ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 10, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

COLLECTION, IDENTIFICATION, MOLECULAR CHARACTERIZATION, AND ANTIOXIDANT ACTIVITY OF NON-GILLED MUSHROOMS COLLECTED FROM NORTH WESTERN HIMALAYAS

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Received: 21 May 2018, Revised and Accepted: 16 June 2018

ABSTRACT

Objective: The objective of present research deals with collection, taxonomic, molecular taxonomy characterization, and antioxidant activity of wild non-gilled mushrooms sample collected from North Western Himalayas.

Methods: The wild non-gilled mushroom samples were characterized for micro, morphological properties, and molecular identification. 1,1 diphenylpicrylhydrazyl (DPPH), nitric oxide (NO_2), hydrogen peroxide (H_2O_2), and free radical scavenging assay were used to evaluate the antioxidant properties of selected wild non-gilled mushrooms.

Results: The obtained results revealed that on the basis of microscopic and morphological identification all the four non-gilled mushrooms samples were tentatively identified as 2/15 (*Auricularia* sp.1), 6/15 (*Auricularia* sp.2), 32/15 (*Ganoderma* sp.), and 132/15 (*Trametes* sp.). The colony, mycelial, and basidiospore morphology of fungal isolate were evidence of Basidiomycetes family. All these four non-gilled mushrooms were sequenced. Sequences were submitted to National Center for Biotechnology Information (NCBI), and their accession number was MF770159 (2/15), MF774107 (6/15), MF770158 (32/15), and MF770160 (132/15). The phylogenetic analysis of 18S ribosomal deoxyribonucleic acid (18S rDNA) sequence of four non-gilled mushrooms was compared with other species and identified as *Auricularia polytricha, Ganoderma lucidum, Auricularia auricula-judae,* and *Trametes elegans.* The four non-gilled mushrooms extract exhibited DPPH, NO_2, and H_2O_2 free radical scavenging activities. *G. lucidum* methanolic extract has the highest effect on free radicals in comparison to hexane effect.

Conclusion: All the isolated four non-gilled mushrooms showed good antioxidant potential, and it can be concluded that these mushrooms are not only consumed but also have a wide range of medicinal properties which must further explode for future use.

Keywords: Non-gilled mushrooms, Phylogenetic tree, 18S r deoxyribonucleic acid, Antioxidant activity.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i10.27423

INTRODUCTION

Mushroom belongs to a cluster of plants recognized as fungus. These are unit of natural ecosystems that have an essential role in the health-maintaining process. Medicinal mushrooms are either collected from nature or cultivated. They introduced a lot of conferments especially in the consumption of food, in bioremediation, in a cure of diseases, antioxidant, and therapeutic diseases [1]. Antioxidants are compounds which terminate the action of free radicals, thereby protecting the body from oxidative damages [2]. These free radicals are independent chemicals with one or more unpaired electrons and are responsible for biological injury and contribute too many non-communicable diseases [3]. They are constantly formed in the human body during energy production, in the mitochondrial electron transport chain, phagocytizes, arachidonic acid metabolism, ovulation, fertilization, and in xenobiotic metabolism [4]. *Ganoderma* and *Trametes* species were studied for the screening of their therapeutic activities. These studies have shown the enormous pharmacological potential of these mushrooms especially for the management of various types of cancers. One of the most accepted possible mechanisms behind anticancer effects has been suggested to be the free radical scavenging activity. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species are mainly implicated in deoxyribonucleic acid (DNA) damage ultimately leading to uncontrolled cell proliferation. In the living system, nature has provided an endogenous defense mechanism as antioxidants which scavenge the harmful free radicals [5]. The consumption of dietary antioxidants would help to prevent free radical oxidative damage by inhibiting the initiation step or interrupting the propagation step of oxidation damage [6]. Natural antioxidants are characterized into plant and fungal extracts such as spices (rosemary, thyme, marjoram, oregano, sage, basil, pepper, clove, cinnamon, and nutmeg), flavonoids, ubiquinol (fully reduced form of coenzyme Q10), glutathione, zinc (Zn), selenium (Se), Vitamin A (including carotenoids), Vitamin C, and Vitamin E (including tocopherols and tocotrienols) [7]. Synthetic phenolic antioxidants include butylated hydroxyanisole, butylated hydroxytoluene, and others, for example, propyl gallate, and tert-butyl hydroquinone, ethoxyquin that all effectively inhibit oxidation [8]. Many researchers were studied that vitamins and polyphenols from planted sources including fruits, vegetables, grains, roots, tea, coffee, and the wine showed good antioxidant activities, and could protect mitochondria from dysfunction and apoptosis. Recently, it was found that wild mushrooms possessed vitamins and polyphenol compounds and exhibits strong antioxidant activities [9]. Therefore, it is necessary to find new potent antioxidants from other mushroom sources. The present study was undertaken to collect, to identify along with the molecular characterization of non-gilled mushrooms and to determine their effects as antioxidant compounds.

