Chief Editor
Dr. A. Singaraj, M.A., M.Phil., Ph.D.
Editor
Mrs.M.Josephin Immaculate Ruba

EDITORIAL ADVISORS

1. Prof. Dr.Said I.Shalaby, MD,Ph.D.
   Professor & Vice President
   Tropical Medicine,
   Hepatology & Gastroenterology, NRC,
   Academy of Scientific Research and Technology,
   Cairo, Egypt.
2. Dr. Mussie T. Tessema,
   Associate Professor,
   Department of Business Administration,
   Winona State University, MN,
   United States of America,
3. Dr. Mengsteab Tesfayohannes,
   Associate Professor,
   Department of Management,
   Sigmund Weis School of Business,
   Susquehanna University,
   Selinsgrove, PENN,
   United States of America,
4. Dr. Ahmed Sebihi
   Associate Professor
   Islamic Culture and Social Sciences (ICSS),
   Department of General Education (DGE),
   Gulf Medical University (GMU),
   UAE.
5. Dr. Anne Maduka,
   Assistant Professor,
   Department of Economics,
   Anambra State University,
   Igbariam Campus,
   Nigeria.
6. Dr. D.K. Awasthi, M.SC., Ph.D.
   Associate Professor
   Department of Chemistry,
   Sri J.N.P.G. College,
   Charbagh, Lucknow,
   Uttar Pradesh. India
7. Dr. Tirtharaj Bhoi, M.A, Ph.D,
   Assistant Professor,
   School of Social Science,
   University of Jammu,
   Jammu, Jammu & Kashmir, India.
8. Dr. Pradeep Kumar Choudhury,
   Assistant Professor,
   Institute for Studies in Industrial Development,
   An ICSSR Research Institute,
   New Delhi- 110070, India.
9. Dr. Gyanendra Awasthi, M.Sc., Ph.D., NET
   Associate Professor & HOD
   Department of Biochemistry,
   Dolphin (PG) Institute of Biomedical & Natural Sciences,
   Dehradun, Uttarakhand, India.
10. Dr. C. Satapathy,
    Director,
    Amity Humanity Foundation,
    Amity Business School, Bhubaneswar,
    Orissa, India.

ISSN (Online): 2455-7838
SJIF Impact Factor (2016): 4.144

EPRA International Journal of

Research & Development

(IJRD)

Monthly Peer Reviewed & Indexed
International Online Journal

Volume:1, Issue:10,December 2016

Published By :
EPRA Journals

CC License

SJIF Impact Factor: 4.144 ISSN: 2455-7838(Online)
EPRA International Journal of Research and Development (IJRD)
Volume: 1 | Issue : 10 | December | 2016

NUTRACEUTICAL FORMULATION OF CORDYCEPS TUBERCULATA CULTURED MYCELIUM AS A NEW BIO-PRODUCT FOR HUMAN HEALTH

Sapan Kumar Sharma[1]
[1]Department of Plant Pathology, CSK, Himachal Pradesh Agriculture University, Palampur 176 062, HP, India.

ABSTRACT

Genus Cordyceps Fr. is widespread and contains many medicinal species. Some species are used in Traditional Chenese Medicine to cure several diseases. However, nutraceutical formulations of only few Cordyceps species have been commercialized. Under present studies dried mycelial formulation prepared from C. tuberculata collected Northwestern Himalayan regions were checked for nutritive and medicinal composition. Standard methods were followed for preparations of dried mycelial formulations and for analysis of nutritive and chemical constituents. The mycelial formulations were found to be richer in protein (18.50 %), carbohydrates (33.12 %), phenolics (40.18 mg/g), and carotenoids (β-carotene, lycopene) and anthocynadins. The mycelial formulation showed significant antioxidant activities measeared through EC50 values. Present studies will open the way for the large scale commercial exploitations of these formulation for nutritional and nutraceutical therapy and use in pharmaceutical industries.

