Original Article

Nutritional and biochemical analysis of *Craterellus odoratus*: rare Chanterelle Basidiomycetes from Similipal Biosphere Reserve of Odisha

Sushri Shant Tripathy, Ashutosh Rajoriya, Nibha Gupta*

Plant Pathology and Microbiology Division, Regional Plant Resource Centre, Bhubaneswar -751015 Odisha, India

Keywords: *Mushrooms, Proximate, Chanterelles, Antioxidant, Minerals*

Abstract

The present study confirms the occurrence of *Craterellus odoratus* in different forest of Odisha. The fresh fruit bodies of this mushroom was collected and analysed for proximate content and antioxidant properties. A good amount of protein, carbohydrate, reducing and non reducing sugars, phenolics, flavonoids, β-carotene, lycopene were found in the fresh fruiting bodies of *Craterellus odoratus*. Analysis of mineral content exhibited a good amount of iron content (6490 ppm), calcium (10.20%), Sodium (0.12%), Phosphorus (0.29%) and magnesium (9.54%). Carbohydrate content especially non reducing type was analyzed in dried mushrooms and it was 34.19%. The presence of β-carotene and lycopene is also confirmed and it was 0.017 and 0.016 mg/gm respectively. Overall the analysis shows the usefulness of *C. odoratus* as a good source of minerals and antioxidants and further exploration as therapeutic component.

***Corresponding author:**
Nibha Gupta, Phone: 0674-2557925, email: nguc2003@yahoo.co.in

Date of Submission: Dec 17, 2014 **Date of Acceptance:** Jan 17, 2014 **Date of Publishing:** Jan 20, 2015

Introduction

Mushrooms are good source of protein, vitamins, fats, carbohydrates, amino acids minerals and considered as dietary supplements [1-5]. Edible mushrooms are not only the source of nutrients but also reported as therapeutic agents, useful in preventing diseases such as cardiovascular diseases, hypertension, hypercholesterolemia and cancer. The preventive properties of mushrooms towards tumor, viral and immunological disorders are due to antioxidant compounds and exopolysaccharides[6-12].

Chanterelles belongs to Basidiomycetes[13] and are of ectomycorrhizal in nature[14]. The word "chanterelle" is derived from the Greek "kantharos" meaning "cup," "goblet," or "drinking vessel," a reference to their funnel like shapes [15]. The present study is based on occurrence, nutritional and biochemical analysis of *C. odoratus* which is first ever report from Odisha, India.

Materials and Methods

Collection and identification: Healthy, fresh and succulent samples were collected from tropical moist deciduous and semi evergreen forests from Similipal Biosphere Reserve during July-September 2011-12. Macroscopic and microscopic examination of pileus, stipe, veil, ring, volva, lamellae and gills etc. were made to identify species according to the specific procedure. All of the assays were performed using the entire mushroom fruiting body including stipe. The dried procured edible mushrooms were cleaned and subsequently dried in the oven at 50^{o}C. Dried mushrooms were ground to fine powder (100mesh) and stored in air tight plastic containers in room temperature for further analysis.

Proximate analysis: Estimation of protein was done at 595nm using Bradford[16] method. The values were expressed in mg per gram of dried sample.Estimation of total carbohydrate was done by phenol sulphuric acid method [17-18]. Glucose was taken as standard and expressed in terms of gm/ 100 gm of DW basis. Estimation of reducing sugar was done by following dinitrosalicylic acid method [19]. Estimation of non reducing sugar was done by taking the difference between total carbohydrates and reducing sugar. The value was expressed in gm/100gm dry weight of the sample [20].

Minerals: Estimation of Ca and Mg was done by titration method taking EDTA as titrate. Estimation of P was done by Vanado phospho molybdate method. Estimation of Sodium was done by flame photometry[21] and estimation of Zn, Cu, Co and Cr was done by Atomic Absorption Spectrophotometer (Analytik Jena) by following the method of Verma and Kesharvani [22].

Antioxidant assay:

Preparation of extracts: 1gm of each dried mushroom sample was mixed with 10ml of methanol. Samples were stirred for 15 minutes for effective extraction and centrifuged at 3000g for 20 minutes. Supernatants were referred to as methanolic extract which was kept at 4^{o}C until analysis.

