Current Research in Environmental & Applied Mycology 6 (4): 334–343 (2016) ISSN 2229-2225

www.creamjournal.org **Article** CREAM

Copyright © 2016 Online Edition

Doi 10.5943/cream/6/4/12

Molecular, physical and biochemical characterization of an edible mushroom, *Psathyrella spadicea* (P. Kumm.) Singer, from cold desert of Ladakh, India.

Yangdol R[1], Sharma YP[1], Bhattacherjee S[2] and Acharya K[2]

[1]*Department of Botany, University of Jammu, Jammu, India- 180006.*
[2]*Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, West Bengal, India. 700019.*

Yangdol R, Sharma YP, Bhattacherjee S, Acharya K 2016 – Molecular, physical and biochemical characterization of an edible mushroom, *Psathyrella spadicea* (P. Kumm.) Singer, from cold desert of Ladakh, India. Current Research in Environmental & Applied Mycology 6(4), 334–343, Doi 10.5943/cream/6/4/12

Abstract

The present investigation focuses on taxonomic identification based on morphological and molecular data, along with the biochemical, physical, and antioxidant activity of *Psathyrella spadicea*. The methanolic extract of this edible mushroom was analyzed for *in vitro* antioxidant activity in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (EC_{50}= 1. 31 mg/ml) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (7.188 TE/mg) radical scavenging activity and total antioxidant capacity (15µg AAE/mg). Fluorescence analysis exhibited significant variation against treated reagents. Furthermore, High performance liquid chromatography (HPLC) profile of the mushroom extract indicated the presence of 15 phenolic compounds in this species. Hence, the studied wild macrofungus holds an elite position as food supplement in the nutrient deficient populations of the remote areas of Ladakh.

Key words – Antioxidant property – HPLC – Taxonomy – Ladakh

Introduction

Mushrooms have been recognized as unconventional form of prime edibles because of their flavour, texture and gastronomic delicacy. Besides their high nutritive value, mushrooms have become attractive as functional foods and as a source of physiologically beneficial medicines (Nandi et al. 2012, Chatterjee et al. 2014, Maity et al. 2014, 2015, Mallick et al. 2015, Khatua et al. 2015). In recent times, changes in lifestyle have lead to increase in oxidative stress and enhanced predisposition to a number of degenerative diseases such as diabetes, lungs diseases, neurological disorders, cardiovascular problems, ageing, rheumatoid arthritis etc. Because of the tremendous side effects of synthetic antioxidants, compounds from natural sources with antioxidant potentials are being sought. Food fortification with natural antioxidants is becoming one of the most appealing modes of modern diet and healthcare. Among them, mushrooms or their derivatives or extracts occupy an elite position to perform such functions (Chatterjee et al. 2011, 2014, Khatua et al. 2013, Chatterjee & Acharya 2016).

The genus *Psathyrella* (Fr.) Quel. (Psathyrellaceae, Agaricales) is cosmopolitan in distribution and represented by 400 dark-spored taxa (Kirk et al. 2008). Out of these taxa, only 7 species including *P. atroumbonata, P. candolleana, P. coprinoceps, P. hymenocephala, P. piluliformis, P. rugocephala* and *P. spadicea,* are known to be edible, and are commonly consumed by the people of Congo and

Submitted 23 August 2016, Accepted 15 December 2016, Published online 24 December 2016
Corresponding Author: Y.P. Sharma – e-mail – yashdbm3@yahoo.co.in

Malaysia. However, there are no reports found related to their nutritional and medicinal values. During foray at Leh district of Ladakh region of Jammu and Kashmir (India), an edible species of *Psathyrella* (*P. spadicea*) was collected. Here an attempt has been made to understand the morphological, anatomical and molecular features along with the biochemical, physical and antioxidative properties of *P. spadicea*.

Materials & Methods

Morphological and molecular identification

Fresh basidiocarps of *Psathyrella spadicea* were collected from Nimoo village, Leh district of Ladakh Province of Jammu and Kashmir, India during the month of September in 2014. The morphological and ecological characters were noted in the field. Microscopic features were described from dried material by free-hand sections of basidiocarps using 3% KOH and then staining with 1% aqueous Congo red. Line drawings of microscopic features were made with the aid of Camera lucida and measurements were recorded for each character for description of average dimensions. The voucher specimens have been deposited in the Herbarium of Botany (HBJU) and Calcutta University Herbarium (CUH), Kolkata, India. Facesoffungi number of the species has also been included (Jayasiri et al. 2015).

