

Total Phenolic Contents and Antioxidant Effects of Infusion And Decoction From *Lepista sordida* (Schumach.) Singer

Krishnendu ACHARYA[°], Sandipta GHOSH^{*}, Rhituparna BISWAS^{*}

Total Phenolic Contents and Antioxidant Effects of Infusion And Decoction From Lepista sordida (Schumach.) Singer

Lepista sordida (Schumach) 'dan İnfüzyon ve Dekoaksiyonun toplam fenolik içeriği ve antioksidan etkileri

SUMMARY

Traditionally mushrooms have been used as an essential part of human health and nutrition as well as resource of bioactive metabolites. *Lepista sordida* is one of the well-known medicinal mushrooms, however there is no report regarding its bioactivity of aqueous extracts. The present study first aims at investigating its mycochemical composition and free radical scavenging efficacy of aqueous formulations prepared from dried powdered wild basidiocarps. Fresh fruiting bodies of *L. sordida* were collected, dried and two aqueous fractions; infusion and decoction were obtained from powdered fruiting bodies following standard protocols. The extracts were subjected for phytochemical analysis and determination of free radical scavenging potentials in different in vitro systems that includes DPPH and ABTS radical scavenging assays, reducing power assay, chelating ability and total antioxidant activity. Phytochemical composition revealed that infusion fraction was the most enriched preparation with high content of phenolics (6.35 µg/mg of extract) and ascorbic acid (7.72 µg/mg of extract). Furthermore, in vitro experiments for antioxidant activity also validate the quantitative data. Antioxidant screening showed that infusion extract had noticeable radical scavenging activity in concentration dependent manner while decoction exhibited moderate activity. Total antioxidant capacity was found to be similar in both extracts. Altogether results provide a supplementary idea about therapeutic application of this taxon on large scale basis that it could be explored as powerful alternatives of synthetic antioxidants.

Key Words: Antioxidant, decoction, infusion, macrofungi, phytochemicals, phenolics.

ÖZET

Geleneksel olarak, mantarlar insan sağlığı ve beslenmesinin önemli bir parçası olmanın yanı sıra biyoaktif metabolitlerin bir kaynağı olarak kullanılmıştır. *Lepista sordida*, iyi bilinen tıbbi mantarlardan biridir, bununla birlikte sulu ekstraktların biyoaktivitesi ile ilgili herhangi bir rapor bulunmamaktadır. Bu çalışmanın amacı, kuru toz halinde basidiokarplardan hazırlanan sulu formülasyonların fitokimyasal kompozisyonu ve serbest radikal süpürme etkinliğini araştırmaktır. *L. sordida*'nın taze meyve kısımları toplandı, kurutuldu ve standart protokollere göre toz haline getirildikten sonra sulu fraksiyon; infüzyon ve dekoaksiyon elde edildi. Ekstraktlarda fitokimyasal analiz ve farklı in vitro sistemlerde DPPH ve ABTS testleri şelat yeteneği ve toplam antioksidan aktivite testleri ile serbest radikal süpürücü potansiyelleri tayin edilmiştir. Fitokimyasal kompozisyon, en yüksek infüzyon fraksiyonunun fenolik içeriğinin (6.35 µg / mg özüt) ve askorbik asitin (7.72 µg / mg özüt olduğunu ortaya çıkarmıştır. Ayrıca, in vitro antioksidan aktivite deneyleri de niceliksel verileri doğrulamaktadır. Antioksidan tarama, infüzyon özütün konsantrasyona bağlı olarak belirgin bir radikal süpürme aktivitesine sahipken, kaynatmanın orta derecede aktivite gösterdiğini gösterdi. Toplam antioksidan kapasitesinin her iki ekstraktta da benzer olduğu bulunmuştur. Sonuçlar, bu tür sentetik antioksidanların güçlü alternatifleri olarak araştırılabileceği, geniş ölçekte terapötik uygulanması hakkında bir fikir vermektedir.

Anahtar Kelimeler: Antioksidan, kaynatma, demleme, makrofungus, fitokimyasallar, fenolik.