Free radical scavenging activity: The DPPH activity was estimated in the methanolic extracts by a colorimetric method[23]. 1ml of methanolic extract was added with 2 ml of DPPH solution (1:2) and incubated for 30 min. in dark after vigorous mixing. Absorbance was measured at 517 nm and scavenging activity of each extract on DPPH radical was calculated. Estimation of FRAP assay was done by the method followed by Benzie and Strain [24] and Athavale *et al*. [25]

Reducing power ability: Each mushroom extract (0.5-4mg/ml) in methanol (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer (pH-6.6) and 2.5ml of 1% potassium ferricyanide, and the mixture was incubated at 50^{0}C for 20 minutes. After 2.5ml of 10% trichloroacetic acid was added, the mixture was centrifuged at 2000 rpm for 10 minutes. The upper layer (5ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was taken at 700nm (Analytik jena) spectrophotometer. Ec 50 value was calculated in mg/ml at 0.5 optical density against reagent blank [26].

Antioxidant components: The total phenolic content was determined through Folin phenol method given by Singleton & Rossi [27]. Gallic acid served as standard. The flavonoid content was estimated by alumi-

nium chloride method given by Chang *et al*. [29] and expressed in terms of quercetin equivalents per gram. The concentration of β-carotene and Lycopene in mushroom extracts was estimated spectrophotometrically following the method of Nagata and Yamashita [30] and Barros *et al*. [31]. The quantification of carotenoid in the dried mushroom sample was done by following the method of Arnon[32] and expressed in mg/gm. Estimation of tannins was done by Folin Denis method by Schandrel [33] and absorbance was measured at 760 nm**.** Tannic acid was served as standard and tannin content in the sample was expressed in terms of mg/gm.

Result & Discussion

Species name *Craterellus odoratus*, **Macroscopic characteristics:** These are peculiar varieties of edible mushrooms. Yellowish orange in colour through out the fruiting body. The total fruiting body was funnel shape. Slippery to touch. Gills and pores are absent. Stipe absent. (Fig.1). **Habitat:** The fruiting body with hollow context in a sieve form is present in Baripada, Kaliyani forest, Thick woody Semi green forest Laterite Gregarious, **Distribution:** Distributed in most parts of Europe, North America, Mexico, Zambia in Africa, Italy, Canada, In Asia it is distributed in major parts of China, Malaysia, Japan & Philipines. In India it is distributed in Himalayas, Solan, Jharkhand, Punjab & Odisha. In Baliguda forest & Kalyani forest of Similipal Biosphere and Tiger Reserve.

At 40µg/ml of concentration of the methanolic extract of *C. odoratus* showed 50% scavenging activity (Fig 2). Both type of antioxidant assay showed a very good activity, FRAP assay revealed 2.39±0.09 mg AEAC/gm and DPPH scavenging activity showed 61.68 % RSA. *C. odoratus* contains a good amount of calcium (10.20%) which is needed for muscle, heart and digestive system, builds bone, supports synthesis and function of blood cells[34-35]. A rich iron (6490ppm) content in the mushroom will help to prevent anaemia [36-38]. The outcome of various nutritional, biochemical and mineral analysis revealed the usefulness of *C. odoratus* as good nutraceutical bioagent.

Figure: 1 Field Photograph of *Craterellus odoratus* mushroom

In the present studies, fruit bodies of *C. odoratus* had good amount of protein (3.66±1.15mg/gm) and carbohydrate (35.31g/100g). Microorganisms accumulate several types of carotenoids as a part of their response to various environmental stresses [39]. Colorful carotenes are used as strong antioxidants and as pigments in food. Carotenoids contents imparts the color of mushrooms due to the presence of pigments. *C. odoratus* showed an appreciable amounts of carotenoids i.e. 0.22± 0.05 mg/gm. β-carotene and lycopene are re-

sponsible for the clear vision and plays an important role in the diet, our sample shows comparatively high amount of β-carotene and lycopene than reported by Khatua *et al.* [40]. Flavonoid content (2.56±0.12mg/gm) was even higher as compared to *Cantherelles* sp. as reported by Kumari *et al.*[41] (Table: 1)

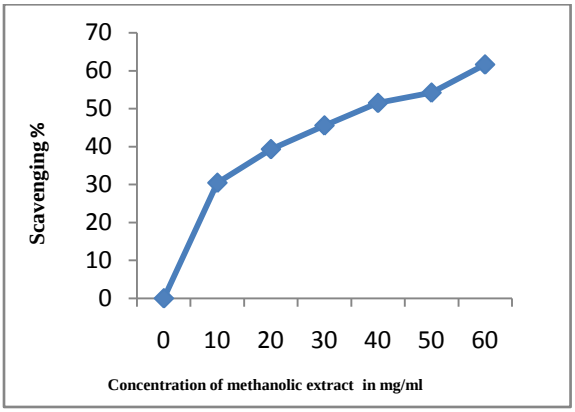

Fig-2: Representing radical scavenging of *C. odoratus*

Table-1: Representing nutritional and antioxidant components in C. odoratus.

