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RESEARCH ARTICLE

Chemical Constituents, Antioxidative and Antibacterial Properties of Medicinal Mushrooms Collected from Similipal Biosphere Reserve, Odisha, India

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Abstract Medicinal mushrooms viz. *Ganoderma applanatum*, *Ganoderma lipsiense*, *Ganoderma chalceum* and *Ganoderma tsugae* are important forest products of Similipal Biosphere Reserve, India. The present study aimed to evaluate the chemical constituents, antioxidant and antibacterial potential of these mushrooms. The chemical constituents were determined in terms of macronutrients, micronutrients and phenolic contents. The macronutrient content of these mushrooms revealed high amounts of protein (17.10–20.50/100 g), carbohydrate (41.80–59.16/100 g) and low fats (1.2–3.0/100 g) and possessed good quantities micronutrients (vitamins, carotenoids). Mushrooms showed strong antioxidant properties due to the presence of high phenol (27.40–220.41 mg catechol/g extract) and flavonoid (3.42–171.44 mg quercetin/g extract) content. All these mushrooms possess moderate antibacterial properties with zone of inhibition ranging from 11.76 to 18.83 mm against five human pathogenic bacteria. Based on their rich bioactive compounds (vitamins and phenolics), antioxidant and antibacterial activity, they might be exploited by pharmaceutical and cosmetic industries.

Keywords Antibacterial · Antioxidant · Bioactive compounds · Nutrients · Vitamins

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Introduction

Mushrooms are important products of forest ecosystem, which grow on the most abundant nutrients such as, cellulose. Mushrooms are defined as a macro-fungus with a distinctive fruiting body which can be either epigeous or hypogeous and large enough to be seen with naked eyes and to be picked up by hand [1]. Mushrooms have a long association with mankind as a source of food and medicine and provide profound biological and economic impact. They have rich nutritional value with high content of proteins, vitamins, minerals, fibers, trace elements and low/no calories and cholesterol and are used as source of food for human consumption [2, 3]. Many of them exhibited therapeutic value and have been used in folk medicines for thousands of years. Some of them are used as nutraceuticals (natural food having potential value in maintaining good health and boosting immune system of the human body) while others can produce potent nutriceuticals (compounds that have medicinal and nutritional attributes and are consumed as medicines in the form of capsules or tablets but not as food) [4, 5]. It is estimated that, out of approximately 15,000 known species in the world, 2000 are safe for human consumption and about 650 of these possess medicinal

properties [6]. Among them, *Ganoderma* species is the most common medicinal/nutriceutical mushrooms which has been used in folk-medicine in China and Japan from ancient times, due to the demonstrated efficiency of it as a popular remedy to treat several diseased conditions, namely chronic hepatitis, arthritis, hypertension, hyperlipidemia, bronchitis, neoplasia, asthma, gastric ulcer, atherosclerosis, diabetes etc. [7]. In India, *Ganoderma lucidum* has been reported to be used by the Baiga tribe of Central India in the treatment of asthma [6]. The extensive range of traditional medical treatments with *Ganoderma* has not been fully substantiated by modern scientific standards.

Over 250 of *Ganoderma* species have been reported to occur throughout the world [8]. Traditionally, *Ganoderma* species were classified into several types based on their colour, including black, red, purple, light black, yellow and white [9]. Each type of *Ganoderma* has its own characteristic biological properties. The commonly used medicinal *Ganoderma* (*G.)* include *G. lucidum*, *G. tsugae*, *G. capense* and *G. applanatum* [6, 10]. Some of the physiological effects and distinctive properties of *Ganoderma* are strain dependent [11]. Besides that, several reports have been made for their distinctive biological properties like antibacterial, antioxidant, anti-inflammatory, anti-proliferative, anti-cancer, anti-tumour, cytotoxic, anti-HIV, hypo-cholesterolemic, anti-diabetic and hepato-protective etc. [6, 10]. Polysaccharides and triterpenes of *Ganoderma* are the major sources of their pharmacological active constituents. Currently more than 100 types of polysaccharides and 130 triterpenoids have been characterized from these mushrooms [12].

Ganoderma has been found to occur widely in India, particularly in the tropical area. Similipal Biosphere Reserve (SBR), Odisha, a tropical forest ecosystem of India is known to be rich in floral diversity including mushrooms. The high humidity level during the monsoon season (June–October) provides ideal atmospheric conditions for the growth of many saprophytic fungi, including *Ganoderma* mushrooms which are saprophytic fungi deriving their nutrition from dead decaying wood of the trees and their trucks. Despite the high diversity of *Ganoderma* species grown in the Similipal forest area, no attempt has been made to evaluate their medicinal properties. Therefore, an attempt has been made to compare chemical composition and bioactive potentials (antioxidant and antibacterial) of some important *Ganoderma* species from SBR.