Received: 02.11.2017

Revised: 29.12.2017

Accepted: 02.01.2018

^{*} Molecular and Applied Mycology and Plant Pathology Laboratory, Department of Botany, University of Calcutta, 35, Ballyunge Circular Road, Kolkata 700019, West Bengal, India

[°] Corresponding Author; Krishnendu ACHARYA
E-mail; krish_paper@yahoo.com

INTRODUCTION

From ancient times, macrofungi (popularly known as mushrooms) are recognized as nature's ultimate "superfood" that constitute a massive source of bioactive compounds including phenols, polysaccharides, terpenes, tocopherols etc (Kalac, 2013). Discovery of their therapeutic activities is now being a diversified area of research, for their exploitation in medicinal field as a potent nutraceutical. Mushrooms are eminently appreciated to all over the world due to its high nutritional value along with their unique taste and flavour. Many European and Asian countries have also started paying attention towards the usage of mushroom in commercial basis to fulfill the crisis of healthy food as they are enriched with high quality fibers, carbohydrates, proteins, minerals and many other bioactive secondary metabolites (Barros et al., 2008; Acharya et al., 2016b).

In recent times, tissue injuries and illnesses induced by free radicals have become a major part of our common health problems. Although all the living organisms are well-protected against such injuries with their in-built radical scavenging systems including antioxidant enzymes, vitamins etc., sometimes the balance deteriorates which results in oxidative stress (Halliwell, 1996). Therefore, it is a high time to explore new dietary supplements with potent antioxidant activity as well as few or no side effects from natural resources that could be beneficial to combat such disorders. Among them, mushrooms have become fruitful resources of natural antioxidant compounds (Stojkovic et al., 2017; Acharya et al., 2016d).

Lepista sordida (Schumach.) Singer is a well-known delicious member of agaric group (Family-Tricholomataceae), distributed mainly in Asian and European countries. Commonly it is called flesh-brown blewit, implying their brown shaded pileus cover easily distinguishable between *L. nuda* and *L. personata* (Thongbai et al., 2016). Several literatures have reported remarkable biological activities of this mushroom such as antitumor, antimicrobial, antioxidant activities etc in both *in vitro* and *in vivo* experiments (Thongbai et al., 2016). Lepistol and lepestol, two anticancer diterpenoids have been isolated from submerged mycelial culture that influences differentiation of human leukemia cells (Thongbai et al., 2016). In addition, Luo et al. had evaluated immunomodulatory efficacy of a water soluble polysaccharide purified from cultivated species of China that exhibited strong activating effect on macrophages by upsurging the expression of

NO and TNF- α (Luo et al., 2012). Merely no studies have been documented regarding pharmacological activities of wild species and its different formulations especially the most commonly used herbal preparations; infusion and decoction. Hence the present study has been attempted to evaluate different major mycochemicals present in *L. sordida* decoction and infusion along with antioxidative potentials of this two fractions.

MATERIAL AND METHODS

Materials

Folin-Ciocalteu reagent, 2, 6-dichlorophenol indophenol dye (DCPIP), acetone, hexane, methanol, aluminium nitrate, sodium acetate, sodium carbonate (Na_2CO_3), oxalic acid, trichloroacetic acid (TCA), sulphuric acid (H_2SO_4), sodium sulphate, ammonium molybdate, potassium ferricyanide, sulphuric acid, ferrozine, ferric chloride (FeCl_3), ferrous chloride (FeCl_2), 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, ascorbic acid, ethylenediaminetetraacetic acid (EDTA), BHA, Trolox, gallic acid, quercetin, were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All chemicals were of analytical reagent grade.

Instrument:

Bio-Rad iMark™ Microplate Reader (USA) was used for absorbance measurement of 96 well plate. The instrument consists of an eight-channel, vertical path length photometer that reads at specific wavelengths such as 415, 450, 490, 595, 655, and 750 nm in 96-well microtiter plate.