For molecular identification, genomic DNA was extracted from the dried basidiocarps following the method of Dutta et al. (2014). PCR amplification of internal transcribed spacer (ITS) region 1 and 2, and the 5.8S of the rDNA, was performed using universal primer pair ITS1 and ITS4 (White et al. 1990). The DNA fragments were amplified on an Applied Biosystems 2720 automated thermal cycler, following Dutta et al. (2015). PCR products were purified using QIAquick® Gel Extraction Kit (QIAGEN, Germany) and was subjected to automated DNA sequencing on ABI3730xl DNA Analyzer (Applied Biosystems, USA) using primers identical with amplification for ITS rDNA region. The newly generated sequence was then deposited in GenBank (www.ncbi.nlm.nih.gov).

Organoleptic and Fluorescence analysis of the powdered sample

The dried mushroom sample was ground to a fine powder using an electric blender, sieved through 160 mesh and stored in an air tight container. For organoleptic studies, various properties like colour, odour and taste of powdered sample were evaluated. Fluorescence analysis was performed using standard pharmacopoeial method (Kokashi et al. 1958). A small amount of dried, sieved powder of the mushroom sample was placed on a clean microscopic slide and freshly prepared reagent solution (1-2 drops) was added to it, kept for 1-2 min. Then the slide was placed inside the UV viewer chamber and viewed in daylight, long (365 nm) and short (254 nm) UV light respectively. The colours observed in various reagents (Mayer's, Hager's, Dragendorff's, phluroglucinol, Barfoed's reagents) under different wavelength of radiation were carefully recorded. Colour terms and codes were recorded as per Kornerup & Wanscher (1978).

Preparation of methanolic extract

The lyophilized powder (1 g) was extracted by overnight stirring with 50 ml of methanol and subsequently separated by Whatman no. 41 filter paper. The residue was then re-extracted with 30 ml of methanol and the pooled methanolic extracts were evaporated at 40 °C (Rotavapor R3 Buchi, Switzerland) to reduce volume. The methanolic extract was stored at -20 °C in dark bottle until analysis. The percentage yield extracts were calculated based on dry weight as:

$$\text{Yield (\%)} = (W_1 \times 100) / W_2$$

Where W_1 = weight of extract after solvent evaporation; W_2 = Weight of the minced mushroom

Quantitative estimation of phytochemicals from methanolic extract

The amount of total phenolic compounds in the dried methanolic mushroom extract was determined by Folin-Ciocalteu reagent. Gallic acid was used as standard in order to create a calibration

curve by plotting absorbance versus concentration (Singleton & Rossi 1965). The results were expressed as µg of gallic acid equivalents per mg of dry extract. Total flavonoid content was estimated by using aluminium nitrate and potassium acetate (Park et al. 1997). Quercetin (5–20 µg/ml) was used to obtain the standard curve. The results were expressed as µg of quercetin equivalents per mg of extract. β-carotene and lycopene contents were estimated by measuring absorbance at 453, 505 and 663 nm (Nagata & Yamashita 1992). The amount of ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye (Rekha et al. 2012).

High performance liquid chromatographic (HPLC) profile of methanol soluble extract

For HPLC analysis, 10 mg dried extract was dissolved in 1 ml of HPLC grade methanol and then filtered through a 0.2 µm membrane, 20 µl of the filtrate was analysed by HPLC (Khatua et al. 2015). Separation was performed with Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) using a flow rate of 0.8 ml/min at room temperature. The mobile phase consisted of eluent A (acetonitrile) and eluent B (aqueous phosphoric acid solution, 0.1% v/v). Elution was carried out by using a gradient procedure: 0-5 min, 5% A; 5-10 min, 15% A; 10-15 min, 40% A; 15-20 min, 60% A; 20-22 min, 90% A. The compounds were identified by their UV spectra recorded with diode array detector and retention time of authenticate standards (gallic acid, chlorogenic acid, vanillic acid, *p*-coumaric acid, ferulic acid, myricetin, salicylic acid, quercetin, cinnamic acid, pyrogallol and kaempferol).

Determination of antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the extracts was performed following the methodology of Shimada et al. (1992). The reaction mixture (2 ml) consisted of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.101 mM) and various concentrations of the methanolic extract. The solution mixture was shaken and kept in dark for 30 min. and then absorbance was measured at 517 nm against blank. EC_{50} value is the effective concentration at which DPPH radicals were scavenged by 50 %. Ascorbic acid was used for comparison. Degree of scavenging activity was calculated by the following equation:

$$\text{Scavenging effect (\%)} = \{(A_0 - A_1)/A_0\} \times 100$$

A_0 was absorbance of the control and A_1 was absorbance in the presence of sample.