Material and Methods

Samples

Four wild mushroom species viz., *Ganoderma applanatum* (Pers.) Pat., *Ganoderma lipsiense* (Batsch) Atk., *Ganoderma chalceum* (Cooke) Steyaert and *Ganoderma tsugae* were collected from the forest of Similipal Biosphere Reserve, Odisha, India between July and December of 2011. Further, the collected samples (fresh and dried) were preserved in 4 % formaldehyde and in paper or polythene bags respectively and numbered for future use. Each of the collected samples were also wrapped in wax paper and brought to the laboratory for identification using standard manuals [13–15]. All the identified specimens were deposited in the Herbarium of Department of Biotechnology, College of Engineering and Technology, Bhubaneswar, Odisha, India.

Chemicals

2,2-Azinobis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS), 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), butyl hydroxytoluene (BHT), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), catechol, quercetin, oxalic acid and ascorbic acid were purchased from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals were of analytical grade and obtained from either Sigma or Merck.

Macronutrients

Samples were analyzed for their chemical composition (moisture, protein, carbohydrate, fat and ash) using the Association of Official Analytical Chemists (AOAC) procedures [16]. Protein content (N $\times$ 6.25) of the samples was estimated by the macro-Kjeldahl method; fat was determined by extracting a known weight of powdered sample with petroleum ether using a Soxhlet apparatus; the ash content was determined by incineration at 600 $\pm$ 15 °C. Carbohydrates were calculated as under:-

$$\text{Carbohydrates} = 100 - (\text{g protein} + \text{g fat} + \text{g ash})$$

The reducing sugar was determined by using dinitrosalicylic acid (DNS) method. Starch was estimated by using the Anthrone reagent method (200 mg anthrone in 100 mL of ice cold 95 % sulphuric acid) and total free amino acid content of mushrooms was estimated by Ninhydrin method [17]. Total energy was calculated according to the following equations: -

$$\text{Energy (kcal)} = 4 \times (\text{g protein} + \text{g carbohydrate}) + 9 \times (\text{g fat})$$

Micronutrients

Thiamine content in the defatted powdered samples of the mushrooms was determined by the method of Sadashivam and Manikam [17]. The thiamine content was calculated using the calibration curve, prepared from thiamine HCl and the results were expressed as mg thiamine/g of extract.

The riboflavin content of the mushroom samples was determined by the methods of Okwu and Josiah [18]. The riboflavin content was calculated using the calibration curve, prepared from riboflavin and the results were expressed as mg riboflavin/g of extract. Ascorbic acid of mushrooms was determined using the method described by Barros et al. [19]. The ascorbic acid content was calculated using the calibration curve, prepared from L-ascorbic acid and the results were expressed as mg ascorbic acid/g of extract. β-Carotene and lycopene of the mushrooms were determined according to the method of Barros et al. [19]. Contents of β-carotene and lycopene were calculated according to the following equations:

$$\beta\text{-carotene}\ (\mathrm{mg/100\,mL}) = 0.216\,A_{663} - 0.304\,A_{505} + 0.452\,A_{453}$$

$$\text{Lycopene}\ (\mathrm{mg/100\,mL}) = 0.0458\,A_{663} + 0.372\,A_{505} - 0.0806\,A_{453}$$

The results were expressed as µg carotenoid/g of extract.

Evaluation of Bioactive Components

Preparation of Mushroom Extracts

A fine dried powder of mushrooms (5 g) was stirred with 100 mL of solvent (ethanol, methanol and water) at room temperature at 150 rpm for 24 h and filtered through Whatman No. 4 paper. The solvent extracts were evaporated at 35 °C by vacuum distillation, re-dissolved in respective solvent at 50 mg/mL concentration and stored at 4 °C for further analysis according to Singdevsachan et al. [20]. Percentage yield was calculated from the dry extract powder. Different concentrations were prepared in respective solvents for various antioxidant and antibacterial screening assays.

Phytochemical Screening

All the three extracts of studied mushrooms were subjected to preliminary phytochemical screening for identification of various classes of active chemical constituents using the standard methodology of Harborne [21]. The presence of different phytochemicals in the mushroom species were evaluated as follows: "+" indicates presence of phytochemicals in low amount, "++" moderate, "+++" high, and "−" indicates absence of phytochemicals.