Mushroom collection

Fresh fruiting bodies of wild *L. sordida* were collected from Gangetic plains of Murshidabad district of West Bengal, India. After collection they were brought to the laboratory and key identifying morphological features were determined with the help of standard literatures (Pegler, 1986). A voucher specimen was preserved in CUH herbarium (CUHAM165) following the protocol of Pradhan et al. (2015). Fruiting bodies were dried by a field drier at 40°C for overnight and then blended through mixer grinder to obtain fine powder and stored in fresh container for further use.

Extraction procedures

Decoction was prepared according to protocol by Guimarães et al. (2012). Briefly, 0.1 g dried powder was added in 20 mL distilled water and boiled for 5 min on heating plate. The mixture was then left to stand for 5 min after that it was filtered through Whatman filter paper. The filtrate was stored at 4°C

for experimental analysis, not more than 2 d.

To prepare infusion extract, 0.1 g dried powder was mixed in 20 mL boiling distilled water and the mixture was maintained for 5 min and then filtered using Whatman filter paper (Guimarães et al., 2012). The filtrate was stored in similar condition.

Phytochemicals estimation

Freshly prepared extracts; decoction and infusion were subjected for quantification of major phytochemicals present in fruiting bodies of this mushroom. Phenol content was determined using Singleton and Rossi protocol with the help of Folin-Ciocalteu reagent where gallic acid used as standard (Singleton and Rossi, 1965). Flavonoid content was measured as per the protocol of Adebayo et al. using quercetin (5–20 µg/mL) as standard (Adebayo et al., 2012). Estimation of ascorbic acid, β-carotene and lycopene contents was performed according to our previous publication (Khatua et al., 2015).

Antioxidant assays

DPPH radical scavenging assay

DPPH radical scavenging potentiality of extracts was evaluated following the method of Pereira et al. (2012). Values were represented with respect to ascorbic acid standard as percentages of DPPH radical discoloration at 575 nm. EC₅₀ values were calculated indicating the concentration where 50% DPPH radicals were scavenged. Percentage of scavenging activity was determined using the following equation: Scavenging percentage (%): $[A_{\text{DPPH}} - A_{\text{Sample}}] \times 100$. A_{DPPH} is the absorbance of DPPH solution and A_{Sample} is the absorbance of the solution containing various concentrations of extract.

Reducing power assay

For determination of reducing power ability of the extracts, the following method was employed (Khatua et al., 2017). 10 µL sample solution with different concentrations of extracts (500–4500 µg/mL) was mixed with 25 µL Na-phosphate buffer (0.2 M, pH 6.6) and freshly prepared 25 µL potassium ferricyanide solution (1%) in a 96 well microtiter plate. After 20 min incubation at room temperature, 85 µL water and 8.5 µL FeCl₃ were added in each well and optical density was measured at 750 nm through microplate reader as mentioned in previous assay. As positive control, ascorbic acid was used. EC₅₀ was evaluated at the concentrations exhibiting 0.5 absorbance value.

ABTS radical scavenging assay

ABTS radical scavenging activity of the mushroom extracts was spectrophotometrically estimated according to the method of Khatua et al. (2017).

ABTS^{•+} reactive cation radical was formed by mixing equal amount of 7 mM ABTS dissolved in water and 2.45 mM potassium persulfate and then the mixture was stored for 12–16 h in dark condition at room temperature. Before use, ABTS^{•+} solution was diluted to obtain an absorbance of 0.700 at 734 nm. Reaction sets (0.2 mL) were prepared for various concentrations of extract (20–160 µg/mL) along with respective amounts of ABTS^{•+} solution in a 96 well plate and incubated for 10 min in dark. Absorbance was taken at 750 nm in microplate reader. Trolox was used as standard and ABTS^{•+} radical scavenging activity (%) and EC₅₀ was determined from calibration curve.

Chelating ability of ferrous ions

Ferrous ion (Fe²⁺) chelating ability of the extracts was measured following the protocol of Khatua et al., (2017). Reaction mixtures (0.2 mL) comprised of 5 µL FeCl₂ (3 nM) and different concentrations of each extract (100–300 µg/mL) followed by corresponding volumes of water. Reaction was commenced after addition of 10 µL ferrozine (0.12 nM). After 10 min incubation period, the absorbance of testing solutions was measured at 595 nm in microplate reader and EC₅₀ values were calculated. EDTA used as standard. Results were represented as percentage of suppression of Fe²⁺–ferrozine complex formation.