METHODS

Collection of samples

Fruiting body of four non-gilled mushrooms was collected from the North Western Himalayas and marked as collection number 2/15, 6/15, 32/15, and 132/15. These fruiting bodies were taken to the laboratory in sterile polybags. Each sample after cleaning with sterile water was cut across the pileus region with the help of sterilized blade to obtain bits (1–2 mm) of tissue, which was dipped in 0.1% mercuric chloride solution using sterile forceps for 10–15 s. Then, bits were transferred on to the plates of malt extract agar (MAE).

Macroscopic and microscopic identification
The identification of the fruiting body of non-gilled mushrooms was based on macroscopic traits such as shape, size, a color of pileus, presence or absence of stripe and microscopic traits spores, and clamp connection.

Molecular identification
The pure fungal cultures were subcultured under sterile conditions in 250 mL flasks containing 100 mL of malt extract broth at 27°C for 8 days. The growing mycelial in MEA was then filtered through Whatman filter paper No.1 and used for DNA extraction.

Isolation of genomic DNA
Total DNA was extracted according to the methods described by Rajesh *et al*. and Moncalvo *et al*. [10,11] with some modification. A mass of 150 mg of biomass was suspended in 500 µL of extraction buffer (200 mm tris aminomethane hydrochloride (Tris-HCl), pH 8.0, 100 mm sodium chloride (NaCl), 25 mm ethylenediaminetetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate, and vortexes and incubated at 65°C in warm water bath for 25 min. Afterward, the samples were cooled on ice for 5 min. The resulting suspension was extracted with 0.5 mL of phenol: 0.5 mL of chloroform:isoamyl alcohol (24:1) by vortexing for 1 min then tubes were incubated for 15 min at room temperature and followed by centrifugation at 12,000 rpm for 25 min in a microcentrifuge. The upper phase was transferred to a new Eppendorf tube. Then, 350 µL chloroform:isoamyl alcohol (24:1) was added to this Eppendorf tube, mixed by gently inverting and spun at 12,000 rpm for 10 min. The genomic DNA was precipitated from the supernatant by adding 0.54 volume of isopropanol and incubated overnight then the supernatant was discarded. The nucleic acid pellet was washed using 70% ethanol, air dried and resuspended in 50 µL of TE buffer (10 mm Tris-HCl, pH 8.0, and 1 mm EDTA). Then, DNA samples were stored at 20°C.