Determination of total antioxidant capacity (TAC)

The assay was carried out as described by Prieto et al. (1999) with little modification. The reaction mixture consisted of 0.3 ml sample solution and 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate). Tubes were capped and incubated at 95°C for 90 min. Samples were cooled at room temperature and absorbance was read against a blank at 695 nm. Concentrations of ascorbic acid (1–30 µg/ml) were used to obtain a standard curve. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid (AAE).

ABTS radical scavenging assay

The free radical-scavenging activity was determined by 2,2' -azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation decolorization assay described by Re et al. (1999). The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution and kept in the dark at room temperature for 12–16 hr. The working solution was prepared by dilution to an absorbance of 0.700 ± 0.02 at 734 nm. After addition of 3 ml of diluted $ABTS^{\cdot+}$solution to 20 µl of methanolic extract, the absorbance was taken exactly after 6 min. of initial mixing. The experiment was performed in triplicates and results were expressed in µM Trolox per mg of the methanolic extract.

Results & discussion

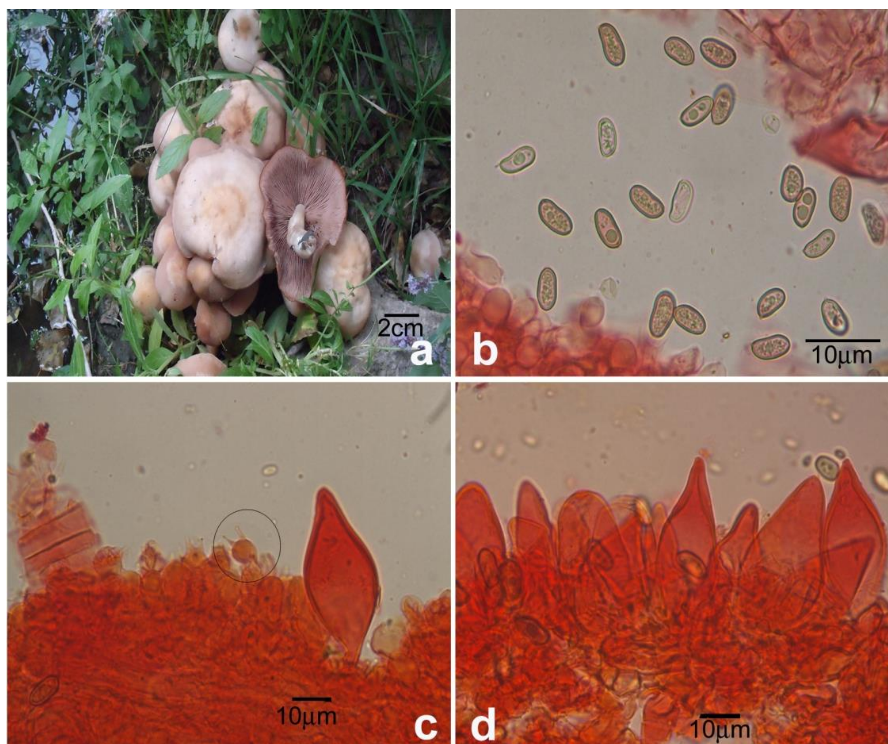

Fig. 1 – *Psathyrella spadicea*. **a**. Carpophores growing in natural habitat. **b**. Basidiospores. **c**. A basidium (encircled). **d**. Pleurocystidia.

Psathyrella spadicea (P. Kumm.) Singer, Lilloa 22: 468 (1951) [1949] Figs 1 – 2

Faces of fungi number: FoF 02698

Synonymy: *Drosophila spadicea* (P. Kumm.) Quel., *Enchir. fung.* (Paris): 116 (1886)

Agaricus hygrophilus Pers., *Mycol. eur.* (Erlanga) 3: 113 (1828)

Drosophila spadicea var. *hygrophila* (Pers.) Quel., *Enchir. fung.* (Paris): 117 (1886)

Current name: *Homophron spadiceum* (P. Kumm.) Örstadius & E. Larss., in Örstadius, Ryberg & Larsson 2015

Pileus convex at first, expanding to broadly convex at maturity, 1.0–9.0 cm in diameter, hygrophanous, deep brown with vinaceous tint when young, turning brownish pink to creamish at maturity, margin inflexed, veil absent **Lamellae** adnate, unequal, crowded, unforked, pale ochre-beige with pinkish shade **Stipe** equal to slightly tapering upward, 2.5–7.0 × 2.3–5.2 cm, central, solid, twisted, light creamy **Odour** pleasant **Taste** mild **Annulus** Absent.