S. no	Parameters	Values
1	Protein (mg/g)	3.66 ± 1.15
2	Carbohydrate (g/100g)	35.31 ± 5.33
3	Reducing Sugar (mg/g)	10.80 ± 2.33
4	Non Reducing Sugar (g/100g)	34.19 ± 5.50
5	Total Phenolics (g/100g)	0.13 ± 0.01
6	Carotenoids (mg/g)	0.22 ± 0.05
7	AEAC(Ascorbic acid Equivalent Antioxidant capacity) mg/gm	0.63 ± 0.02
8	DPPH Scavenging (%)	61.68 %
9	IC 50 (µg/ml)	40.00
10	FRAP (AEAC mg/gm)	2.39±0.09
11	Flavonoids (mg/gm)	2.56±0.12
12	Tannins (mg/gm)	2.95±0.36
13	β-carotene (mg/gm)	0.017±0.006
14	Lycopene (mg/gm)	0.016±0.006
15	Phosphorous (%)	0.29
16	Sodium (%)	0.12
17	Calcium (%)	10.20
18	Magnesium (%)	9.54
19	Iron(ppm)	6490
20	Zinc (ppm)	1.10

Acknowledgements

Authors are highly thankful to Dr. N.S. Atri, Professor and Head, Department Of Botany, Patiala University, Punjab, Dr. Kaviyarasan, Dept of Botany CAS, Madras University for identity verification of the specimen. We are thankful to the Division forest offices of Odisha for providing permission and logistic support in the field.

Funding

Forest and Environment department, Govt. of Odisha.

Competing Interests

None declared.

References

1. Chang ST, Bushwell JA. Mushroom nutraceuticals world, J. Microbiol. Biotechnol. 1996; 12:473-6.
2. Jiskani MM. Energy potential of mushrooms. The DAWN economic and business review, 2001. P.IV. 15-21
3. Buigut SK. *Mushroom production in suitable small scale farming system-oppurtunities and constraints: a survey of Uasin Gishu district. In proceedings of the horticulture seminar on sustainable horticulture production in the tropics at Jomo Kenyatta University of Agriculture and technology*, Juja, Kenya 3rd-6th October, 2001. Eds. Wesonga JM, Losenge T, Ndung'u CK, Fricke A, Hau B, Stutzel H, 2002. Pp. 1-5.
4. Boa E. Wild Edible Fungi: *A Global Overview of Their Use and Importance to People*. Non –wood forest products; Rome 2004; Series no. 17. FAO.
5. Kalač P. Chemical composition and nutritional value of European species of wild growing mushrooms: A review, Food Chemistry. 2009, 113, 9–16.
6. Jones K. Maitake: A potent medicinal food. Alternative Comparative Therapy, Dec 1998. 420-429.
7. Brandt CR, Piraino F. Mushroom antivirals. Recent Res Dev Antimicrob Agents Chemother., 2000, 4, 11–26.
8. Reshetnikov SV, Wasser SP, Tan KK. Higher Basidiomycetes as a source of antitumour and immunostimulating polysaccharides (Review), International Journal of Medicinal Mushrooms., 2001:3;361-94.
9. Mau JL, Chang CN, Huang SJ, Chen CC. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food chemistry, 2004; 87: 111-8.
10. Barros L, Venturini B A, Baptista P, Estevinho L M and Ferreira I C F R, Chemical composition and biological properties of portugese wild mushrooms: A