Extracted genomic DNA by agarose gel electrophoresis
Tris-acetate-ethylene (TAE) diamine tetraacetic acid buffer was prepared. For this, 500 mg of agarose was dissolved in 50 mL of ×0.5 TAE working buffer prepared from ×50 TAE stock solution (240 g Tris base, 57.1 mL glacial acetic acid 100 mL 0.5 M EDTA). Then, the mixture was heated up to agarose gel was dissolved. The agarose solution was cooled at 60°C after that 10 mg/mL ethidium bromide solution was added to the buffer (concentration of 0.5 µg/mL). The solution was poured into the gel. The gel casting tray was sealed on both sides with tape and agarose was poured into the tray. The comb was placed in the gel and allowed to solidify at room temperature for 30–35 min. After solidification, the comb and the tape were removed. The gel tray was placed in the electrophoresis tank. A volume of 2 µL DNA, 1 µL tracking dye, and 7 µL distilled water were mixed well and loaded in well with the help of micropipette. The gel was run at 50 volts for 45–60 min. The electrophoresis was stopped when the samples had run the required distance and gel was visualized under the ultraviolet (UV) illuminator and photographed under UV rays.

Polymerase chain reaction (PCR) amplification
5.8 S rRNA gene was amplified by PCR using universal primer pair of 0.5 µM of each primer, i.e. ITS1 (5'- TCCGGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC -3'). The 50 µL reaction mixture contained the following components: 1 µL template DNA, ×1 PCR buffer, 2.5 units of Taq DNA polymerase, 0.2 mM of deoxyribonucleotide triphosphate mixture, 1.5 mM magnesium chloride ($Mgcl_2$), and sterile water 31.5 µL. Mix the contents for 1–2 s thoroughly. The tubes were placed in thermocycler block. The PCR program was carried out in the following manner of 30 cycles with an initial denaturation step at 94°C for 5 min, followed by denaturation step at 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 2 min. A volume of 5 µL of PCR product with 5 µL of loading dye was mixed and loaded on a 1.5 % agarose gel and PCR product were resolved at 50 V for 45 min. The gel was visualized under UV light and photographed under the gel doc imaging system.

5.8S rRNA Gene sequencing
PCR product was sent to the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, for sequencing.

Phylogenetic tree
The 18rDNA nucleotide sequence was used to carry out Basic local alignment search tool (BLAST). BLAST of the obtained sequence was performed with a database of the gene bank. Based on maximum identity score first, 10 were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using ribosomal database project database and the phylogenetic tree was constructed using Mega 6 software.

1,1 diphenylpicrylhydrazyl (DPPH) free radical scavenging activity
The DPPH free radical scavenging activity assays were performed according to the procedure described by Hung and Morita, Tibuhwa [12,13] with some modification. Ascorbic acid was used as a standard control. A measure of 0.5 mL of the extract was mixed with 0.5 mL of 0.1Mm methanolic solutions containing DPPH radical that is very stable. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm against blank. A lower absorbance represents a higher DPPH scavenging activity. The percentage of DPPH radical scavenging activity was calculated using the following equation:

DPPH free radical scavenging activity (%) = (1-As/Ac) ×100

Ac = absorbance of control containing DPPH solution

As = absorbance of extract solution containing DPPH

Nitric oxide (NO_2) radical scavenging activity
The analog of the extract of macrofungi with NO_2 was determined by nitrite detection method. NO_2 ions were produced using the Griess Illosvoy reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extracts and the mixture was incubated at 25°C for 5 h. After the incubation of 5 h at 37°C, 0.5 mL of Griess reagent (α-naphthyl ethylenediamine dihydrochloride) 0.1% in water and sulfanilic acid 1% in phosphoric acid (H_3PO_4), and 5% was added into 1.0 mL sulfanilic acid. The UV absorbance was measured at 546 nm. The NO_2 radicals scavenging activity was calculated according to the following equation [14].

Hydrogen peroxide (H_2O_2) free radical scavenging activity
H_2O_2 (40 mM) solution was prepared in phosphate buffer (7.4 pH). The absorption at 230 nm was measured by a spectrophotometer to determine the concentration of the H_2O_2. The extracts (10–100 µg/mL) were added to the H_2O_2 solution. After 10 min of incubation, the absorbance was determined at 230 Nm. Phosphate buffer was used as a blank. Ascorbic acid was used as standard curve [15,16].