Total Phenols and Flavonoid Content

The total phenol content in the mushroom extracts were estimated by a colorimetric assay using Folin–Ciocalteau's phenol reagent (FCP) according to the method of Rath et al. [22]. Phenolic content was expressed as mg catechol/g of extract. Flavonoid contents of mushroom extracts were determined by a colorimetric method described by Barros et al. [19]. Flavonoid contents were calculated using a standard calibration curve, prepared from quercetin. The flavonoid contents were expressed as mg quercetin/g of extract.

Total Antioxidant Capacity

The total antioxidant capacity of the solvent extracts of mushrooms were determined by the method of Prieto et al. [23]. The assay is based on the reduction of molybdenum (VI)–molybdenum (V) by the mushroom extracts and its subsequent formation of a green phosphate/molybdenum (V) complex at acidic pH. An aliquot (0.1 mL) of the mushroom extracts was combined to 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min. After, the mixture had cooled to room temperature; the absorbance of each solution was measured at 695 nm against an appropriate blank. The antioxidant capacity was expressed as mg catechol/g of extract.

ABTS Radical Scavenging Activity

The ABTS free radical scavenging activity of the mushroom extracts was determined as per the standard method of Thaipong et al. [24]. The radical scavenging activity was calculated as per following equation.

$$\text{Radical scavenging activity}\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity of the mushroom extracts was determined by the standard method of Patra et al. [25]. The radical scavenging activity was calculated as per following equation:-

$$\text{Radical scavenging activity}\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

H_2O_2 Scavenging Activity

The ability of mushroom extracts to scavenge hydrogen peroxide (H_2O_2) were determined according to standard

method of Rath et al. [22]. The percentage scavenging activity was calculated as per following equation.

$$\text{Scavenging activity }(\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Metal Chelating Activity

The ferrous ion metal chelating activity of solvent extracts of mushrooms were assessed as described by Patra et al. [25]. The metal chelating effect of the mushroom extracts were calculated as per following equation.

$$\text{Metal chelating effect }(\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Thin Layer Chromatography (TLC) Analysis for Antioxidant Constituents

About 2 μg of mushroom extracts (ethanol and methanol) and standard catechol were loaded on TLC plates (Merck, 20 × 20 cm^2). Based on previous literature, different solvent systems were tested for the detection of phenolic compounds in the mushroom extracts and the best solvent system (toluene: ethyl acetate: acetic acid, 5:5:1) was selected for further analysis. The plates were developed in toluene: ethyl acetate: acetic acid (5:5:1) to separate various constituents mainly phenolic compounds present in the extracts. The developed plates were air dried and observed (under visible and UV light). After this examination, the antioxidant constituents were analyzed by DPPH technique [26, 27]. For this 0.05 % of DPPH solution in methanol was sprayed on the surface of developed TLC plates and incubated for 10 min at room temperature. The active antioxidant constituents of the mushroom extracts were detected as yellow spots produced by reduction of DPPH by resolved bands against purple background on the TLC plates, the Rf value of the bands were also determined.

Antibacterial Study

Antibacterial assay of mushroom samples was performed in three different extracts (ethanol, methanol and aqueous) after redissolution in DMSO at a concentration of 50 mg/mL.

Microorganisms and Culture Conditions

Five pathogenic bacteria, *Staphylococcus aureus* (MTCC 1144), *Vibrio cholerae* (MTCC 3904), *Pseudomonas aeruginosa* (MTCC 1034), *Bacillus subtilis* (MTCC 7164) and *Escherichia coli* (MTCC 1089) used in the study, were obtained from Institute of Microbial Technology (IMTECH), Chandigarh. Organisms were maintained on nutrient agar (Hi-Media, India) slopes at 4 °C and subcultured before use. Active cultures for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of nutrient broth (NB) that were incubated with agitation for 24 h at 37 °C.

Determination of Antibacterial Activity

In vitro antibacterial activity of crude extract of each solvent was screened by agar well diffusion method [20]. Wells were prepared in seeded nutrient agar (NA) plates. Prior to introduction of test compounds, 0.1 % inoculum suspension was swabbed uniformly and was allowed to dry for 5 min. 100 μL extract (from a concentration of 50 mg/mL with 100 % dimethylsulphoxide) was introduced in the wells. Plates were incubated overnight at 37 °C. Antibacterial spectrum of extract was determined in terms of zone sizes (in mm) around each well. Diameter of zone of inhibition produced by extracts was compared with those of antibiotic Tetracycline (50 μg/mL). Each zone of inhibition was measured three times (in three different plates) and the average was considered.