Total antioxidant activity

The assay was performed as reported by Prieto et al. (1999). Reaction mixture (3.3 ml) contained 0.3 mL sample solution and 3 mL freshly prepared reagent solution (0.6 M sulphuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate). Blank was prepared with the addition of 0.3 mL water in place of sample solution. Tubes were incubated at 95°C for 90 min and after cooling down absorbance were measured at 695 nm against blank. Ascorbic acid (1–30 µg/mL) was used to prepare standard curve and values were expressed as the number of equivalents of ascorbic acid.

Statistical analysis

All assays were expressed as mean values and standard deviation (SD). Data analysis was done using Microsoft Office Excel (Microsoft®, USA). Results were compared by ANOVA to determine variances among samples and values of p ≤ 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

After grinding, dried powder appeared in brown colour, granular and without any significant odour (Figure 1). Traditionally, most of the herbal preparations are relied on the simplest extraction methodologies of infusion and decoction. By these

processes active constituents of herbal drugs easily get incorporated into hot water which is considered as an integral part of Ayurveda therapies. In the present study, two fractions such as decoction and infusion were prepared from dried basidiocarps of *L. sordida* and subjected for quantification of phytochemicals that are major constituents of edible macrofungi. A comparative evaluation regarding different phytochemical contents of these extracts has been presented in Table 1. Results demonstrated that the infusion fraction was comparatively enriched in phenols and ascorbic acid with respect to decoction. The observation was found to be similar as reported by Guimarães et al. (2012) who evaluated chemical composition of infusion and decoction fractions obtained from wild German. They have found phenolics as major bioactive compound of infusion fraction, although decoction also contained noticeable amount of phenols. Quantity of different phytochemicals is less in decoction than infusion indicating infusion is a better extractive method instead of boiling in water. Similar observation was reported in earlier publication by Fotakis et al. (2016) who demonstrated that increasing boiling time in decoction preparation reduce metabolites concentration as well as their relative biological activity. However phenol content

was remarkably higher than methanolic fractions obtained from dried basidiocarps of *Macrocybe crassa* which account for 3.31 ± 0.09 μg gallic acid equivalent/mg of extract (Acharya et al., 2015b). Phenolics are ubiquitous in all plants as well as mushrooms, therefore, an integral part of human diet. High content of phenolics and its derivatives in mushrooms are usually associated with significant health promoting benefits. Similarly infusion contained higher amounts of flavonoids than decoction which are considered as one of the bioactive compounds of mushrooms having remarkable antioxidant and anti-inflammatory properties (Akyüz, 2013). As it is known to all that, several medicinal benefits of mushroom such as antioxidant, anti-inflammatory, immunomodulatory potentialities exhibit positive correlation with polyphenols, flavonoids and ascorbic acid contents (Walter and Marchesan, 2011). Being dietary carotenoids, lycopene and β -carotene are mostly studied for their unique role as radical scavenger and besides that they are also reported to reduce chances of oxidative stress related disorders (Fiedor and Burda, 2014). Therefore, aforementioned results are indicative to the therapeutic potentials of this macrofungus.

Table 1: Quantitative estimation of phytochemicals decoction and infusion, extracted from powdered basidiocarps of *L. sordida*.

| Phytochemicals ($\mu\text{g}/\text{mg}$) | Decoction | Infusion |
|--|------------------------|------------------------|
| Phenol | 3.725 ± 0.01^a | 6.35 ± 0.02^b |
| Flavonoid | 0.132 ± 0.42^a | 0.22 ± 0.15^b |
| Ascorbic acid | 2.76 ± 0.05^a | 7.275 ± 0.09^a |
| β carotene | 0.00219 ± 0.12^a | 0.003094 ± 0.02^b |
| Lycopene | 0.0004619 ± 0.01^a | 0.0006727 ± 0.21^a |

Results are mean \pm SD, n=3 per group. Within the same row, different letters are significantly different at $p \leq 0.05$.