RESULTS

Identification
Four non-gilled fruiting bodies were identified on the basis of taxonomic and molecular identification.

Macroscopic identification
Fruiting bodies of non-gilled mushrooms were based on microscopic traits such as traits, size, and color of pileus, and presence or absence of stripe (Table 1).

Microscopic identification
Four pure cultures were obtained on MEA. These four pure cultures were carried out for microscopic study which included clamp connection and spores (Fig. 1). On the basis of macroscopic and microscopic identification, the samples were tentatively identified 2/15 (*Auricularia* sp.1), 6/15 (*Auricularia* sp.2), 32/15 (*Ganoderma* sp.), and 132/15 (*Trametes* sp.).

Table 1: Morphological identification of non-gilled mushrooms fruiting bodies

Sample	Fruiting bodies	Morphological identification
2/15		2–4 cm cup or fan-shaped, elastic, and gelatinous. Creamish brown, lower surface is gelatinous, brown color, upper surface is soft, silky, pinkish brown, and rubber-like body
6/15		Fruiting bodies were 3–6 cm, fan-shaped, smooth curved, elastic, gelatinous, and upper surface is dark brown in color or lower surface is brown in color, stripe is absent, Gills absent
32/15		Fruiting bodies were large. Cap: Circular or semicircular, kidney-shaped 5–8 cm broad. Upper surface smooth or tough, orange-red or reddish black in center, light yellow toward the margin, the cap is tough or woody. Stalk 6–8 cm
132/15		Short stripe such as base, pileus up to 11 cm wide, woody, upper surface is dark brown, and inner surface is creamish, color, and smooth

2/15: *Auricularia auricular-judae*, 6/15: *Auricularia polytricha*, 32/15: *Ganoderma lucidum*, 132/15: *Trametes elegans*

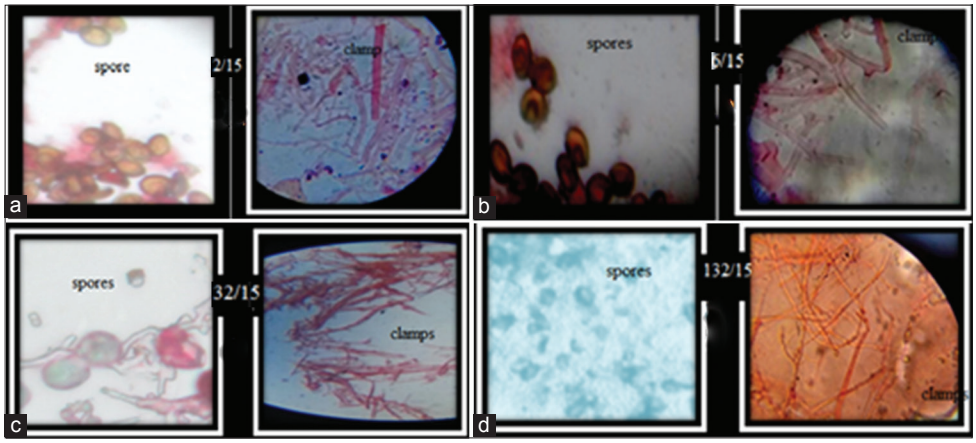

Fig. 1: Endospore structure and clamp connection (a) 2/15: *Auricularia* sp.1, (b) 6/15: *Auricularia* sp.2, (c) 32/15: *Ganoderma* sp., and (d) 132/15: *Trametes* sp.