Basidia clavate, 17.6–25.6 × 5.6–7.2 µm, hyaline, thin walled, 4-spored, sterigmata (1.6–2.4 µm long) **Basidiospores** ellipsoidal to broadly ellipsoidal, 8.0–9.6 × 3.2–4.8 µm, a_vL= 8.8, a_vW= 4.0, Q= 2.5–2.0, thick walled, apiculate, without germ pore, sometimes phaseoliform, pale beige with pink tint (in Congo red), mono to- multiguttulate, olive green oil droplets **Pleurocystidia** muricate and thick walled, 35.2–52.8 × 14.4–18.4 µm, hyaline, sub-acute apex, abundant **Cheilocystidia** not observed **Hymenophoral trama** regular **Pileipellis** a cutis of somewhat inflated to cylindrical, 8.0–18.0 µm wide, hyaline, septate, branched, clamp connections absent **Stipitipellis** composed of 6.4–18.0 µm, hyaline, thin walled, septate, unbranched, clamp connection absent.

Habit and Habitat – gregarious to caespitose, humicolous, at the base of *Populus nigra.*

Collection examined – India, Jammu and Kashmir, Leh district, Nimoo village, 10th September 2014, Rigzin Yangdol and Y. P. Sharma, voucher no. HBJU– 498 (CUH AM031).

Edibility – Edible in the study area.