comprehensive study, Journal of agricultural and food chemistry, 2008, 56: 3856-3862.
11. Riberio B, Lopes R, Andrade P B, Seabra R M, Goncalves R F, Baptista P, Quelhas I and Valentao P, Comparative study of phytochemicals and antioxidant potential of wild edible mushroom cap and stipes, Food chemistry, 2008, 110, 47-56.
12. Ferreira I C F R, Barros L and Abreu R M V. Antioxidants in Wild Mushrooms. Current Medical Chemistry, 2009, 16(12), 1543–1560.
13. Alexopoulos C J, Mims S W, Blackwell M. *Introductory mycology*. 4th Ed; John Wiley and Sons. New York. 1996, Inc. 869 .
14. Pilz D, Molina R and Liegel L. Biological productivity of chanterelle mushrooms in and near the Olympic Peninsula Biosphere Reserve. In: Liegel, L.H., comp. The biological, socioeconomic, and managerial aspects of chanterelle mushroom harvesting: the Olympic Peninsula, Washington State, U.S.A. Ambio Special Report 9. Stockholm, Sweden: Royal Swedish Academy of Sciences, 1998, 8–13.
15. Persson O and Mossberg B. The chanterelle book. Berkeley, CA: Ten Speed Press. 1997; 120 p.
16. Bradford M M, A Rapid and sensitive method for the quantification of Microgram quantities of the protein utilizing the principle of protein dye binding, Anal. Biochem, 1976, 72, 248-258.
17. Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F. Colorimetric method for determination of sugars and related substances, Analytical Chemistry, 1956, 28, 350–356.
18. Hedge J E and Hofreiter B T, In: Carbohydrate Chemistry, 17 (Eds. Whistler R.L. and. Be Miller, J.N.), Academic Press, New York. 1962.
19. Miller G I, Use of Dinitrosalicylic acid reagent for determination of reducing sugars, Anal. Chem., 1972, 31, 426.
20. Nazarudeen A. Nutritional composition of some lesser known fruits use by ethinic communities and local folks of Kerala, Ind. j. traditional Knowl, 2010, 9(2), 398-402.
21. Bray R H and Kurtz L T, Determination of total, organic and available forms of phosphorus in soils. Soil science, 1945, 59, 39-45.
22. Verma A and Kesharvani G P, Mineral content of edible mushrooms, Indian journal of nutrition and diet, 1987, 24, 241-245.
23. Chan E W C, Lim Y Y and Omar M, Antioxidant and antibacterial activity of leaves of *Etlingera* Species (Zingiberaceae) in Peninsular Malaysia. Food Chemistry, 2007, 104 (4), 1586-1593.
24. Benzie I F F and Strain J J, The Ferric Reducing Ability of Plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP Assay. Analytical Biochemistry, 1996, 239, 70-76.
25. Athavale A, Jirankalgikar N, Nariya P and Des S. Evaluation of in-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. International journal of pharmaceutical sciences and research, 2012, 3(8), 2543-2549.
26. Oyaizu M, Studies on products browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japan journal of Nutrition, 1986, 44, 307-315.
27. Singleton V L and Rossi J A. Colorimetric of total Phenolics with Phospomolybdic acid reagents, American Journal of Enology and Viticulture, 1965, 16, 144-158.
28. Harris L J and Ray S N, Determination of plasma Ascorbic acid by 2, 6-dichorphenol indophenols titration. Lancet, 1935, 1: 462.
29. Chang C, Yang M, Wen H and Chern J, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, Journal of Food Drug Analaysis, 2002, 10, 178-182.
30. Nagata M and Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids Tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 1992, 39(10), 925-928.
31. Barros L, Ferreira M J, Queiros B, Ferreira I C F R and Baptista P, Total phenols, ascorbic acid, B-carotene and lycopene in portuguese wild edible mushrooms and their antioxidant activities, Food chemistry, 2007, 103, 413-419.
32. Arnon D I, Copper enzymes in isolated chloroplast, polyphenol oxidase in *Beta vulagris*, Plant Physiology., 1949, 24: 1-15.
33. Schanderl S H, In: Method in food analysis academic press, New York, 1970, 709.
34. Kanis J A and Passmore R. Calcium supplementation of the diet. Br Med J. 1989, 298, 205-208.
35. Horowitz M, Wishart J M , Need A G, Morris H A and Nordin B E C. Effects of norethisterone on bone related biochemical variables and fore arm bone mineral in postmenopausal osteoporosis. Clin Endocrinol, 1993, 39, 1-5.
36. Senger J M and Weiss R J. Hematologic and erythropoietin responses to iron dextran in the hemodialysis environment, Anna J, 1996, 23, 319–323

37. Spinowitz B S, Kausz A T, Baptista, J *et al*., Ferumoxytol for treating iron deficiency anemia in CKD, J Am Soc Nephrol., 2008, 19, 1599–1605.
38. Silverberg D S, Blum M, Agbaria Z et al. The effect of i.v. iron alone or incombination with low-dose erythropoietin in the rapid correction of anemia of chronic renal failure in the predialysis period, Clin Nephrol, 2001, 55, 212–219.
39. Bhosale P, Environmental and cultural stimulants in the production of carotenoids from microorganisms, Applied Microbiology and Biotechnology, 2004,63: 351-61.
40. Khatua S, Roy T and Acharya K. Antioxidant and free radical scavenging capacity of phenolic extract from *Russula laurocerasi*, Asian J Pharm Clin Res, 2013, 6 (4), 156-160.
41. Kumari D, Reddy M S and Upadhyay R C. Antioxidant activity of three species of wild mushroom genus *Cantherellus* collected from north-western Himalaya, India. International journal of agriculture and biology, 2011, 13(3), 415-418.