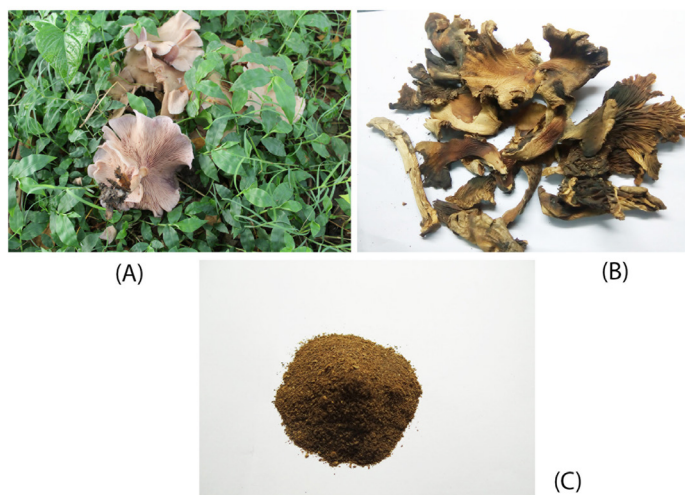


Figure 1: *L. sordida* (A) Fresh basidiocarps (B) Dried basidiocarps (C) Powder

Thereafter free radical scavenging potential of *L. sordida* infusion and decoction was demonstrated using five different *in-vitro* systems such as DPPH and ABTS radicals scavenging activity, chelating activity, reducing power assay and total antioxidant activity.

DPPH radical scavenging assay is recognized as one of the easiest method to detect antioxidant potentiality of the examined compounds. The principle of this assay is usually laid on the hydrogen/electron donating ability of the antioxidant compounds to the reactive free radical, DPPH for stabilization that resulted in the decolourization of the violet coloured DPPH solution (Acharya et al., 2015a). The present examination revealed that infusion fraction showed highest radical scavenging activity ($p < 0.05$) at the rate of 20.53%, 51.33% and 71.86% in 120 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$ and 280 $\mu\text{g/mL}$ concentrations respectively probably due to high ascorbic acid and phenolic content of this fraction whereas decoction extract showed moderate scavenging activity (Figure 2A). EC_{50} values are presented in Table 2. Although these fractions have demonstrated good radical scavenger of DPPH radical with EC_{50} values significantly lower than methanolic extract of *L. sordida* (EC_{50} - 9.82 mg/mL) (Heleno et al., 2010), *Schizophyllum commune* (EC_{50} - 1.07 mg/mL) (Acharya et al., 2016a) and *Lentinus sajor-caju* (EC_{50} - 0.43 mg/mL) (Acharya et al., 2017). However, in contrast an intracellular polysaccharide was isolated from mycelial culture of this fungus which also showed remarkable DPPH radical scavenging potentials and increment in the level of antioxidant enzymes after dose dependent application on experimental mice model (Zhong et al., 2013).

Reducing power of the tested fractions was assessed by measuring the ability of antioxidant compounds to transform Fe^{2+} - Fe^{3+} and thereby acting as reductones (Mitra et al., 2014). The present examination revealed that infusion extract (EC_{50} - 3010 \pm 2.5 $\mu\text{g/mL}$) showed

better reducing capability than decoction (EC_{50} - 3440 \pm 1.75 $\mu\text{g/mL}$) (Figure 2B). Similar result was also observed by another research group who evaluated antioxidant activity of similar fractions obtained from *Achillea millefolium* representing infusion acted as better reductones than methanol and decoction extract (Dias et al., 2013).

ABTS radical scavenging method is based on the ability of antioxidant compounds to reduce $\text{ABTS}^{+\cdot}$ that reflected on the visible discoloration of the green coloured $\text{ABTS}^{+\cdot}$ solution, equally relevant for both lipid soluble as well as water soluble antioxidant compounds. As shown in Figure 2C, decoction exhibited highest scavenging potentials compared to the infusion extract. Herein, decoction extract remarkably quenched about 74% $\text{ABTS}^{+\cdot}$ at 100 $\mu\text{g/mL}$ whereas infusions scavenged only about 57% radical at 80 $\mu\text{g/mL}$ concentrations ($p < 0.01$). Although the observations well related with the high level of phenols in the corresponding extract.

