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Nutraceutical composition of wild species of genus *Lentinus* Fr. from Nothern India

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Abstract

Neutraceutical composition of wild edible fungal species of genus *Lentinus* viz. *L. sajor-caju*, *L. connatus*, *L. torulosus*, *L. cladopus* and *L. squarrosulus* was determined. Nutraceuticals composition was done with the use of HPLC-ELSD, GC-MS, UPLC and standard quantitative methods. Sugars, fatty acids, ascorbic acid, carotenoids, lycopene, phenolic compounds and amino acids were analyzed. All the species composed of three types of sugars evaluated, sucrose (0.488-4.941 %) was found to be predominated over glucose and xylose. Among fatty acids, SFA content ranged from 26.76–57.36 %, MUFA 16.57–67.35% and PUFA 0.70–1.45 %. Ascorbic acid content ranged from 0.42–0.49 mg/100g, β-carotene 0.08–0.22 µg/100g, lycopene content 0.032–0.086 µg/100g, phenolic compounds 6.39–20.11 mg/100g of gallic acid. Amongst amino acids, aspartic acid 0.25–0.37%, arginine 0.21–0.29 %, alanine 0.09–0.15 %, proline 0.01–0.06 % .and tyrosine amount ranged 0.16–0.24 %.

Key words – Carotenoids – *Lentinus* – nutraceutical – wild mushrooms

Introduction

Wild mushrooms are one of the valued non timber minor forest products. They provide local population with seasonal food, medicine and also serve as an alternative source of income (Sysouphanthong *et al.*, 2010). Mushrooms have long been valued as highly nutritious tasty food items by many societies throughout the world (Breene, 1990; Chang & Miles, 1992; Manzi *et al.*, 1999). Wild mushrooms are becoming more and more important in our diet for their nutritional qualities (Breene, 1990; Crisan & Sands, 1978). In addition to their nutritional value, many edible mushrooms have long been investigated for their pharmaceutical constituents (Bobek *et al.*, 1995; Bobek & Galbavay, 1999). At present, over 270 species of mushroom are reported with known therapeutic properties (Ying *et al.*, 1987; Hobbs, 1995; 2003; Ooi & Liu, 1999; Wasser & Weis, 1999 a, b; Gunde - Cimerman, 1999). Medicinal mushroom extracts were considered as important remedies for the prevention and treatment of many diseases for thousands of years especially in the Oriental regions (Wasser & Weis, 1999a; Kidd, 2000; Israilides & Philippoussis, 2003). Many of them are rich source of various bioactive molecules having anticancer and immunomodulatory potential. Such compounds are increasingly used in many parts of the world to help support immune function in cancer patients during radio and chemotherapy and are reported to prolong

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survival times in some types of cancer (Mizuno, 1995). Previous studies have shown that *Lentinus* Fr. mushrooms can lower both blood pressure and free cholesterol in plasma, as well as accelerate accumulation of lipids in the liver, by removing them from circulation (Kabir and Kumura, 1989). Nucleic acids from *Lentinula edodes* (Berk.) Sing. have been reported to initiate significant platelet agglutinating inhibitory effects (Hokama and Hokama, 1981).

Nutraceutical aspects of mushrooms with respect to their antioxidant (Mau *et al.*, 2005), antitumor (Wasser and Weis, 1999a), and antimicrobial properties (Smânia *et al.*, 1995) are now well established facts. Besides pharmacological utility (Lindequist *et al.*, 2005), wild mushrooms are an important part of our diet due to their proximate composition with high amount of protein and low fat/energy contents (Diéz and Alvarez, 2001). Some examples of nutritive nutraceuticals or ‘‘functional food ingredients” present in mushrooms are dietary fibers, polyunsaturated fatty acids (PUFA), proteins, peptides, amino acids, keto acids, minerals, antioxidative vitamins and other antioxidants (Andlauer and Fürst, 2002).

Genus *Lentinus* Fr. belongs to family Polyporaceae and is recognized by as many as 40 species the world over (Kirk *et al.,* 2008). The genus *Lentinus* Fr. is characterized by the presence of dimitic and amphimitic hyphal systems (Moser, 1978; Kühner, 1980; Pegler, 1983; Singer, 1986) with the fascicles of sterile hyphae coming out from the hymenium surface (hyphal pegs). This genus also shares these features with other genera of family Polyporaceae (Pegler, 1983; Corner, 1981; Singer, 1986). Many of its species are edible except those with tough and leathery texture.