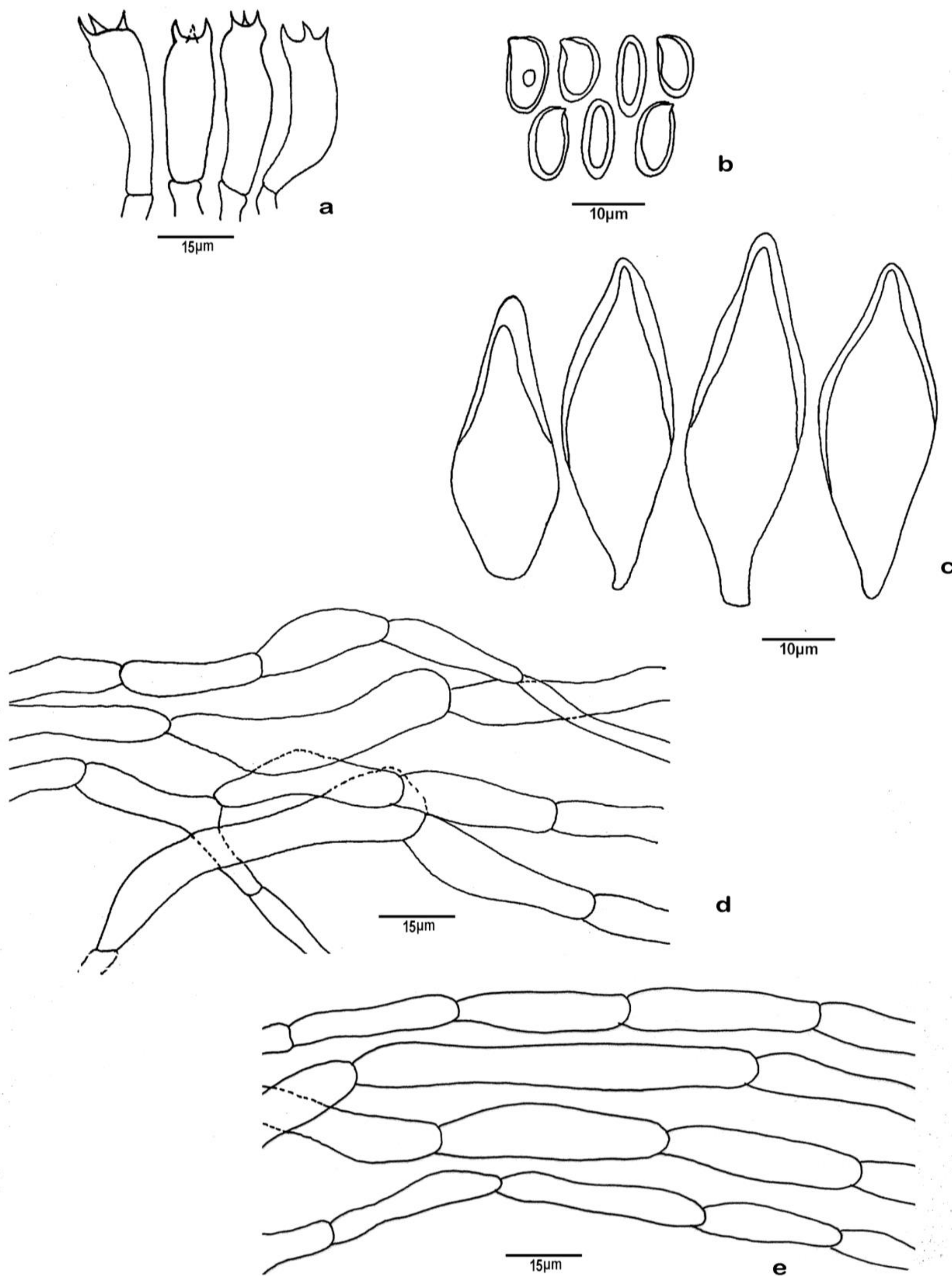

Fig. 2 – Line drawings of *Psathyrella spadicea*. **a**. Basidia **b**. Basiospores **c**. Pleurocystidia **d**. Pileipillis **e**. Stipitipellis.

Result of NCBI BLAST search

Based on megablast in NCBIs GenBank database using the ITS (KU928134) sequence, the closest hits using the nrDNA ITS sequence had highest similarity to *Psathyrella spadicea* (GenBank AB594840, Identities = 547/548(99%), Gaps = 0/548(0%) deposited from Japan; GenBank JF908635, Identities = 544/548(99%), Gaps = 0/548(0%) deposited from Italy; GenBank FN396132, Identities = 544/548(99%), Gaps = 0/548(0%) and GenBank FN396134, Identities = 544/548(99%), Gaps = 0/548(0%) deposited from Hungary).

Table 1 Fluorescence analysis of dry powder. Numbers in bracket indicate colour codes were as indicated by Kornerup & Wanscher (1978)

Treatment	Visible light	Long UV (365 nm)	Short UV (254 nm)
Blank	Dark brown / Chocolaty brown (7F5)	Blackish brown (9F5)	Blackish brown (9F5)
H_2O	Dark brown / Coffee brown (9D6)	Blackish brown (9F5)	Blackish brown (9F6)
Mayer's Reagent	Rusty brown (9D6)	Black (8F4)	Greyish brown (7F3)
Hager's Reagent	Brown (7F4)	Dark brown (7F4)	Violet brown (10F5)
Dragendroff's Reagent	Brown (7E8)	Dark brown (7F4)	Greyish brown (7F3)
Phloroglucinol	Reddish brown (8D7)	Dark brown (9F5)	Reddish brown (9E5)
Barfoed's Reagent	Dark brown (9F5)	Dark brown (7F4)	Dark ruby (12F5)

Organoleptic evaluation of the powdered sample

Organoleptic study was conducted with the dried sieved powder. The powder was dark-brown in colour, flavourless, tasteless, and of granular texture.

Fluorescence analysis

Fluorescence analysis is useful in identification of authentic samples and recognizing adulterates and therefore is an important pharmacognostic character. Various chemical constituents only fluoresce upon the application of exciting rays, which do not visibly fluoresce in day light. In addition there are certain chemicals that could be present in drug which exhibits fluorescence in UV light after treatment with different reagents. The drug was allowed to react with different reagents which resulted in fluorescence in UV light by various chemical constituents present in it. As a result fluorescence analysis displayed an array of colours that could be employed for identification of probable classes of compounds in the mushroom (Bhattacharya et al. 2009, Sonibare & Olatubosun 2015). In the present study, powder drug was treated with 5 different chemical reagents which gave characteristics colours when seen under UV light (365 nm and 254 nm) and was compared with colours observed under ordinary light (Table 1).

Quantitative estimation of phytochemicals from methanolic extract

The methanolic extract of the mushroom sample was found to contain phenol as much as 1.5 ± 0.208 µg gallic acid equivalent/mg of extract. Total flavonoid content was determined by using quercetin as standard. The extract contained flavonoid as 3.49 ± 0.450 µg quercetin equivalent/mg of extract. Very negligible amount of β-carotene and lycopene were found such as 0.02 ± 0.005 µg/mg and 0.01 ± 0 µg/mg of the extract respectively. The amount of ascorbic acid found was 2.2 ± 0.424 µg/mg of extract.