Ferrous ion (Fe^{2+}), being a strong transition metal, possesses the ability of single electron donation thereby stimulates free radical formation and also regarded as powerful prooxidant in comparison with other metal ions (Acharya et al., 2016c). Chelators are secondary antioxidants capable of stabilizing metal ions and cause breakage in free radical chains. In present experiment chelating activity of extracts were evaluated by ferrozine assay. Activity was based on colour reduction from violet to yellow colour in the presence experimental chelating agents which was compared against standard, EDTA. As shown in representation, with increasing concentrations of each extract, chelating potentials of both extracts were markedly increased (Figure 2D). Although decoction extract exhibited highest chelating activity (69.8%) at the maximum concentration of 700 $\mu\text{g/mL}$ and at the same concentration infusion fraction chelated metal ion about 57%.

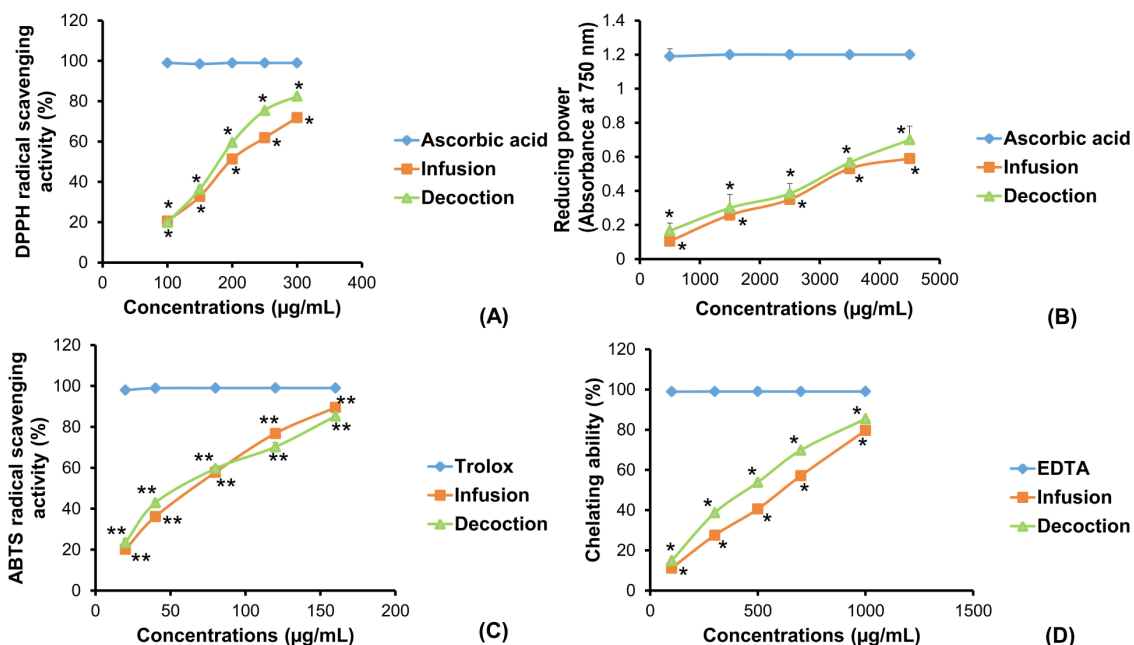


Figure 2: Antioxidant activity (A) DPPH radical scavenging activity (B) Reducing power (C) ABTS radical scavenging activity (D) Chelating activity. Data shown mean and S.D. of 4 experiments with *p<0.05 compared with control, **p<0.01 compared with control, n=5 per group.

Table 2: EC₅₀ values (µg/ml) of *in vitro* antioxidant assays.

| Antioxidant assays | Decoction | Infusion |
|-------------------------------|------------------------|-----------------------|
| DPPH radical scavenging assay | 210±2.01 ^a | 200±1.5 ^b |
| Chelating assay | 410±1.2 ^a | 610±0.54 ^a |
| Reducing Assay | 3440±1.75 ^a | 3010±2.5 ^a |
| ABTS radical scavenging assay | 50±0.08 ^a | 60±1.08 ^a |

Results are mean±S.D., n=5. Within the same row, different letters are significantly different at p≤0.05.