Under present investigations the evaluation for nutraceutical components of five wild species of *Lentinus* namely, *Lentinus sajor-caju* (Fr.) Fr., *L. connatus* Berk., *L. torulosus* (Pers. : Fr.) Lloyd, *L. cladopus* Lév and *L. squarrosulus* Mont. was compared and presented in this manuscript.

Materials and Methods

Mushrooms

All the five fully mature samples were collected from various localities of North West India (Table 1). The samples were dried at 45 ^{0}C and preserved in cellophane bags in air tight conditions with small amount of 1-4 para dichlorobenzene in porous packets to keep them insect free for further investigations. Taxonomic identification was done following the description given by Pegler (1983). The samples were deposited at the herbarium of Department of Botany, Punjabi University Patiala (Punjab) India under PUN (Holmgren *et al.*, 1990).

Sugar composition

Free sugars were determined by high performance liquid chromatography coupled to a evaporative light scattering detector (HPLC-ELSD) following the method given by Bhandri *et al.* (2008). Dried sample (0.1g) was extracted with 2.5 ml of 70% aqueous methanol followed by 1.5 ml of 70 % aqueous methanol and then 1 ml of 70% aqueous methanol. This extract was centrifuged at 4000 rpm at 4ºC for 10 min. Supernatant was collected and volume made up to 5 ml with 70% methanol. The extract was passed through Millipore filter (0.45 µm) prior to injection on the HPLC.

LC instruments and chromatographic conditions

LC analysis was performed on a Waters Analytical HPLC system equipped with 600 quaternary gradient pump, 2424 ELSD, column oven, autosampler and controlled by Empower 2 software. Separation was achieved on a LichroCART-NH_2 column (250 mm × 4.6 mm, 5 µm particle size) maintained at 40ºC with a mobile phase flow rate of 0.8 mL/min. The mobile phase contained acetonitrile (ACN): water (77:23) in isocratic elution. The drift tube temperature was set at 70 ºC and nebulizing gas (N_2) flow rate was 2.0 spatial light modulator (SLM)

Fatty acid composition

Reagent preparation – Four reagents were prepared to saponify the cells, esterify extract and base wash the fatty acid extracts.

Reagent1 - In 50% methanol (300ml) added 45g NaOH pellets to the solution with stirring. Stirred till the pellets get dissolved.

Reagent 2 - Added 2 ml of 6N HCl to methanol with stirring.

Reagent 3 - Added 25ml methyl tert-butyl ether (MTBE) added to hexane and stirred well.

Reagent 4 - Added 3ml 10% NaOH to the water while stirring.

Method – Reagent 1 (1 ml) was added in 100 mg dried powdered of each mushroom sample, vortexed for 1 min and left at 100 ºC for 5 min. Again vortexed for 1 min and left again for 100 ºC for 25 min on water bath. Reagent 2 (2 ml) was added for methylation, vortexed for 1min and heated at 80 ºC on water bath. Extraction of fatty acids was done by adding 1.25 ml of reagent 3 and solution was shaken for 10 min. Upper layer was removed. 3ml of reagent 3 was added. $2/3^{rd}$ top phase was removed and transferred into GC vial and injected.

Antioxidant composition

Ascorbic acid

Reagents – 3% meta-phosphoric Acid (H_3PO_4). Ascorbic acid standard L- ascorbic acid in 3% (H_3PO_4). Dye solution was prepared by dissolving 50 mg of the sodium salt of 2, 6 dichlorophenol- indophenol in approximately 150 ml of hot glass distilled water counting 42 mg sodium bicarbonate. Cooled and diluted with glass distilled water to 200 ml. Stored in a refrigerator and standardized every day.

Procedure – 5 ml of standard ascorbic acid solution was taken and to this 5 ml of (H_3PO_4) was added. Microburette was filled with the dye and titrated with dye solution to a pink colour which persisted for 15 seconds. The dye factor was determined i.e. mg of ascorbic acid per ml of the dye using formula: 0.5/ titrate.