Molecular identification

In the present study, DNA was extracted from the fungal mycelium of four non-gilled mushrooms isolates was amplified with ITS 1 and ITS 4 primers. The size of the DNA of four mushrooms samples was approximately 500 bp (Fig. 2). PCR products were sent to PGMIER Chandigarh, for sequencing. 18S DNA nucleotide sequence was obtained by sequencing the PCR product shown in Table 2 and analyzed for BLAST. According to the blast, samples (6/15, 32/15, 2/15, and 132/15) they mostly resembled with *Auricularia auricula-judae* (2/15), *Auricularia polytricha* (6/15), *Ganoderma lucidum* (32/15), and *Trametes elegans* (132/15). The aligned nucleotide sequence of samples was submitted to NCBI and nucleotide sequence is provided with gene bank accession no. MF770159, MF774107, MF770158, and MF770160**.** The phylogenetic tree was constructed (Fig. 3) of all the four isolated non-gilled mushrooms and revealed that all the samples are closely related fungi.

DPPH free radical scavenging activity

The antioxidant activity of methanolic and hexane extracts was expressed as inhibitory concentration (IC_{50}) values of DPPH. Both the extracts of *G. lucidum* showed good IC_{50} value. However, the methanolic extract of *G. lucidum* showed the higher effect on DPPH having IC_{50} value 140.85±0.64 in comparison to hexane extract of *G. lucidum* having IC_{50} value 429.9±2.16 followed by *A. polytricha* having IC_{50} value 203.52±1.10 for methanolic extract and 587.53±0.92 for hexane extract. Both the extracts of *T. elegans* having lower IC_{50} value 287.08±2.53 for methanol extract and 1079.31±2.15 for hexane extract as shown in Tables 3 and 4.

NO_2 free radical scavenging activity

NO_2 scavenging effect increased with the increasing concentrations of extract. Our study suggests that all the extracts inhibited NO_2 production. NO_2 having IC_{50} value 365.16±0.23 of *G. lucidum* in the methanolic extract was highest in compared to hexane extract of *G. lucidum* 584.76±0.32 and lower in *T. elegans* having IC_{50} value 667.02±1.99 for methanol extract and 1589.85±1.63 for hexane extract as shown in Tables 3 and 4.

H_2O_2 free radical scavenging activity

Tables 3 and 4 showed the H_2O_2 scavenging effect by measuring the inhibition of the degradation of 2-deoxyribose by free radical generated