Total antioxidant capacity of the fractions was evaluated on the basis of phosphomolybdenum method by measuring the intensity of green phosphate /Mo (V) complex which is formed due to reduction of Mo (VI) to Mo (V) by the antioxidant compounds. Total antioxidant capacity of infusion and decoction extracts of *L. sordida* was investigated and compared against ascorbic acid. Extracts exhibited 1.5±0.61 µg AAE/mg of extract and 1.8±1.05 µg AAE/mg of extract total antioxidant activity in decoction and infusion respectively.

CONCLUSION

Thus the above investigation highlights *L. sordida* as a valuable species due to its significant nutraceutical composition along with remarkable radical scavenging potentiality. However decoction method exhibited moderate activity indicating phenolic compounds may be affected by this procedure. The investigation adds to the existing knowledge of this mycotaxon and recommends its application in the development

of crude drug formulation that could be successfully exploited for treatment of oxidative disorders.

CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

Acharya, K., Bera, M., Tarafder, E., Dasgupta, A. (2015a), Pharmacognostic standardization of *Ganoderma lucidum*: A commercially explored medicinal mushroom, *Der Pharmacia Lettre*, 7, 7, 175-181.

Acharya, K., Ghosh, S., Dutta, A.K. (2016a), Pharmacognostic standardization based on physicochemical and molecular parameters of a medicinal mushroom *Schizophyllum commune*, *Orient Pharm Exp Med*, 16, 259-266.

Acharya, K., Ghosh, S., Ghosal, S. (2016b), Pharmacognostic standardization of a widely explored medicinal mushroom, *Pleurotus ostreatus*, *Der Pharmacia Lettre*, 8, 6, 66-72.