High performance liquid chromatographic (HPLC) profile of methanolic extract

HPLC is an efficient technology for the preliminary separation, identification and quantification of constituents in a mixture. Fig. 3 represents a typical HPLC molecular profile of the methanolic extract from *Psathyrella spadicea.* The chromatogram indicated presence of at least 15 phenolic components (Table 2). Out of these, only two phenolics *viz.*, ferulic acid (11.85 min) and pyrogallol (16.094 min) (Fig. 3) were identified by comparing with previously run standards (Khatua et al. 2015).

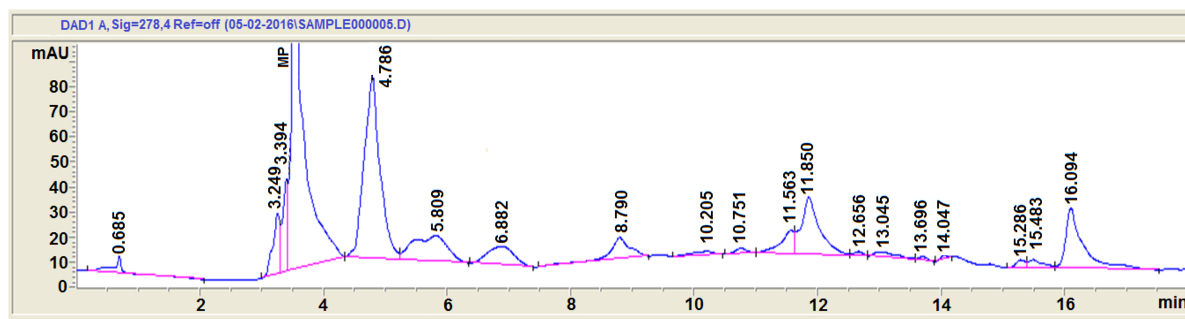

Fig. 3 – Enlarged HPLC chromatogram of methanolic extract from *Psathyrella spadicea*. (MP = Mobile Phase)

Table 2 HPLC chromatogram of methanolic extract from *Psathyrella spadicea* at 278 nm

Peak no.	R_f (min)	Area (mAU)	Height (mAU)
1	4.786	1303.95	73.068
2	5.809	391.506	10.189
3	6.882	218.271	7.358
4	8.790	189.784	8.459
5	10.205	38.353	1.444
6	10.751	24.696	1.995
7	11.563	141.627	9.164
8	11.850	460.141	22.734
9	12.656	16.034	1.617
10	13.045	54.176	2.1
11	13.696	15.775	1.633
12	14.047	12.611	1.606
13	15.286	33.905	3.225
14	15.483	57.051	3.323
15	16.094	482.416	24.26

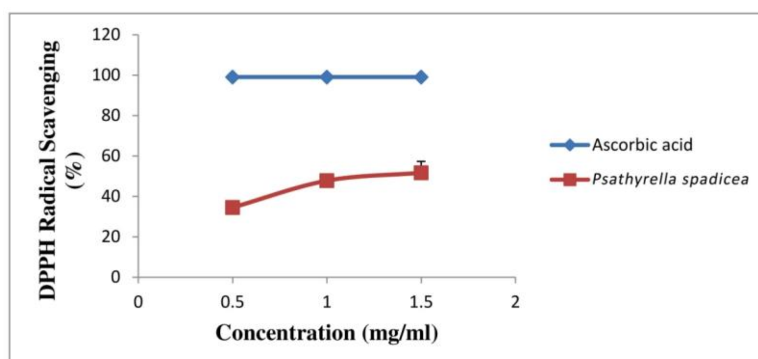

Fig. 4 – DPPH radical scavenging activity of methanol extract from *Psathyrella spadicea*. Results are presented as the mean ± SD of three separate experiments, each in triplicate.

Antioxidant capacity of methanolic extract

In order to determine the antioxidant activity the methanolic extract was investigated by three different in-vitro antioxidant assay systems. DPPH is a stable free radical that does not disintegrate in methanol. The efficacy of the extract depends on the ability of antioxidant compounds present in the extract to lose hydrogen and the structural conformation of this compound (Shimada et al. 1992, Fukumoto & Mazza 2000). DPPH free radical can easily receive an electron or hydrogen from antioxidant molecules to form a stable diphenylhydrazine molecule (Soares et al. 1997). Results indicated that with increasing concentration of the methanolic extract an increasing rate of discolouration signifies to the decreasing quantity of the DPPH radical in the environment. Optical density was measured at 517 nm. The antioxidant activity of the methanolic extract was compared with ascorbic acid. EC_{50} value of the methanolic extract was found to be at 1.31 mg/ml (Fig. 4). Recent investigation on EC_{50} values of DPPH radical scavenging activity by the methanolic extracts of *Laetiporus sulphureus* (Acharya et al. 2016), *Grifola frondosa* (Acharya et al. 2015a), *Lentinula edodes* (Acharya et al. 2015b), *Macrocybe crassa* (Acharya et al. 2015c), were 0.11 mg/ml, 0.66 mg/ml, 1.25 mg/ml and 2.45 mg/ml respectively.