Sample was prepared by taking 10 g of sample grounded in meta- phosphoric acid and made the volume upto 100 ml. Titrated after filtration till pink colored appeared.

$$\text{Mg of ascorbic acid per 100g or ml} = \frac{\text{Titrate} \times \text{Dye factor} \times \text{Vol. made}}{\text{Aliquot of extract} \times \text{wt. of sample}} \times 100$$

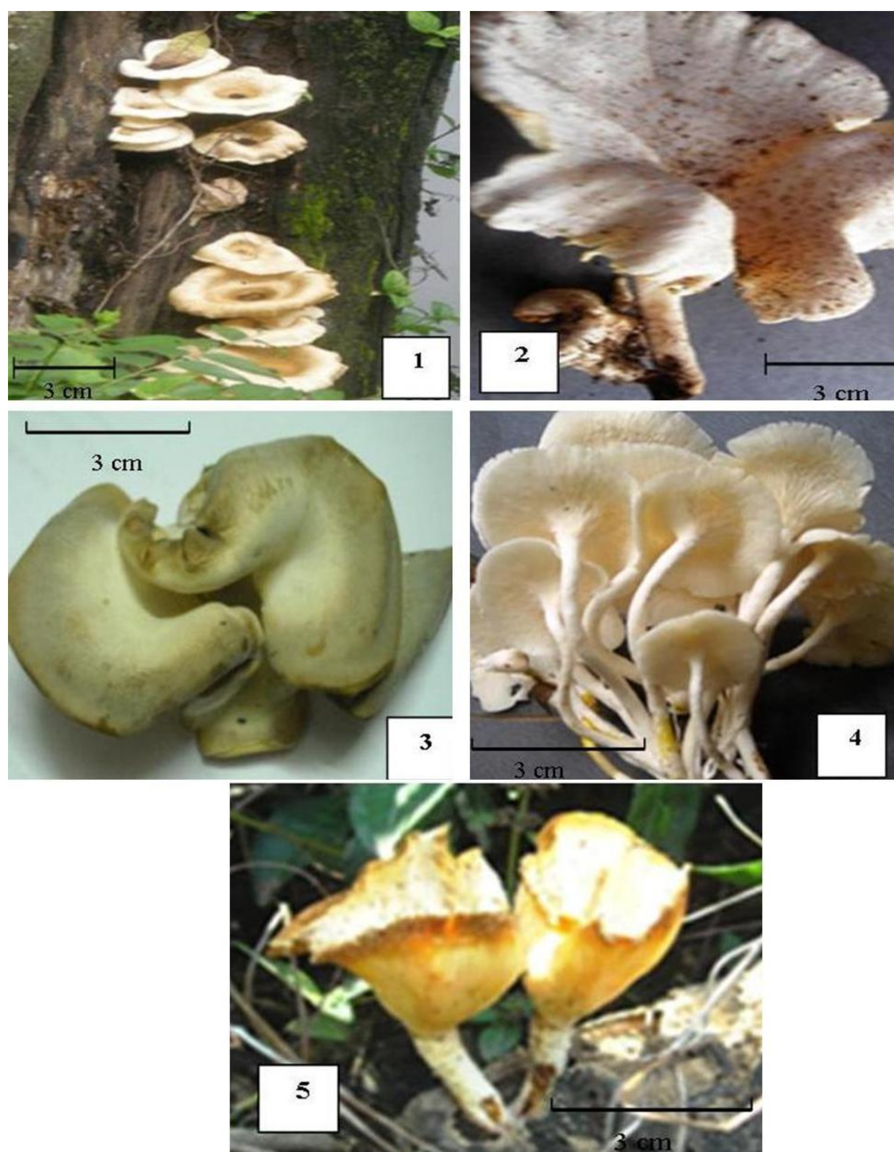
β-carotene and lycopene composition

Sample preparation – The fruiting bodies were air dried and powered before analysis. The dried sample (~5) were extracted by stirring with 100 ml of methanol at 25 ºC at 150 rpm for 24 h and filtered through Whatman No.4 paper. The residue was then extracted with two additional 100 mL portions of methanol, as described earlier. The combined methanolic extracts were evaporated at 42 ºC to dryness and redisolved in methanol at a concentration of 50 mg/ml, and stored at 4 ºC for further use.

Determination of β-carotene and lycopene – β-carotene and lycopene were estimated on the basis of the method described by Nagata and Yamashita (1992) . The dried methanolic extract (100 mg) was vigorously shaken with 10 mL of acetone – hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. Contents of β carotene and lycopene were calculated according to following equation: lycopene (mg/100mL) = $-0.0458 \times A663 + 0.372 \times A505 - 0.0806 \times A\ 453$: β-carotene (mg/100mL) = $0.216 \times A663 - 0.304 \times A505 + 0.452 \times A\ 453$.