KEYWORDS: *Cordyceps formulations, Cordyceps species, mycelial cultivation, nutritive and biochemical constituents, antioxidant activities.*

INTRODUCTION

Genus *Cordyceps* Fr. contains more than 400 widespread species distributed throughout the world on higher altitudes (Kobayasi, 1982; Stensrud, Hywel-Jones & Schumache, 2005; Kirk et al., 2008). The genus contains many medicinal species which are used in many parts of the world due to the source of disease combating natural product with tremendous biological activities. Fruitbodies and mycelial extracts of many of its species exhibit different biological activities (Ji et al., 2009). *Cordyceps sinen*sis is widely used species from ancient times. The role it plays to cure many diseases is well understood now. It has been used extensively to cure various types of cancers by inhibiting tumour growth as well as enhancing the immune system (Kiho et al., 1996; Chen et al., 1997; Mizuno, 1999; Kim et al., 2002; Zhao et al., 2002). The active constituents of many of *Cordyceps* species are polysaccharides and their range varies between 3-8 % among all bioactive constituents (Ma et al., 1999; Smith, Rowan & Sullivan, 2002, Lu 2005). Due

to richness with various types of bioactive constituents *Cordyceps* species have become natural remedies for several diseases. The bioactive constituents like cordycepin and others which possess liver protective effects, antioxidative activities, enhances the Tcell and macrophages activity, reduce the level of c-Myc, c- Fos, and VEGF levels in the lungs and liver by exopolysaccharide fraction, and reduce the level of cholesterol and triglyceride (Liu & Fei, 2001; Kim and Yun, 2005; Hsu et al., 2002; Li et al., 2006). Some uncommon cyclic dipeptides, including co- cyclo-[Gly-Pro], cyclo-[Leu-Pro], cyclo-[Val-Pro], cyclo-[Ala-Leu], cyclo-[Ala-Val], and cyclo- [Thr-Leu] and small amounts of polyamines, such as 1,3- diamino propane, cadaverine, spermidine, spermine, and putrescine are also present in these fungi which exhibit multiple pharmacological activities including antitumor, antiinflammatory, mmunopotentiation, hypoglycemic, and hypocholesterolemic effects, protection of neuronal cells against the free radical-induced cellular toxicity, steroidogenesis, and antioxidant activities (Koh et al., 2003; Yu et al., 2004; Zhang et al., 2014; Liu et al., 2015).The commercialized products of few *Cordyceps* species viz. *Cordyceps sinensis*, *C. militaris* are used now for nutritional therapy. There are several other species of this genus which are richer source of biochemical constituents and can be commercialized through different formulations prepared from fruibodies as well as mycelia. Since the species of this genus are rare hence mycelial cultivation is the suitable option to exploit these fungi for commercialization. Present studies have been conducted to evaluate the composition of dried mycelial formulations prepared from *Cordyceps* tuberculata fungi for nutritive and nutraceutical potential.

MATERIALS AND METHODS

Collection, culturing and processing of samples

The species wwas collected from Dalhousie (H.P., India) regions of northwestern Himalayas. After noting the details pertaining to morphology and habitat, culturing of the samples was performed with the tissue culture technique. The freshly infected insect body was washed thoroughly 4 - 5 times with water, dipped into 70 % ethanol for 30 seconds, and then rinsed 3 - 4 times with sterile distilled water. The sterilized fruiting body was cut into small sections and incubated on potato dextrose agar (SD Fine, India) at 25 °C. The isolated pure cultures were maintained at 25 ºC.The samples were then dried in wooden drier at 45 ºC and preserved in air-tight cellophane bags, with a small amount of 1-4-paradichlorobenzene in porous packets to keep them free of insects. The sample was deposited in the Herbarium Department of Botany, Punjabi University Patiala.