Total Antioxidant Capacity was evaluated by phosphomolybdenum method where reduction of Mo(VI) to Mo(V) takes place by the antioxidant compound and the formation of green phosphate/Mo(V) complex at acidic pH. Total antioxidant capacity of the methanolic extract was investigated and compared against ascorbic acid. The extract presented 15 ± 1.44 µg AAE/ mg of extract total antioxidant activity. Antioxidant activity with variable amount has also been reported in several edible mushrooms including *Grifola frondosa* (18.64 ± 5.28 µg AAE/mg of extract), *Macrocybe crassa* (7.4 ± 1.24 µg AAE/mg of extract), *Lentinula edodes* (13.996 ± 0.285 µg AAE/mg of extract), *Laetiporus sulphureus* (18.01 ± 1.55 µg AAE/mg of extract), (Acharya et al. 2015a, Acharya et al. 2015b, Acharya et al. 2015c, Acharya et al. 2016).

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) forms relatively stable radical cation ($ABTS^{\cdot+}$) upon one-electron oxidation and the assay is based on the inhibition of the formation of this $ABTS^{\cdot+}$ by one electron oxidants. The methanolic extract of the sample showed 7.188 ± 1.169 µM Trolox equivalents (TE)/mg of extract antioxidant activity.

Conclusion

This paper reports important diagnostic characters of *Psathyrella spadicea* that may be employed in the authentication of the species. Various parameters such as molecular characterization, HPLC, antioxidant activity, fluorescence analysis were studied. The results indicate that examined mushroom extract exhibited a substantial amount of antioxidant activity with significant amounts of flavonoids, ascorbic acid, β-carotene, total phenolics and high ability to scavenge DPPH radical. Therefore, this mushroom can be used as an easily available source of natural antioxidants and to treat various oxidative stresses related diseases.

Acknowledgement

The authors are grateful to the Head, Department of Botany, University of Jammu, Jammu for providing laboratory facilities. The first author also wishes to acknowledge the financial support received from University Grants Commission (UGC), New Delhi, in the form of Rajiv Gandhi National Fellowship.

References

Acharya K, Bera I, Khatua S, Rai M. 2015a – Pharmacognostic standardization of *Grifola frondosa.* Der Pharmacia Lettre 7(7), 72–78.

Acharya K, Ghosh S, Khatua S, Mitra P. 2016 – Pharmacognostic standardization and antioxidant capacity of an edible mushroom *Laetiporus sulphureus.* Journal fur Verbraucherschutz und Lebensmittelsicherheit 11, 33–42.

Acharya K, Khatua S, Sahid S. 2015b – Pharmacognostic standardization of *Macrocybe crassa*: An imminent medicinal mushroom. Research Journal of Pharmacy and Technology 8(7), 860–866.

Acharya K, Mukherjee S, Mitra P. 2015c – Pharmacognostic standardization of *Lentinula edodes*: A widely explored medicinal mushroom. International Journal of Pharmacognosy and Phytochemical Research 7(4), 866–872.

Bhattacharya S, Zaman MK. 2009 – Pharmacognostical evaluation of *Zanthoxylum nitidum* bark. International Journal of PharmTech Research 1(2), 292–298.

Chatterjee A, Acharya K. 2016 – Include mushroom in daily diet- A strategy for better hepatic health. Food Reviews International 32(1), 68–97.

Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. 2014 – Chemopreventive effect of *Tricholoma giganteum* against benzo[a] pyrene-induced forestomach cancer in Swiss albino mice. International Journal of Pharmaceutical Sciences Review and Research 26(2), 189–196.

Chatterjee S, Saha GK, Acharya K. 2011 – Antioxidant activity of extracts obtained by different fractions from *Tricholoma giganteum* basidiocarps. Pharmacologyonline 3, 8897.

Dutta AK, Chandra S, Pradhan P, Acharya K. 2014 – A new species of *Marasmius* sect. *Sicci* from India. Mycotaxon 128, 117–125.