Table 2: Nucleotide sequences of non-gilled mushrooms

Sample	Sequence
2/15	CGGAGGGGAACCTGCGGGAGGACATTAAAGATTCTAAGGCTTCGTCTTAATTTCGGCTGTGCGCCTCAGGCTGCCACGCCGTTAACCTCTCACACCTGTGCACCTTTTCGGTTTCGGCCGCTTCCGCTTTTA
	CATGCAACCACGAAAAGTCTAGAATGTGACGAGAACTATAAAGTAACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATC
	ATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCATGGAGCATGCCTGTTTGAGTGTCACGTAAACCCTCACCCCTGCGATGTAACAGTCGCCTGCGGTGGACTTGGACCGTGCCGGTAATCGGCTCGTCTTG
	AAATGCATTAGCTGGTGCTTTTAGAGTGCTGGGCGAACGGTGTGATAATTATCTGCGCCGACTGCCCTGGGCCTCTTCAGCGGCGCTGCTTCACAGCCGTCCCTTGTGGACAACTATTTTAAAGCTTTGGCCTCAAATC
	AGGTAGG
6/15	CTTCACCGGGCTGCACGCTGAAGCAAGACCCACACCTGTGCACCTTTTCGGTTGCGGCTTCGGTCGCTGCCGCTTTTTAAATGCAACAACTCAGTCTCGAATGTGAACAAAACCATAAAAAAGAACCACGTTCAACA
	ACGGATCCCCTGGGTCCCCCCTCCATTAAAAAAGCCACGAAATGGCAATAGTAAAGGGAATTGGAAAATTTCGTGGATCCTCCAATCCTTTAACGCCTTTTGGGCCTCCTGGTTTTTCATGGAACCTGGCTGGTTTAA
	TGGTCCGTAAAACCCCCCCCCTGGCAAGTTACCATCGCTCCCGGTGGAATTGGAATGGGGCCGAACCGGGTCCGCCTGGAATTGGATTAACTGGGGCTTTTAAAATGGTGGGCCAACGGGGGGTTATTATCTGGCGCCA
	TTGCCTTAGGCTCCTCCGCCGTGCTGCTTAACACCCTTCCTCTGGCGAACCATTTTTAAAGCTTTGGGCCCCAATCCGGGAAGGATACCCGCTGAA
32/15	TATTGTCTTCCCCTTCCTGGATTTGAGGTCATAAAGCTGTCTTATAAGACGGTTAGAAGCTCGCCAACGCTTCACGGTCGCGGCGTAGACATTATCACACCGAGAGCCGATCCGCAAGGAACCAAGCTAAT
	GCATTTAAGAGGAGCCGACCGATAACGGCCGACAAGCCTCCAAGTCCAAGCCTACAAACCACAAAAGCTTGTAGGTTGAAGATTTCATGACACTCAAACAGGCATGCTCCTCCGGAATACCAAGGAGCGCAAGGTGCGT
	TCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAGCCAAGAGATCCGTTGCTGAAAGTTGTATATAGATGTGTTACATCACAATACACATTCTGTT
	ACTTTATAGAGTTTGTGATAAAGCGCAGGCACAGATACGCTCCGCAAGCCCGGTAAAGAGGCGTGCCTCGCAATCTGAAGCCCACACAGTAAGTGCACACGGTGTAGAGTGGATGAGGCAAGTGCGCCACATGCCTCGGAAGGCC
	AGCTACAACCCGGTCAAAACTCGATAATGATGCCTTCCGCAGGTTCACCTACGG
132/15	TTCGTACGTGACCTGCGGAGGATCATTAACGAGTTCTGAAACGGGTTGTAGCTGGCCTTCCGGGGCATGTGCACACCCTGCTCATCCACTCTACACCTGTGCACTCACTGTAGGTTGGCGTGGGCTTCCTTCGCGGGAAG
	CATTCTGCCGGCCTATGTACACTACAAACACTATAAAGTAACAGAATGTATTCGCGTCTAACGCATCATAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATT
	GTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCCTGGTATATTTCGAAGAACATGCCTGTTGAGTGTCATGGAATTCTCAACCTATAAATCTTTGTGGTTTATGGGCTTGGACTTGGAGG
	CTTGCCGGCCTCAATGGTCGGCTCCTCTTGAATGCATTAGCTTGATTCCGTGCGGATCGGCTCTCAGTGTGATAAATTGTCTACGGCTGTTGGCGAGCTTCTAACCGTCCCGTTGGGACAATTTTACT
	GACATCTGACCTCAAATCAGTAGACGCATT

2/15: *Auricularia auricular-judae*, 6/15: *Auricularia polytricha*, 32/15: *Ganoderma lucidum*, 132/15: *Trametes elegans*

Fig. 2: 18S Polymerase chain reaction replicon of 2/15: *Auricularia auricula-judae*, 6/15: *Auricularia polytricha*, 32/15: *Ganoderma lucidum*, and 132/15: *Trametes elegans*

by Fenton reaction. In the present study, it was observed that effect on H_2O_2 was higher having IC_{50} 368.46±1.26 in *G. lucidum* of methanolic extract and lower 1145.45±1.11 in *T. elegans* of hexane extract.

DISCUSSION

Antioxidants donate an electron to stabilize a free radical. When a free radical is invalidated, it is not capable of damaging our cells. Today science is quite anxious for natural scavengers from natural sources to fight as defense mechanisms against various diseases and ailments. Antioxidants escort our body against free radicals and mushrooms are a rich source of these antioxidants.