Liquid cultivation and formulations

Submerged cultivation of the mycelium was done in a standard basal medium (sucrose 30.0 g/L, yeast powder 5.0 g/L, peptone 5.0 g/L, MgSO4. $7H_2O$1.0 g/L, and KH2PO4 0.5 g/L) (Xu et al., 2009; Qinqin et al., 2012; Sharma, gautam & Atri, 2015). Isolated pure mycelium was inoculated in basal liquid medium in 200 mL baffled flasks. After 15 days mycelium was harvested with the help of Whatman no. 1 filter paper. Briefly, harvested mycelium was washed with ultrapure water and subjected to extraction with hot water (70 °C) for 12 hrs. The extracts were mixed with cyclodextrin in ratio 1 to 0.5-5 (mycelial extract to cyclodextrin ratio). The formulations were prepared by drying the mixture to powder.

Nutritive analysis

Protein contents from all the formulations were estimated using the Kjeldahl method (Atri et al., 2013). Fat content was estimated using a Soxhlet apparatus by extraction of powered samples with petroleum ether. Ash contents were calculated by incineration in silica dishes at 525 ± 20 ^oC containing 5-10 g/sample. Fibres contents were estimated by using the acid-alkali method (1.25 % each). Carbohydrates percentage was calculated by the difference as the total weight - (moisture content + protein content + crude fat + ash content + crude fibres). Minerals were analyzed using Atomic Absorption Spectophotometer (Perkin Elmer Analyst A400).

Biochemical Composition

Bioactive compounds

Dried formulations (∼5 g) were extracted with 100 mL of methanol at 25 ºC (150 rpm) for 24 hours and filtered through Whatman no. 2 filter paper. The residues were again re-extracted with two additional portions of methanol (100 mL). These extracts were evaporated to dryness at 42 ºC, then redissolved in methanol at a concentration of 50 mg/mL, and stored at 4 ^0C. The dried methanolic extracts (100 mg) were shaken vigorously with 10 mL of acetone/hexane mixture (4:6) for 1 min. and filtered. The absorbance of the filtrate was measured at 453, 505, and 663 nm (Nagata & Yamashita, 1992). β-carotene and lycopene contents were estimated using the following equation: Lycopene (mg/100mL) = $(0.0458 \times A663) + (0.372 \times A505) - (0.0806 \times A453)$ β-carotene (mg/100mL) = $(0.216 \times A663) - (0.304 \times A505) + (0.452 \times A453)$.

Phenolic compounds were quantified using Folin and Ciocalteu's phenol reagent. Formulations were mixed with Folin and Ciocalteu's phenol reagent (1 mL). After three min., saturated sodium carbonate solution (1mL) was added to the mixture, and the volume was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min. and the absorbance was read at 725 nm. Gallic acid was used to calculate the standard curve (0.01-0.4 mM; R^2 = 0.9999) and the results were expressed as milligrams of gallic acid equivalents per gram of extract (Singleton & Rossi, 1965; Jagdish et al., 2009).[28-29]

Ascorbic acid contents were quantified using colorimetric assays. Standard ascorbic acid

solution (5 mL/ L-ascorbic acid in 3 % phosphoric acid) was prepared and added to 5 mL of phosphoric acid. A microburette was filled with dye, and the samples were titrated with the dye solution to a pink color, which remained for 15 seconds. The dye factor (milligrams of ascorbic acid per milliliter of dye using formula: 0.5/titrate) was determined. A sample was prepared by taking 10 g of formulatoion grounded in metaphosphoric acid, and the volume was increased up to 100 mL. It was titrated after filtration until a pink color appeared.[25] The amount of ascorbic acid was calculated with the use of the following equation: mg of ascorbic acid per 100 g or mL=titrate × dye factor × vol. made aliquot of extract × wt. of sample × 100.

Anthocyanidins were quantified by using standard protocol (Vamanu & Nita, 2013). Briefly, 0.5 g of formulations were mixed with the solvent (mixture of 85 : 15 (v/v) of ethyl alcohol and hydrochloric acid 1.5 M) followed by ultrasonication for 15 min. and filtration through Whatman filter paper no. 1. Standard solution was prepared with cyanidin chloride (con. 5-15 μg/mL). The absorption was measured at 546 nm. The total quantity of anthocyanidins (expressed in g of cyaniding chloride/100 g extract) = (Ap × mst × f × 100)/(Ast × mp), where Ap is absorption rate of the sample solution; mp is mass of the processed sample, in g; Ast is absorption rate of the standard solution; mst is mass of the processed standard solution, in g; and f is dilution coefficient.