Dutta AK, Wilson AW, Antonin V, Acharya K. 2015 – Taxonomic and phylogenetic study on gymnopoid fungi from Eastern India.I. Mycological Progress 14, 79. DOI 10.1007/s11557-015-1094-3

Fukumoto LR, Mazza G. 2000 – Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry 48(8), 3597–3604.

Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat J, Buyck B, Cai L, Dai YC, Adb-Elsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bakhkali AH, Karunarathna SC, Liu JK, Luangsa-ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo JM, Ghobad-Nejhad M, Nilsson H, Pang KL, Pereira OL, Phillips AJL, Raspe O, Rollins AW, Romero AI, Etayo J, Selcuk F, Stephenson SL,Suetrong S, Taylor JE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen TC, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena RS, Li WJ, Perera RH, Phookamsak R, Silva NI de, Thambugala KM, Tian Q, Wijayawardene NN, Zhao RL, Zhao Q, Kang JC and Promputtha I. 2015 – The faces of fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.

Khatua S, Dutta AK, Acharya K. 2015 – Prospecting *Russula senecis*: A delicacy among the tribes of West Bengal. Peer J 3, e810. DOI 10.7717/peerj.810.

Khatua S, Paul S, Acharya K. 2013 – Mushroom as the potential source of new generation of antioxidant: a review. Research Journal of Pharmacy and Technology 6, 496–505.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008 – Ainsworth and Bisby's Dictionary of the Fungi. 10th ed. CAB International, Wallingford, UK. 771 p.

Kokashi CJ, Kokashi RJ, Slama FJ. 1958 – Fluorescence of powdered vegetable drugs under ultraviolet radiation. Journal of the American Pharmaceutical Association 47, 715–717.

Kornerup A, Wanscher JH. 1978 – Methuen handbook of colour. 3rd ed. Eyre Methuen, London, UK. 252 p.

Maity P, Nandi AK, Sen IK, Pattanayak M, Chattopadhyay S, Dash SK, Roy S, Acharya K, Islam SS. 2014 – Heteroglycan of an edible mushroom *Entoloma lividoalbum*: Structural characterization and study of its protective role for human lymphocytes. Carbohydrate Polymers 114,157–165.

Maity P, Sen IK, Maji PK, Paloi S, Devi KSP, Acharya K, Maiti TK, Islam SS. 2015 – Structural, Immunological, and antioxidant studies of β-glucan from edible mushroom *Entoloma lividoalbum.* Carbohydrate Polymers 123, 350–358.

Mallick S, Dey S, Mandal S, Dutta A, Mukherjee D, Biswas G, Chatterjee S, Mallick S, Lai TK, Acharya K, Pal C. 2015 – A novel triterpene from *Astraeus hygrometricus* induces reactive oxygen species leading to death in *Leishmania donovani*. Future Microbiology 10(5), 763–789.

Nagata M, Yamashita I. 1992 – Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Japanese Society for Food Science and Technology (Nippon Shokuhin Kogyo Gakkaishi) 39, 925–928.

Nandi AK, Sen IK, Samanta S, Maity K, Devi KSP, Mukherjee S, Maiti TK, Acharya K, Islam SS. 2012 – Glucan from hot aqueous extract of an ectomycorrhizal edible mushroom, *Russula albonigra* (Krombh.) Fr.: Structural characterization and study of immunoenhancing properties. Carbohydrate Research 363, 43–50.

Park YK, Koo MH, Ikegaki M, Contado JL. 1997 – Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arquivos de Biologia e Tecnologia 40, 97–106.

Prieto P, Pineda M, Aguilar M. 1999 – Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 269, 337–334.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999 – Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free radical Biology and Medicine 26(9–10), 1231–1237.

Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithra DJ, Vijay KHT, Kekuda TRP. 2012 – Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chemical Science Transactions 1, 303–310.

Shimada K, Fujikawa K, Yahara K, Nakamura T. 1992 – Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40, 945–948.

Singleton VL, Rossi Jr JA. 1965 – Colorimetry of total phenolics with phosphomolybdiophosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144–158.

Soares JR, Dins TCP, Cunha AP, Almeida LM. 1997 – Antioxidant activity of some extracts of *Thymus zygis*. Free Radical Research 26, 469–478.

Sonibare MA, Olatubosun OV. 2015 – Pharmacognostic and free radical scavenging evaluation of *Cyathula prostata* (Blume) L. Pharmacognosy Journal 7(2), 107–116.

White TJ, Bruns T, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. Innis MA, Gelfand DH, Shinsky JJ, White TJ (ed), Academic Press, San Diego. 315–322 p.