Determination of total phenol

Phenolic compounds in mushroom extracts were estimated by colorimetric assay following the method of Singleton and Rossi (1965). Briefly 1 mL of the sample was mixed with 1 mL of



Figs 1–5 – **1** Wild basidiocarp of *Lentinus sajor-caju.* 2 Wild basidiocarp *of L. conatus.* 3 Wild basidiocarp of *L. torulosus.* 4 Wild basidiocarp of *L. cladopus*. 5 *L. squarrosulus*

sample of Folin and Ciocalteu's phenol reagent. After 3 min, 1mL of saturated solution of sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 minutes, after which the absorbance was read at 725 nm. Gallic acid was used to calculate the standard curve (0.01 – 0.4 mM, R^2 =0.9997) and the results were expressed as mg of gallic acid equivalents (GAEs) per g of extract (Fig. 1/F).

Amino acid analysis

Samples were crushed and dried up to constant weight. Dried sample (0.1g) was extracted with 2.5ml of 70% aqueous methanol followed by 1.5ml and 1ml of 70% aqueous methanol. This extract was then centrifuged at 4000 rpm at 4ºC for 10 minutes. Supernatant was collected and volume made up to 5 ml by dissolving 70% methanol. The extract was passed through Millipore filter (0.45 µm). Derivatisation was done with derivatising agent prepared by ethanol: triethylamine : water : phenylisothiocynate (PITC). Mixed 10µl sample in a test tube and dried using vacuum oven. Added 20µl derivatising agent mix with it and re dried it. Then left the sample for 25 minutes at room temperature. Allowed the vial to dry thoroughly to remove all traces of PITC. After proper drying added 1ml ethanol and injected into UPLC.

An Acquity UPLC from Waters India Pvt. Ltd. equipped with PDA (Photodiode array detector) and Pico. Tag column (3.9 × 150 mm) for amino acid analysis was used. Mobile phase A consisted of 0.1 % TEA (triethylamine) in 940 ml water + 60 ml acetonitrile and mobile phase B consisted of 600 ml acetonitrile + 400 ml distilled water were used. Following parameters were used for separation of amino acid.

Table 1 Samples collected from various localities of North West India

Time	Flow	A %	B %
0	1.00	100	0
11	1.00	50	50
20	1.00	100	0

Column temperature was 38 °C and amino acids estimated at 254 nm.

Statistical Analysis

Data was statistically analyzed with ANOVA software.

Result and Discussion

Sugars have a direct relation with various metabolic processes occurring in the body at cellular levels. Sucrose, glucose and xylose are the common and important sugars analyzed in the studied *Lentinus* species. Amongst the *Lentinus* species evaluated *L. cladopus* have more sucrose content (4.941 %) followed by *L. torulosus* (3.671 %), *L. squarrosulus* (0.693 %) and *L. sajor-caju* (0.585 %) whereas *L. connatus* contained least amount of sucrose (0.488 %) in comparison. Glucose content was highest in *L. torulosus* (0.542 %) followed by *L. squarrosulus* var. *squarrosulus* (0.519 %), *L. connatus* (0.515 %) and least amount of glucose content was recorded in *L. sajor-caju* (0.2 %), whereas it was not detected in *L. cladopus. L. connatus* showed maximum percentage of xylose (0.296 %) followed by *L. squarrosulus* (0.035 %) and *L. sajor-caju* (0.011 %). In all the studied species three main sugars detected are glucose, sucrose and xylose (Table 3). These findings are in conformity with the results of Crisan and Sands (1978) for *Agaricus bisporus* in which raffinose, sucrose, glucose, fructose and xylose are reported as the main sugars. As compared in case of *Ganoderma lucidum* (Curtis) P. Karst. and *Coriolus versicolor* (L.) Quél. Mau *et al.* (1998) documented the presence of trehalose and glucose sugars besides the presence of psicose sugar in lowest amount in *Pleurotus eryngii* (DC.) Quél. Aletor (1995) documented trehalose to be the most common sugar in mushrooms. In case of *Agaricus bisporus* (J.E. Lange) Imbach, *A. silvaticus* Schaeff., *A. silvicola* (Vittad.) Peck, *Boletus edulis* (Vittad.) Peck, *Calocybe gambosa* (Fr.) Donk, *Canthrellus cibarius* Cantharellus cibarius, *Craterellus cornucopioides* (L.) Pers. and *Marasmius oreades* (Bolton) Fr.