Antioxidant activities

DPPH scavenging activity was measured by adding DPPH (200 μL) solution at different concentrations (2-10 mg/mL) to the formulations (0.05 mL) dissolved in ethanol. A control was prepared by adding an equal amount of ethanol to ascorbic acid (Vamanu, 2012).The absorbance was read after 20 min at 517 nm and the inhibition was calculated using the formula DPPH scavenging effect (%) = $A0 - AP$ $A0$ × 100 absorbance in the presence of the sample.

ABTS radical scavenging activities of all the dried formulations were measured through spectophotometric method (Li et al., 2011). Briefly, 10 µL of the sample was added to 4 mL of the diluted $ABTS^{\cdot+}$ solution (prepared by adding 7 mM of the ABTS stock solution to 2.45 mM potassium persulfate, kept in the dark, at room temperature, for 12-16 h before use). The solution was then diluted with 5 mM phosphate-buffered (pH 7.4) and absorbance was measured at 730 nm after 30 min. The ABTS radical scavenging activity was calculated as S % = (Acontrol−Asample/A control) ×100.

Reducing power estimation was done by mixing the samples (200 µL) with sodium phosphate buffer (pH 6.6), 1 mM $FeSO_4$, and 1 % potassium ferricyanide and incubated for 20 min. at 50 °C after that trichloroacetic acid was added and the mixtures were centrifuged. Supernatant (2.5 mL) was mixed with an equal volume of water and 0.5 mL $FeCl_3$ (0.1 %). The absorbance was measured at 700 nm (Papuc et al., 2010). Iron chelating activities of the samples were measured by mixing them with 3.7 mL of ultrapure water. The mixture was reacted with ferrous chloride (2 mmol/L, 0.1 mL) and ferrozine (5 mmol/L, 0.2 mL) for 20 min and the absorbance was read at 562 nm with using EDTA as control.The chelating activities were calculated using the formula: chelating activity (%) = [(Ab − As)] × 100, where Ab is the absorbance of the blank and As is the absorbance in the presence of the extract (Oyetayo, Dong & Yao, 2009).

The scavenging activities of superoxide anion radicals were measured following standard method (Marklund & Marklund, 1974). Formulations (0-2.0 mg/mL, 1 mL) and Tris-HCl buffer (50.0 mM, pH 8.2,3mL) were incubated in a water bath at 25 °C for 20 min. and to this pyrogallic acid (5.0 mM, 0.4 mL) was added. HCl solution (8.0 M, 0.1 mL) was added to terminate the reaction after 4 min. The absorbance of the mixture was measured at 320 nm. The scavenging ability if a sample was calculated using the following formula: scavenging ability (%) = (1−Asample/Acontrol) × 100.Where Acontrol is the absorbance of control without the sample and Asample is the absorbance in the presence of the sample.

RESULTS AND DISCUSSION

Nutritive analysis

The nutritive composition of mycelial formulations is shown in table 1. Protein contents of dried mycelial formulations were observed 18.50 ± 3.2 %.Fat contents of the formulations were documented in lesser amounts 0.24 %. In general all the formulations of dried mycelium were found to be richer in protein and low in fat. Crude fibres were found to be 0.80 %. Carbohydrates were found to be major nutritional components in all the formulations. The percentage of carbohydrates was documented 33.12 ± 2.5 %. Minerals composition showed the presence of Fe, Na, K, Ca, Mg and Cu. Amongst all the minerals, Mg was detected in major quantity as compared to other minerals.

