY & Lee TS, Antioxidant activities and tyrosinase inhibitory effects of different extracts from *Pleurotus ostreatus* fruiting bodies. *Mycobiology*, 38 (2010) 295.
13 Indian Pharmacopoeia, (1996) 2nd ed. Government of India, New Delhi 23.
14 Pathania P & Sagar A, Studies on antibacterial activity of *Cordyceps militaris* (L.) Link. *Int J Pharm Bio Sci*, 5 (2014) 61.
15 Balouiri M, Sadiki M & Ibnsouda SK, Methods for *in vitro* evaluating antimicrobial activity: A review. *J Pharm Anal*, 6 (2016) 71.
16 Teh H, Nazni WA, Nurulhusna AH, Norazah A & Lee HL, Determination of antibacterial activity and minimum inhibitory concentration of larval extract of fly via resazurin based turbidometric assay. *BMC Microbiol*, 17 (2017) 36.
17 Makkar HPS, Blummel M, Borowy NK & Becker K, Gravimetric determination of tannins and their correlation with chemical and protein precipitating method. *J Sci Food Agric*, 61 (1993) 161.
18 Jia Z, Tang M & Wu J, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, 64 (1999) 555.
19 Nagata M & Yamashita I, Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon shokuhin Kogyo Gakkaishi*, 39 (1992) 925.
20 Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A & Bekhradnia AR, Determination of antioxidant activity, phenol and flavonoid content of *Parrotia persica* mey. *Pharmacologyonline*, 2 (2008) 560.
21 Iqbal S, Sivaraj C & Gunasekaran K, Antioxidant and anticancer activities of methanol extracts of seeds of *Datura stramonium*. *Free RadicAantioxidants*, 7 (2017) 184.
22 Riaz T, Abasi MA, Urehman A & Shahzadi T, Phyto-chemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscose* Jacq. J Serb *Chem Soc*, **7** (2012) 423.
23 Oyetayo VO, Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. *Braz J Microbiol*, 40 (2009) 380.
24 Huang SJ, Huang LC, Chen CC & Mau JL, Antioxidant properties of *Agaricus blazei*. In: Proceedings of the third international conference on mushroom biology and mushroom products (Eds, Broderick A & Nair T; Sydney, Australia), 1999, 266,.
25 Tsai SY, Tsai HL & Mau JL, Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis. LWT-Food Sci Technol*, 40 (2007) 1392.
26 Li H, Extraction, purification, characterization and antioxidant activities of polysaccharides from *Ramaria botrytis* (Pers.) Ricken. *Chem Cent J*, 11 (2017) 24.
27 Alam N, Yoon KN, Lee JS, Cho HJ & Lee TS, Consequence of the antioxidant activities and tyrosinase inhibitory effects of various extracts from the fruiting bodies of *Pleurotus ferulae*. *Saudi J Biol Sci*, 19 (2012) 111.
28 Li SP, Su ZR, Dong TTX & Tsim KWK, The fruiting body and its caterpillar host of *Cordyceps sinensis* show close resemblance in main constituents and antioxidation activity. *Phytomed*, 9 (2002) 319.
29 Nasim G & Ali M, Estimation of antimicrobial potential of *Ganoderma lucidum* (Leyss. Ex. Fr.) Karst. extracts. *Pak J Bot*, 43 (2011) 183.
30 Kamra A & Bhatt AB, Evaluation of antimicrobial and antioxidant activity of *Ganoderma lucidum* extracts against human pathogenic bacteria. *Int J Pharm Pharm Sci*, 4 (2012) 359.
31 World Health Organization report on infectious diseases. 2000. Overcoming antimicrobial resistance.
32 Peres-Bota D, Rodriguez H, Dimopoulos G, DaRos A, Melot C, Struelens MJ & Vincent JL, Are infections due to resistant pathogens associated with a worse outcome in critically ill patients? *J Infect*, 47 (2003) 307.
33 Pittet D, Infection control and quality health care in the new millennium. *Am J Infect Control* , 33 (2005) 258.
34 Melosilveira RF, Fidelis GP, Viana RLS, Soeiro VC, da Silva RS, Machaido D, Costa LS, Ferriera CV & Rocha HAO, Antioxidant and antiproliferatic activities of methanolic extract from a neglected agricultural product: corn cobs. *Molecules*, 19 (2014) 5360.
35 Ferguson LR, Role of plant polyphenols in genomic stability. *Mut Res*, 475 (2001) 89.
36 Khatun S, Islam A, Akilcioglu U, Guler P & Chatterjee NC, Nutritional qualities and antioxidant activity of three edible oyster mushrooms (*Pleurotus* spp.) *NJAS-Wageningen J Life Sci*, 72-73 (2015) 1.
37 Barros L, Ferreira MJ, Queiros B, Ferreira ICFR & Baptista P, Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem*, 103 (2007) 413.