Barros *et al.* (2008) showed the presence of mannitol and trehalose as the main sugars whereas maltose and melezitose are reported to be present in smaller amounts.

Fatty acids especially polyunsaturated fatty acids are known for their nutraceutical potential. Pelargonic acid (C9:00) composition was found to be highest in *L. connatus* (3.47 %) followed by *L. torulosus* (1.11 %), *L. sajor-caju* (0.99 %) and least amount of this acid was detected in *L. squarrosulus* (0.75 %), whereas it was not detected in *L. cladopus*. The maximum amount of capric acid (C10:00) was detected in *L. connatus* (1.79 %) followed by *L. squarrosulus* (1.64 %), *L. torulosus* (0.30 %), *L. sajor-caju* (0.26 %) and *L. cladopus* (0.21). Lauric acid (C 12:00) was found to be present only in *L. torulosus* (1.72 %) while in all other species evaluated it was not detected. Fatty acid (C 15 :00) was present in highest amount in *L. squarrosulus* (5.82 %) followed by *L. torulosus* (4.47 %), *L. sajor-caju* (4.41 %), *L. connatus* (2.03 %) and *L. cladopus* (1.69 %). Another unsaturated fatty acid (C 16:01) was detected in highest amount in *L. connatus* (2.05 %) followed by *L. squarrosulus* (1.71 %), *L. cladopus* (1.53 %), *L. torulosus* (0.74 %) and *L. sajor-caju* (0.63 %). Maximum amount of palmitic acid (C 16:00) was found in *L. squarrosulus* (45.13 %) followed by *L. torulosus* (41.83), *L. sajor-caju* (41.29 %), *L. cladopus* (22.79 %) while least amount was

Table 2 Associated natural host, location with altitude and forest type of presently investigated *Lentinus* species.

Species	Host	Location	Altitude (m)
L. sajor-caju	*Bauhinia variegate*	Sirmour (Himachal Pradesh)	672
L. connatus	*Mangifera indica*	Chandigarh (Punjab)	200
L. torulosus	*Pinus roxburghii*	Palampur (Himachal Pradesh)	850
L. cladopus	*Albizia chinensis*	Palampur (Himachal Pradesh)	1200
L. squarrosulus	*Albizia chinensis*	Palampur (Himachal Pradesh)	1200

Table 3 Sugar composition in five wild edible *Lentinus* species (%).

Species	Xylose	Glucose	Sucrose
Lentinus sajor-caju	0.011 ± 0.002 a	0.2 ± 0.01 b	0.585 ± 0.01 b
Lentinus conatus	0.296 ± 0.04 b	0.515 ± 0.02 b	0.488 ± 0.02 b
Lentinus torulosus	Nd	0.542 ± 0.001 b	3.671 ± 0.00 c
Lentinus cladopus	Nd	Nd	4.941 ± 0.02 c
Lentinus squarrosulus	0.035 ± 0.001 a	0.519 ± 0.003 b	0.693 ± 0.01 b

nd = not detected
In each row and column different letters mean significant differences ($p<0.05$).