Upon analysis of nutrients, the results obtained for all the formulations showed comparable values similar to other edible and commercially used species viz., *Agaricus bisporus, Boletus edulis, Morchella esculenta, Cordyceps sinensis*, and *Lentinula edodes*. (Manzi, Aguzzi & Pizzoferrato, 2001; Manzi et al., 2004; Krbavcic & Baric, 2004; Agrahar & Subbulakshmi, 2005; Sharma & Gautam, 2015; Sharma, Gautam & Atri, 2015). Results are also in confirmity with several commercially cultivated medicinal fungi with high protein and carbohydrate contents and low fat levels (Diez & Alvarez, 2001; Barros et al., 2008; Atri et al., 2013). The values documented for *Cordyceps tuberculata* were significantly higher than previously analyzed and commercially used species viz., *Agaricus arvensis, Pleurotus cystidiosus, Amanita caesarea, Agaricus campestris, Cantharellus cibarius*, and *Lentinus cladopus* etc. (Sharma and Gautam, 2015; Sharma, Gautam & Atri, 2015). There was no significant difference observed

in fat contents of the mycelial formulations species. Small percentages of crude fibres were also detected from all the formulation which is showing their nutritional values (Sharma & Atri, 2014).

Biochemical composition

β-carotene contents was documented as 0.63 g/100g. Lycopene content in the formulations was documented as 0.33 g/100g. Formulation prepared from the mycelium of *Cordyceps tuberculata* contained highest amount of β-carotene and lycopene (0.45 g/100g).

Phenols were documented in significant amounts from the formulations (40.18 mg/g). Ascorbic acid content of all the formulations was documented as 0.43 mg/100g. Anthocyanidins ducemented as 7.23mg cyanidin chloride/100 g extract in the formulation.

Phenolic compounds in all the mycelial formulations were documented in higher amounts than other bioactive compounds. Presence of high phenolic compounds accounts for the high antioxidant properties of all these formulations (Barros et al., 2007). However, β-carotene, lycopene, and ascorbic acids were detected in minor quantities. Anthocyanidins were detected in appreciable amounts from all these mycelial formulations. The presence of these functional medicinal compounds plays soignificant role similar to beta-glucans, selenium, ganoderic acid, triterpenes, or cordycepin from fruitbodies and mycelia of other species. In many studies it is reported that the amounts of these vary with the type of extraction as ethanolic extract yields higher amounts of anthocyanidins as compared to methanolic, hot water, and cold water extracts (Vamanu & Nita, 2013).

Antioxidant activities of mycelial formulations

Antioxidant properties of all the formulations were expressed as EC_{50} values and are presented in table 4. EC_{50} values obtained for DPPH radical scavenging activity higher effectiveness in antioxidant properties. Mycelial formulation of *Cordyceps tuberculata* (2.98 ± 0.1 mg/mL) showed higher DPPH, ABTS (3.99±0.3 mg/mL), scavenging ability on superoxide radical (1.94 ± 0.7 mg/mL) and iron chelating activities. Better antioxidant properties of mycelial formulations may be attributed due to the presence of higher phenolic compounds, β-carotene, lycopene, ascorbic acids, anthocyanidins, and tocopherol contents in them (Yoon et al., 1994; Klaus & Nksic, 2007).

CONCLUSIONS

The formulations prepared from *Cordyceps tuberculata* showed high nutritive and nutraceutical potential. These investigations have been carried out for the first time. The formulations were observed as richer source of nutritional constituents such as proteins, carbohydrates, minerals. Besides that biochemical composition of present formulations showed richness in carotenoids, ascorbic acids and anthocynadins. These compounds play a vital role in human body and useful for nutritional therapy. Direct use of these formulations can be health promoting with additive effects of all the biochemical constituents. These formulations can be useful for the patients with nutritional deficiency and immune related disorders. Present investigations will lead the way to researches on commercial exploiatations and their use in pharmaceutical industries.