- Acharya, K., Ghosh, S., Khatua, S., Mitra, P. (2016c), Pharmacognostic standardization and antioxidant capacity of an edible mushroom *Laetiporus sulphureus*, *J Verbr Lebensm*, 11, 33-42.
- Acharya, K., Ghosh, S., Kundu, I. (2016d), Pharmacognostic standardization of a well-known edible mushroom, *Volvariella volvacea*, *J Appl Pharm Sci*, 6,11, 185-190.
- Acharya, K., Ghosh, S., Saha, T. (2017), Physicochemical characterization and antioxidant property of powdered basidiocarp of wild *Lentinus sajor-caju*, *Int J ChemTech Res*, 10, 1, 126-1327.
- Acharya, K., Khatua, S., Sahid, S. (2015b), Pharmacognostic standardization of *Macrocybe crassa*: An imminent medicinal mushroom, *Research J Pharm and Tech*, 8, 7, 860-866.
- Adebayo, E.A., Oloke, J.K., Ayandele, A.A., Adegunlola, C.O. (2012), Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius*-LAU 09 (JF736658), *J Microbiol Biotech Res*, 2, 366-374.
- Akyüz, M. (2013), Nutritive value, flavonoid content and radical scavenging activity of the truffle (*Terfezia boudieri* Chatin), *J Soil Sci Plant Nutr*, 13, 1, 143-151.
- Barros, L., Cruz, T., Baptista, P., Estevinho, L.M., Ferreira, I.C.F.R. (2008), Wild and commercial mushrooms as source of nutrients and nutraceuticals, *Food Chem Toxicol*, 46, 2742-2747.
- Dias, M.I., Barros, L., Dueñas, M., Pereira, E., Carvalho, A.M., Alves, R.C., Oliveira, M.B.P.P., Santos-Buelga, C., Ferreira, I.C.F.R. (2013), Chemical composition of wild and commercial *Achillea millefolium* L. and bioactivity of the methanolic extract, infusion and decoction, *Food Chem*, 141, 4152-4160.
- Fiedor, J., Burda, K. (2014), Potential role of carotenoids as antioxidants in human health and disease, *Nutrients*, 6, 2, 466-488.
- Fotakis, C., Tsigrimani, D., Tsiaka, T., Lantzouraki, D.Z., Strati, I.F., Makris, C., Tagkouli, D., Proestos, C., Sinanoglou, V.J., Zoumpoulakis, P. (2016), Metabolic and antioxidant profiles of herbal infusions and decoctions, *Food Chem*, 211, 963-971.
- Guimarães, R., Barros, L., Dueñas, M., Calhella, R.C., Carvalho, A.M., Santos-Buelga, C., Queiroz, M.J.R.P., Ferreira, I.C.F.R. (2012), Infusion and decoction of wild German chamomile: Bioactivity and characterization of organic acids and phenolic compounds, *Food Chem*, 136, 947-954.
- Halliwell, B. (1996), Antioxidants in human health and disease, *Ann Rev Nutr*, 16, 33-50.
- Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Ferreira, I.C.F.R. (2010), Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity, *Food Chem*, 119, 1443-1450.
- Kalac, P. (2013), A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms, *J Sci Food Agric*, 93, 209-218.
- Khatua, S., Dutta, A.K., Acharya, K. (2015), *Russula senecis*: a delicacy among the tribes of West Bengal, *PeerJ*, 3:e810. doi:10.7717/peerj.810.
- Khatua, S., Ghosh, S., Acharya, K. (2017), A simplified method for microtiter based analysis of in vitro antioxidant activity, *Asian J Pharmacol*, 11, 2, S327-S335.
- Luo, Q., Sun, Q., Wu, L., Yang, Z. (2012), Structural characterization of an immunoregulatory polysaccharide from the fruiting bodies of *Lepista sordida*, *Carbohydr Polym*, 88, 820-824.
- Mitra, P., Sarkar, J., Mandal, N.C., Acharya, K. (2014), Phytochemical analysis and evaluation of antioxidant efficacy of ethanolic extract of *Termitomyces medius*, *Int J Pharm Sci Rev Res*, 27, 261-6.
- Pegler, D.N. (1986), Agaric flora of Sri Lanka. Kew Bulletin Additional Series XII, Royal Botanic Gardens, Kew.
- Pereira, E., Barros, L., Martins, A., Ferreira, I.C.F.R. (2012), Towards chemical and nutritional inventory of Portuguese wild edible mushrooms in different habitats, *Food Chem*, 130, 394-403.
- Pradhan, P., Dutta, A.K., Acharya, K. (2015), A low cost long term preservation of macromycetes for fungarium, *Protoc Exch*, doi:10.1038/protex.2015.026.
- Prieto, P., Pineda, M., Aguilar, M. (1999), Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal Biochem*, 269, 2, 337-41.
- Singleton, V.L., Rossi, J.J. (1965), Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am J Enol Viticult*, 16, 144-158.
- Stojkovic, D.S., Kovacevic-Grujicic, N., Reis, F.S., Davidovic, S., Barros, L., Popovic, J., Petrovic, I., Pavi, A., Glamoclija, J., Ciric, A., Stevanovic, M., Ferreira, I.C.F.R., Sokovic, M. (2017), Chemical composition of the mushroom *Meripilus giganteus*

- Karst. and bioactive properties of its methanolic extract, *LWT - Food Science and Technology*, 79, 454-462.
- Thongbai, B., Wittstein, K., Richter, C., Miller, S.L., Hyde, K.D., Thongklang, N., Klomklung, N., Chukeatirote, E., Stadler, M. (2016), Successful cultivation of a valuable wild strain of *Lepista sordida* from Thailand, *Mycol Progress*, doi:10.1007/s11557-016-1262-0.
- Walter, M., Marchesan, E. (2011), Phenolic compounds and antioxidant activity of Rice, *Braz Arch Biol Technol*, 54, 2, 371-377.
- Zhong, W., Liu, N., Xie, Y., Zhao, Y., Song, X., Zhong, W. (2013), Antioxidant and anti-aging activities of mycelial polysaccharides from *Lepista sordida*, *Int J Biol Macromol*, 60, 355-359.