detected in *L. connatus* (14.25 %). Unsaturated fatty acid C 17:01 was detected in highest amount in *L. squarrosulus* (2.01 %) followed by *L. torulosus* (1.05 %) and *L. cladopus* (1.02 %). As compared, in *L. connatus* and *L. sajor-caju* 0.69 % of this fatty acid was recorded. Maximum amount of C17:03 OH fatty acid was found in *L. squarrosulus* (1.45 %) followed by *L. sajor-caju* (1.31 %), *L. torulosus* (1.30 %), *L. cladopus* (0.76 %) and *L. connatus* (0.70 %). Fatty acid C18:1 cis9 was maximum in *L. cladopus* (47.87 %) followed by *L. squarrosulus* and *L. connatus* (23.38 %), *L. sajor-caju* (13.9 %) and minimum amount of this acid was detected in *L. torulosus* (13.56 %). Stearic acid (C 18:00) was found to be maximum in *L. torulosus* (7.37 %) followed by *L. sajor-caju* (6.94 %), *L. connatus* (5.51), *L. squarrosulus* (4.02 %) and *L. cladopus* (2.07 %). The amount of fatty acid C 19:01 was maximum in *L. cladopus* (16.93 %) followed by *L. connatus* (6.6 %), *L. sajor-caju* (1.05 %) and least was detected in *L. torulosus* (0.31 %) whereas in *L. squarrosulus* it was not detected at all. Fatty acid C 20:01 was found to be present only in *L. connatus* (2.41 %) and *L. torulosus* (2.27 %) whereas in all other species it was either absent or not detected at all. As it is quite clear from the data that these mushrooms are rich in unsaturated fatty acids especially monounsaturated fatty acids with maximum percentage in *L. cladopus* (67.35 %) followed by *L. connatus* (32.72 %), *L. squarrosulus* (27.1 %), *L. torulosus* (17.93 %) and *L. sajor-caju* (16.27 %) (Table 4). Maximum amount of saturated fatty acid was found in *L. squarrosulus* (57.36 %) followed *L. torulosus* (56.8 %), *L. sajor-caju* (53.89 %), *L. connatus* (27.05 %) and *L. cladopus* (26.76 %) (Table 4). Polyunsaturated fatty acids were found maximum *L. squarrosulus* (1.45 %) followed by *L. sajor-caju* (1.31 %), *L. torulosus* (1.3), *L. cladopus* (0.76 %) and while least amount of this fatty acid was detected in *L. connatus* (0.70 %) (Table 4). The presence of these three types of fatty acids i.e. SFA MUFA and PUFA in wild edible mushrooms, namely *Agaricus bisporus*, *A. silvaticus*, *A. silvicola*, *Boletus edulis*, *Calocybe gambosa*, *Canthrellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* has also been demonstrated by Barros *et al.* (2008). Wani *et al.* (2010) documented that the fats present in mushrooms are dominated by unsaturated fatty acids. As documented by Barros *et al.* (2008) in these species the percentage of SFA ranged from 14.52 - 22.63 %, MUFA ranged from 1.52 – 59.85 % while the percentage of PUFA ranged from 23.79 – 76.95 %. In comparison in the presently evaluated species of *Lentinus* much higher percentage of SFA (26.76 – 57.36 %) and MUFA (16.27 – 67.35%) have been evaluated. The presence of palmitic acid (C 16:00) in the presently studied *Lentinus* species ranged from 14.25 – 45.13 %. These findings are also supported by Longvah and Deosthale (1998) while working on

Scizophyllum commune Fr. and *Lentinula edodes* (Berk.) Pegler in which the presence of linoleic acid (65 %), palmitic acid (20 %) and oleic acid (10 %) have been documented, although the amount reported is slightly less than evaluated in the presently studied species. Similar observations are available for other mushrooms as well (Senatore *et al.*, 1988). Diéz and Alvarez (2001) while working on *Tricholoma* reported the presence of oleic acid (57 %) and linoeic acid (28 %). Dominance of unsaturated fatty acids over saturated fatty acids in mushrooms has found support from number of workers including Diéz and Alvarez (2001), Longvah and Deosthale (1998) and Mauger *et al.* (2003). The results achieved presently confirm this above generalization with regard to the dominance of unsaturated fatty acids in mushrooms (Table 4).

Table 4 Fatty acid composition of five wild edible *Lentinus* species (%).

Fatty acids	*L. sajor-caju*	*L.connatus*	*L. torulosus*	*L. cladopus*	*L. squarrosulus*
C9:00	0.99 ± 0.01 a	3.47± 0.02 b	1.11 ± 0.2 a	nd	0.75 ± 0.001 a
C10:00	0.26 ± 0.04 a	1.79 ± 0.02 a	0.30 ± 0.02 a	0.21 ± 0.02 a	1.64 ± 0.00 a
C12:00	Nd	Nd	1.72 ± 0.01	nd	nd
C15:00	4.41 ± 0.01 b	2.03 ± 0.01 a	4.47 ± 0.00 b	1.69 ± 0.01 a	5.82 ± 0.00 c
C16:01	0.63 ± 0.02 a	2.05 ± 0.2 a	0.74 ± 0.02 a	1.53 ± 0.002 a	1.71 ± 0.001 a
C16:00	41.29 ± 0.4 e	14.25 ± 0.2 c	41.83 ± 0.1 e	22.79 ± 0.1 d	45.13 ± 0.02 e
C17:01	0.69 ± 0.01 a	0.69 ± 0.01 a	1.05 ± 0.002 a	1.02 ± 0.01 a	2.01 ± 0.002 a
C17:03 OH	1.31 ± 0.02 a	0.7 ±0.002 a	1.3 ± 0.00 a	0.76 ± 0.03 a	1.45 ± 0.001 a
C18:1 cis9	13.9 ± 0.00 c	23.38 ± 0.1 d	13.56 ± 0.01 c	47.87 ± 0.1 e	23.38 ± 0.3 d
C18:00	6.94 ± 0.004 c	5.51 ± 0.02 c	7.37 ± 0.002 c	2.07 ± 0.002 a	4.02 ± 0.01 b
C19:01	1.05 ± 0.01 a	6.6 ± 0.02 c	0.31 ± 0.001 a	16.93 ± 0.2 d	nd
C20:01	Nd	2.41 ± 0.04 b	2.27 ± 0.02 a	nd	nd
SFA	53.89 ± 0.3 e	27.05 ± 0.1 d	56.8 ± 0.1 e	26.76 ± 0.1 d	57.36 ± 0.2 e
MUFA	16.27 ± 0.1 d	32.72 ± 0.3 f	17.93 ± 0.2 d	67.35 ± 0.2 e	27.1 ± 0.1 d
PUFA	1.31 ± 0.04 a	0.70 ± 0.2 a	1.3 ± 0.01 a	0.76 ± 0.001 a	1.45 ± 0.00 a