REFERENCES

1. Agrahar-Murugkar D, Subbulakshmi G. 2005. Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chemistry* **89**: 599-603.
2. Atri NS, Sharma SK, Joshi R, Gulati A, Gulati A. 2013. Nutritional and neutraceutical composition of five wild culinary-medicinal species of genus *Pleurotus* (Higher Basid-iomycetes) from Northwest India. *International Journal of Medicinal Mushrooms* **15**:49-56.
3. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira ICFR. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology* **46**:2742-2747.
4. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry* **103**:413-419.
5. Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Analytical Biochemistry* **239**:70-76.
6. Chen YJ, Shiao MS, Lee SS, Wang SY. 1997. Effect of Cordyceps sinensis on the proliferation and differentiation of human leukemic U937 cells. *Life Sciences* **60**:2349–59.
7. D'ıez VA, Alvarez A. 2001. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chemistry* **75**:417-422.
8. Hsu TH, Shiao LH, Hsieh C, Chang DM. 2002. A comparison of the chemical composition and bioactive ingredients of the Chinese medicinal mushroom Dong Chong Xia Cao, its counterfeit and mimic, and fermented mycelium of *Cordyceps sinensis*. *Food Chemistry* **78**:463–9.
9. Huang DJ, Ou B, Prior RL. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural Food Chemistry* **53**:1841-1856.
10. Jagadish LK, Venkata Krishnan V, Shenbhagaraman R, Kaviyarasan V. 2009. Comparitive study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.Lange) Imbach before

and after boiling. *African Journal Biotechnology* **8**:654-661.
11. Ji DB, Ye J, Li CL, Wang YH, Zhao J, Cai S. 2009. Antiaging effect of *Cordyceps sinensis* extract. *Phytotherapy Resesearch* **23**:116–22.
12. Kanu PJ, Zhu K, Baby Kanu J, Zhou H, Qian H, Zhu K. 2007. Biologically active components and nutraceuticals in sesame and related products: a review and prospect. *Trends in Food Science and Technology* **18**:599-608.
13. Kiho T, Yamane A, Hui J, Usui S, Ukai S. 1996. Polysaccharides in fungi, XXXVI. Hypoglycemic activity of a polysaccharide (CS-F30) from the cultural mycelium of Cordyceps sinensis and its effect on glucose metabolism in mouse liver. *Biological and Pharmaceutical Bulletin* **19**:294–6.
14. Kim HO, Yun JW. 2005. A comparative study of the production of exopolysaccharides by two entomopathogenic fungi *Cordyceps militaris* and *Cordyceps sinensis* in submerged mycelial cultures. *Journal of Applied Microbiology* **99**:728–38.
15. Kim SW, Hwang HJ, Xu CP, Na YS, Song SK, Yun JW. 2002. Influence of nutritional conditions on the mycelia growth and exopolysaccharide production in *Paecilomyces sinclairii*. *Letters in Applied Microbiology* **34**:389–93.
16. Kirk PM, Cannon P, David JC, Stalpers JA. 2008. Ainsworth and Bisby's dictionary of the fungi. 10th ed. Wallingford, Oxon: CAB International.
17. Klaus A, Niksic M. 2007. Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. *Zbornik Matice srpske za prirodne nauke* **113**:219–26.
18. Kobayasi Y. 1982. Keys to the taxa of the genera *Cordyceps* and *Torrubiella*. *Transactions of the Mycological Society of Japan* **23**:329-64.
19. Koh JH, Kim JM, Chang UJ, Suh HJ. 2003. Hypocholesterolemic effect of hot-water extract from mycelia of Cordyceps sinensis. *Biological and Pharmaceutical Bulletin* **26**:84–7.
20. Krbavciˇc IP, Baric IC. 2004. Influence of deep fat frying on some nutritional parameters of novel food based on mush-rooms and fresh soft cheese. *Food Chemistry* **84**: 417-419.
21. Li P, Huo L, Su W, et al. 2006. Free radical-scavenging capacity, antioxidant activity and phenolic content of Pouzolzia zeylanica. *Journal of Serbian Chemical Society* **76**:709–17.
22. Li PL, Huo L, Su W et al. 2011. Free radical-scavenging capacity, antioxidant activity and phenolic content of *Pouzolzia zeylanica*. *Journal of Serbian Chemical Society* **76**:709-717.
23. Liu JL, Fei Y. 2001. Enhancement of Cordyceps taii polysaccharide and Cordyceps pruinosa polysaccharide on cellular immune function in vitro. *Immunology Journal* **17**:189–91.
24. Liu RM, Zhang XJ, Liang GY, Yang YF, Zhong JJ, Xiao JH. 2015. Antitumor and antimetastatic activities of chloroform extract of medicinal mushroom Cordyceps taii in mouse models. *BMC Complementary and Alternative Medicine* **15**:1–13.
25. Lu C. 2005. Preventive activity of Cordyceps polysaccharides on non alcoholic steatohepatitis and their partial mechanisms of action, MSc Thesis. Anhui, PR China: Anhui Medical University. 1–56.
26. Ma X, Qiu DK, Xu J, Li JQ, Zeng MD. 1999. Effect of Cordyceps polysaccharideliposome on transforming growth factor b-1 in the experimental liver fibrotic rats. *Chinese Journal of Gastroenterology* **4**:205–6.
27. Manzi P, Aguzzi A, Pizzoferrato L. 2001. Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry* **73**:321-325.
28. Manzi P, Marconi S, Aguzzi A, Pizzoferrato L. 2004. Commer-cial mushrooms: nutritional quality and effect of cooking. *Food Chemistry* **84**:201-206.
29. Marklund S, Marklund G. 1974. nvolvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**:469-474.
30. Mizuno T. 1999. Medicinal effects and utilization of Cordyceps (Fr.) Link (Ascomycetes) and Isaria Fr. (Mitosporic fungi) Chinese caterpillar fungi, "Tochukaso,". *International Journal of Medicinal Mushrooms* **1**:251–62.
31. Nagata M, Yamashita I. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaishi* **39**:925-928.
32. Oyetayo VO, Dong C, Yao Y. 2009. Antioxidant and antimicro-bial properties of aqueous extract from *Dictyophora indusiata*. *The Open Mycology Journal* **3**: 0-26.
33. Papuc C, Crivineanu M, Goran G, Nicorescu V, Durdun N. 2010. Free radicals scavenging and antioxidant activity of European mistletoe (*Viscum album*) and European birthwort (*Aristolochia clematitis*). *Revista de Chimie* **61**:619-622.