DPPH is a stable free radical scavenger with an absorption band around 515–528 nm, that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and thus DPPH reagent used for evaluation of antioxidant activity of compounds. Antioxidants reduce the DPPH radical to a yellow colored compound (DPPH), and the degree of the reaction will depend on the hydrogen donating ability of the compounds [17]. In the present study, results revealed that scavenging effects of methanol extracts and hexane extracts on DPPH radical increased with increased concentrations. However, methanol extracts of *G. lucidum* show higher scavenging activity on DPPH radical than hexane extracts. Another study also found that the *Auricularia judae* showed a high scavenging activity at 93.33% followed by *Polyporus tenuiculus* with 90.71% of methanolic extract [18]. However, the lowest effective concentration of EC_{50} has observed in *A. judae* 0.08 mg/ml showed strongest. Methanolic extracts from *Tarchonanthus camphoratus* and *Agaricus blazei* at 2.5 mg/mL showed excellent scavenging effects (96.3–99.1 and 97.1%) [19]. According to Kamiyama *et al.* showed that *Trametes versicolor* of acetone extract showed highest scavenging activity at 54.9±0.1% at 500 µg/mL than methanol extract at 40.0±0.2% against DPPH radical [20]. Scavenging activity of medicinal mushrooms including *G. lucidum*, *G. lucidum antler,* and *Ganoderma tsugae* of methanolic extract exhibited a strong reducing power of 1.62, 2.28, and 2.38 at 4.0 mg/mL [21].

NO_2 is an effective pleitropic inhibitor of physiological processes present in mammalian cells such as smooth muscles and an essential bioregulatory molecule required for neural signal transmission, immune response, control vasodilatation, and control of blood pressure [22]. NO_2 is free radical that has an unpaired electron. NO_2 in higher concentration can be a toxic product, peroxynitrite ($ONOO^-$). Hence, inhibition of NO_2 is must be done. Reducing the NO_2 generation in the digestive tract was observed to be effective in preventing the reactions of nitrate with amines and amides to form carcinogenic nitrosamines and nitrosamides. Scavenging activity of *G. lucidum* extract of NO_2 could play an essential role against nitrosamine mediated carcinogenesis [23]. The present study finds that all the four non-gilled

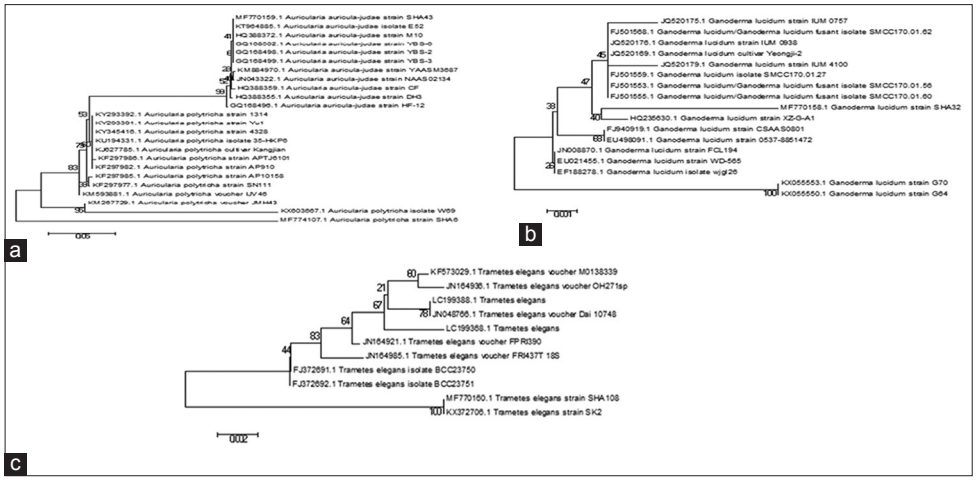

Fig. 3: Phylogenetic tree derived from partial 18S rDNA gene sequence of (a) *Auricularia polytricha* and *Auricularia auricula-Judea*, (b) *Ganoderma lucidum*, (c) *Trametes elegans*