nd = not detected
In each row and column different letters mean significant differences ($p<0.05$).

Ascorbic acid content has a direct relation with the antioxidant potential. All the five wild *Lentinus* species have sufficient amount of ascorbic acid content (Table 4). The highest amount of ascorbic acid was documented in *Lentinus torulosus* (0.49 mg /100g) followed by *L. squarrosulus* (0.48 mg/100g), *L. cladopus* (0.46 mg /100g), *L. connatus* (0.45 mg /100g) and least amount was documented in *L. sajor-caju* (0.42 mg /100g) (Table 5).

Table 5 Ascorbic acid, carotenoids and Phenolic compounds composition of five wild edible *Lentinus* species

Species	Ascorbic acid (mg / 100 g)	β-carotene (µg/100 g)	Lycopene (µg/100 g)	Phenolic compounds mg / 100 g of gallic acid
L. sajor-caju	0.42 ± 0.003 a	0.22 ± 0.04 a	0.086 ± 0.005 b	8.83 ± 0.2 c
L. connatus	0.45 ± 0.001 a	0.12 ± 0.06 a	0.047 ± 0.003 b	6.39 ± 0.3 c
L. torulosus	0.49 ± 0.003 a	0.12 ± 0.03 a	0.049 ± 0.007 b	20.11 ± 0.32 c
L. cladopus	0.46 ± 0.003 a	0.21 ± 0.07 a	0.081 ± 0.006 b	17.48 ± 0.22 c
L. squarrosulus	0.48 ± 0.002 a	0.08 ± 0.3 b	0.032 ± 0.7 b	8.83 ± 0.5 c

In each row and column different letters mean significant differences ($p<0.05$).

Among all the five *Lentinus* species, *Lentinus sajor-caju* contained maximum amount of β-carotene (0. 22 µg/100 g) followed by *L. cladopus* (0.21 µg/100 g) while *L. connatus* and *L. torulosus* contained comparatively less amount of β-carotene (0.12 µg/100 g). Minimum amount of this antioxidant has been detected in *L. squarrosulus* (0.08 µg/100 g). The amount evaluated is much less in comparison to 15.11 - 33.78 µg/100 g β-carotene and 5.41 – 13.04 µg/g lycopene

evaluated by Barros *et al.* (2007) in *Lactarius piperatus*. While working with number of other mushrooms including *Agaricus bisporus*, *A. silvaticus*, *A. silvicola*, *Boletus edulis*, *Calocybe gambosa*, *Canthrellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* Barros *et al.* (2008) documented β-carotene amount ranging from 1.95 – 13.56 µg/g and lycopene content ranging from 0.54 – 5.53 µg /g which is substantially on the higher side in comparison to their range in different species of *Lentinus* (Table 4).

Amongst the analyzed samples of different *Lentinus* species, maximum amount of lycopene was documented in *L. sajor-caju* (0.086 µg/100 g) followed by *L. cladopus* (0.081 µg/100 g), *L. torulosus* (0.049 µg/100 g) and *L. connatus* (0.047 µg/100 g). In comparison least amount of lycopene was detected in *L. squarrosulus* (0.032 µg/100 g) (Table 4).