34. Qinqin XU, Zhenhua LIU, Yisheng SUN, Zhongjie DING, Longxian LÜ, Yongquan LI. 2012. Optimization for Production of Intracellular Polysaccharide from Cordyceps ophioglossoides L2 in submerged culture and its antioxidant activities in vitro. *Biotechnology and Bioengineering* **20**:294–301.
35. Reis FS, Pereira E, Barros L, Sousa MJ, Martins A, Ferreira ICFR. 2011. Biomolecule profiles in inedible wild mushrooms with antioxidant value. *Molecules* **16**:4328–4338.
36. Sharma SK, Atri NS. 2014. Nutraceutical composition of wild species of genus *Lentinus* Fr. from Nothern India. *Current Researches in Environmental and Applied Mycology* **4**:11-32.
37. Sharma SK, Gautam N, Atri NS. 2015. Optimized extraction, composition, antioxidant and antimicrobial activities of exo and intracellular polysaccharides from submerged culture of *Cordyceps cicadae*. *BMC Complementary and Alternative Medicine* **15**:1-10.
38. Sharma SK, Gautam N. 2015. Chemical bioactive and antioxidant potential of twenty wild culinary mushroom species. *Biomed Reserach International* **2015**:1-12.
39. Shrama SK, Gautam N, Atri NS. 2015. Optimization, composition and antioxidant activities of exo and intracellular polysaccharides in submerged culture of Cordyceps gracilis (Grev.) Durieu & Mont. *Evid Based Complementary and Alternative Medicine* **2015**:1-8.
40. Singleton VL, Rossi JA. 1965. Colorimetric detection of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **16**:144-158.
41. Smith JE, Rowan NJ, Sullivan R. 2002. Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. *Biotechnology Letters* **24**:1839–45.
42. Stensrud Ø, Hywel-Jones NL, Schumache T. 2005. Towards a phylogenetic classification of Cordyceps: ITS nrDNA sequence data confirm divergent lineages and paraphyly. *Mycological Research* **109**:41–56.
43. Vamanu E, Nita S. 2013.Antioxidant capacity and the cor-relation with major phenolic compounds, anthocyanin, and tocopherol content in various extracts from the wild edible *Boletus edulis* mushroom*. BioMed Research International* **2013**:1-11.
44. Vamanu E. 2012. Biological activities of the polysaccharides pro-duced in submerged culture of two edible *Pleurotus ostreatus* mushrooms. *BioMed Research International* **2012**:1-8.
45. Xu Q, Lü L, Chen S, Zheng G, Zheng J, Li Y. 2009. Isolation of Cordyceps ophioglossoides L2 from fruit body and optimization of fermentation conditions for its mycelial growth. *Chinese Journal of Chemical Engineering* **17**:278–85.
46. Yagi Y, Satou M, Ishikura T. 1985. Powdery mushroom extract composition. *Japanese patent* JP60186260.
47. Yoon SY, Eo SK, Kim YS, Lee CK, Han SS. 1994. Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics. *Archives of Pharmaceutical Ressearch* **17**:438-42.
48. Yu R, Song L, Zhao Y, Bin W, Wang L, Zhang H, et al. 2004. Isolation and biological properties of polysaccharide CPS-1 from cultured *Cordyceps militaris*. *Fitoterapia* **75**:465–72.
49. Zhang DW, Wang ZL, Qi W, Zhao GY. 2014. The effects of Cordyceps sinensis phytoestrogen on estrogen deficiency-induced osteoporosis in ovariectomized rats. *BMC Complementary and Alternative Medicine* **14**:484.
50. Zhao CS, Yin WT, Wang JY, Zhang Y, Hong Y, Cooper R, et al. 2002. CordyMax™ Cs-4 improves glucose metabolism and increases insulin sensitivity in normal rats. *Journal of Alternative and Complementary Medicines* **8**:309–14.