Table 3: IC_{50} value of methanol extract of different non-gilled mushroom cultures for DPPH, NO_2, and H_2O_2 free radical scavenging activity

Isolates	IC_{50} value (mg/mL) for the methanol extract		
	IC_{50} (DPPH)	IC_{50} (NO_2)	IC_{50} (H_2O_2)
Auricularia auricula-judae	245.72±1.02	599.11±1.30	828.38±0.54
Auricularia polytricha	203.52±1.10	457.74±1.91	457.74±1.91
Ganoderma lucidum	140.85±0.64	365.16±0.23	368.46±1.26
Trametes elegans	287.08±2.53	667.02±1.99	1089.5±0.41

Values are means of triplicate determinations±standard deviation
IC_{50}: Inhibitory concentration, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, NO_2: Nitric oxide, H_2O_2: Hydrogen peroxide

Table 4: IC_{50} value of hexane extract of different non-gilled mushroom cultures for DPPH, NO_2, and H_2O_2 free radical scavenging activity

Isolates	IC_{50} value (mg/mL) for the hexane extract		
	IC_{50} (DPPH)	IC_{50} (NO_2)	IC_{50} (H_2O_2)
Auricularia auricula-judae	793.63±2.45	1006.02±1.86	843.69±0.64
Auricularia polytricha	587.53±0.92	915.38±1.03	663.43±1.91
Ganoderma lucidum	429.9±2.16	584.76±0.32	514.29±0.41
Trametes elegans	1079.31±2.15	1589.85±1.63	1145.45±1.11

Values are means of triplicate determinations±standard deviation.
IC_{50}: Inhibitory concentration, DPPH: 2,2-diphenyl-1-picrylhydrazyl, NO_2: Nitric oxide, H_2O_2: Hydrogen peroxide

mushrooms inhibited the NO_2 production but methanol show more effectiveness in antioxidant activity than hexane extract. NO-scavenging dose-response curve of cold water, hot water, and methanolic extracts that exhibitory concentration 50 value for hot water 14-fold greater than that of methanolic extract of *Pleurotus squarrosulus* [24]. The chloroform extract of *G. lucidum* showed scavenging activity of the extract ($IC_{50:}$ 21.6±1.5 µg/mL) against NO_2 [23].

H_2O_2 is a biologically material, non-radical oxidizing species may be formed in tissues through oxidative processes. H_2O_2 generates hydroxyl radicals (•OH) resulting in the initiation and propagation of lipid peroxidation. The ability of the extracts to quench (•OH) seems to be directly related to the prevention of the lipid peroxidation [25]. Methanolic extract of *Agaricus bisporous* showed good H_2O_2 scavenging activity, but it was less effective than standard ascorbic acid and appears to be a moderate scavenger of active, reactive oxygen species [26].

The present study showed that both methanolic and hexane extracts of four non-gilled mushrooms mycelial cultures showed free radical scavenging activity. Methanolic and hexane extracts showed maximum scavenging activity in *G. lucidum*. However, in comparison to hexane the methanolic extract shows more scavenging activity.

CONCLUSION

According to this study, these four non-gilled mushrooms may be beneficial to the antioxidant protection system of the human body against oxidative damage. Both the methanolic and hexane extracts used against different free radical systems showed excellent radical scavenging potential which is indicative of their antioxidant activity. This study innovated the potential antioxidative role of non-gilled mushrooms in free radical systems. *G. lucidum* could evolve to be a better source of antioxidants. Therefore, these non-gilled mushrooms can be used as an easily available source of natural antioxidants and as promising food supplements in an effort to curtail nutrition insecurity.

ACKNOWLEDGMENT

The authors express their sincere thanks to the Department of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Solan, India, for support and corporate facilities.

AUTHOR'S CONTRIBUTIONS

The experimental part of the work and writing of the manuscript was done by the first author Shaveta Singh under the supervision of author Dr. Astha Tripathi.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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