Amongst the *Lentinus* species evaluated for the presence of phenolic compounds *L. torulosus* (20.11 µg/100 g) contained maximum amount of phenolic compound followed by *L. cladopus* (17.48 µg/100 g) whereas in *L. sajor-caju* and *L. squarrosulus* the amount is much less (8.83 µg/100 g) in comparison. Least amount of phenolic compounds was detected in *L. connatus* (6.39 µg/100 g) (Table 4).

The net amount of phenolics evaluated presently in wild *Lentinus* species was much less in comparison to 39.16 mg evaluated by Omar *et al.* (2010) in the mycelial extract of *Lentinus squarrosulus*. The range of 1.70 – 3.94 mg/g phenolic compounds have been documented in number of wild edible mushrooms, namely *Agaricus bisporus*, *A. silvaticus*, *A. silvicola*, *Boletus edulis*, *Calocybe gambosa*, *Canthrellus cibarius*, *Craterellus cornucopioides* and *Marasmius oreades* by Barros *et al.* (2008), which is substantially on the higher side in comparison to the amount of phenolics in the presently evaluated Indian species (Table 5).

Out of the five wild edible species of *Lentinus* evaluated for the presence of amino acids, aspartic acid content was maximum in *L. squarrosulus* (0.37 %) followed by *L. sajor-caju* (0.33 %), *L. cladopus* (0.31 %) and *L. connatus* (0.28 %). Least amount of this amino acid was documented in *L. torulosus* (0.25 %). The amount of arginine was maximum in *L. torulosus* (0.29 %) followed by *L. connatus* (0. 27 %), *L. sajor-caju* (0.25 %) and *L. cladopus* (0.24 %). In comparison, minimum amount of arginine was documented in *L. squarrosulus* (0.21 %). Maximum amount of alanine was recorded in *L. torulosus* (0.15 %) followed by *L. connatus* (0.13 %), *L. sajor-caju* (0.12 %) and *L. cladopus* (0.11 %). As compared minimum amount of alanine was documented in *L. squarrosulus* (0.09 %). The amount of proline was maximum in *L. squarrosulus* (0.06 %) followed by 0.04 % in *L. torulosus* and *L. cladopus*, 0.03 % in *L. sajor-caju* and minimum in *L. connatus* (0.01). Tyrosine was maximum in *L. cladopus* (0.24 %), followed by *L. torulosus* (0.21 %), *L. squarrosulus* and *L. connatus* (0.19 %). Least amount of tyrosine was evaluated in *L. sajor-caju* (0.16 %). While working on the wild edible *Boletus* species Valery *et al.* (2010) documented the dominance of aspartic acid over other amino acids which are in conformity with the present observations. In comparison, Bano *et al.* (1981) reported the dominance of arginine in *Pleurotus* species. Similar results were shown by Dembitsky *et al.* (2010) while working on wild edible mushrooms of genus *Boletus* (Table 6).

Table 6 Percent amino acid composition of five wild *Lentinus* species

Species	Aspartic acid	Arginine	Alanine	Proline	Tyrosine
L. sajor-caju	0.33 ± 0.002 a	0.25 ± 0.001 a	0.12 ± 0.002 b	0.03 ± 0.002 c	0.16 ± 0.001 a
L. connatus	0.28 ± 0.001 a	0.27 ± 0.003 a	0.13 ± 0.001 b	0.01 ± 0.00 c	0.19 ± 0.002 a
L. torulosus	0.25 ± 0.003 a	0.29 ± 0.004 a	0.15 ± 0.001 b	0.04 ± 0.00 c	0.21 ± 0.001 a
L.cladopus	0.31 ± 0.004 a	0.24 ± 0.002 a	0.11 ± 0.001 b	0.04 ± 0.001 c	0.24 ± 0.001 a
L. squarrosulus	0.37 ± 0.002 a	0.21 ± 0.001 a	0.09 ± 0.001 c	0.06 ± 0.001 c	0.19 ± 0.001 a

In each row and column different letters mean significant differences ($p<0.05$).

Conclusion

From the studies undertaken on the five wild culinary species of *Lentinus* it has become clear that these are as good in their nutraceutical composition as are other edible commercially grown mushrooms such as *Agaricus bisporus* (Lange) Imbach*, Pleurotus ostreatus* (Jacq.) P. Kumm., *Lentinus* edodes (Berk.), etc. Although differences were observed in the net amount of individual mushroom components, however, each of the species exhibited richness in one or the other nutraceutical component.

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