Table 1: Nutritive composition of mycelial formulations of *Cordyceps tuberculata* (mean±SD).

Chemical constituents	Formulation Composition (%)
Protein	18.50 ± 3.2^{b}
Crude fat	1.41 ± 0.0^{a}
Fibres	0.80 ± 0.0^{a}
Ash	0.60 ± 0.0^{a}
Carbohydrates	33.12 ± 2.5^{b}

Values are expressed as mean ±SE and different letters represent the significant difference in each column with $p \leq 0.05$.

Table 2. Minerals content of *Cordyceps tuberculata* formulation

Minerals	(mg/100g)
Fe	6.3 ± 1.3 [b]
Na	1.93 ± 0.2[a]
K	1.2 ± 0.0 [a]
Ca	5.2 ± 1.1 [b]
Mg	5.2 ± 1.3 [b]
Cu	1.5 ± 0.0[a]

Table 3: Biochemical composition of mycelial formulations *Cordyceps tuberculata*

Bioactive molecules	Formulation
β-carotene(g/100 g)	0.63 ± 0.0[a]
Lycopene(g/100 g)	0.33 ± 0.0[a]
Phenolic compounds (mg/100 g of gallic acid)	40.18 ± 1.2[c]
Ascorbic acid (mg/100 g)	0.43 ± 0.0[a]
Anthocyanidins (mg cyanidin chloride/100 g extract)	7.23 ± 2.1[b]

Values are expressed as mean ±SE and different letters represent the significant difference in each column with $p \leq 0.05$. ND=not detected

Table 4: EC50 values of different antioxidant assays

Antioxidant Assays	EC50 values
DPPH radical scavenging activity(mg/mL)	3.24 ± 0.4
ABTSmg/mL)	3.99 ± 0.3
Reducing power(mg/mL)	4.16 ± 0.5
Fe^{2+} chelating activity (mg/mL)	3.22 ± 0.4
Scavenging on superoxide anion radical (mg/mL)	2.45 ±0.2
FRAP (mol Fe^{2+} quivalents/g DW)	2.60 ± 0.3