Statistical Analysis

For each one of the mushroom species three samples collected from different areas of Similipal Biosphere Reserve were analyzed and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD). The means of all the parameters were examined for significance by analysis of variance (ANOVA) and in case of significance mean separation were accomplished by GenStat discovery (Edition 3) statistical software package. Differences were considered significant at a probability level of $p < 0.05$ with Duncan's multiple range test analysis.

Results and Discussion

Macronutrient Contents

The results of macronutrient composition and estimated energetic value (expressed on dry weight basis) of four *Ganoderma* sp. are shown in Table 1. Moisture content ranges between 42.85 % fw in *G. lipsiense* to 53.08 % fw in *G. applanatum*, which is comparatively less than other mushroom species (70–96 %) in the world [28, 29]. This variability of moisture content is species dependent and on

other parameters such as environmental temperature, relative humidity during growth and relative amount of metabolic water that may be produced or utilized during storage [30]. The highest level of protein was found in *G. tsugae* (20.50/100 g dw). Carbohydrates, calculated by difference, were also the abundant macronutrient and ranged from 41.80/100 g dw in *G. applanatum* to 59.16/100 g dw in *G. tsugae*. Reducing sugars are only a small part of carbohydrate contents, being polysaccharides such as chitin and starch. Reducing sugar content varied between 2.35/100 g dw in *G. tsugae* to 4.51/100 g dw in *G. applanatum* whereas starch content as found between 3.04/100 g dw in *G. tsugae* and 6.08/100 g dw in *G. lipsiense* (Table 1). Total free amino acid content ranged from 2.61/100 g dw in *G. lipsiense* to 4.45/100 g dw in *G. chalceum*. The fat contents were found between 1.2/100 g dw in *G. tsugae* and 3.0/100 g dw in *G. lipsiense*. The ash content varied between 9.13/100 g dw in *G. tsugae* and 15.2/100 g dw in *G. applanatum*. The *G. lipsiense* showed the highest energetic contribution (284.6 kcal/100 g dw) mainly due to its higher fat values. Results of studied mushrooms have been compared and found that all these mushrooms contain good quantities of macronutrients. The results of macronutrient contents are in agreement with different studies reported by several authors [28, 29].

Micronutrient Contents

Micronutrients such as vitamins and carotenoids in the selected mushroom species were determined and the results are presented in Table 2. Ascorbic acid contents in *Ganoderma* were more abundant than those of other vitamins (thiamine and riboflavin) and carotenoids (β-carotene and lycopene). *G. chalceum* showed high ascorbic acid content (128.08 mg/g dw) than the other mushroom species studied. However the thiamine content were found between 0.06 mg/g dw in *G. applanatum* to 0.77 mg/g dw in *G. lipsiense* whereas riboflavin was not found in all the four species (Table 2). In case of carotenoids, β-carotene ranged from 0.75 μg/g dw in *G. chalceum* to 2.3 μg/g dw in *G. lipsiense* and lycopene content varied between 0.02 μg/g dw in *G. chalceum* and 0.8 μg/g dw in *G. lipsiense*. Among the four species studied, *G. chalceum* showed maximum micronutrient content. The findings of the micronutrient contents of studied wild *Ganderma* species are in the agreement with the earlier published data for different edible and non-edible mushroom species [31, 32]. Presence of vitamins and carotenoids in the studied wild mushrooms could play a protective role in diseases related to oxidative stress, such as cancer and cardiovascular diseases [33].

Preliminary Screening of Phytochemicals

The preliminary qualitative phytochemical analysis in the four studied mushroom species showed the presence of active compounds such as phenols, flavonoids, alkaloids, steroids, terpenoids, saponins, carbohydrate, glycoside and tannin (Table 3). Among the three different solvent systems, methanol extract showed the presence of most of the phytochemicals. Among the four studied mushroom species, *G. applanatum* showed maximum number of phytochemicals. Recently, the phenolic compounds have attracted much interest among the scientists because various in vitro and in vivo studies have suggested that they possess a variety of beneficial biological properties like anti-inflammatory, antitumor, antioxidant and antimicrobial activities [34].

Yield of Extracts

A sequential extraction with ethanol, methanol and water was performed in order to obtain extracts with low

Table 1 Macronutrient contents and estimated energetic value of four *Ganoderma* species

Macronutrients	*G. applanatum*	*G. lipsiense*	*G. chalceum*	*G. tsugae*
Moisture (%)	$53.08 \pm 2.00^{b,*}$	42.85 ± 1.22^{c}	48.06 ± 2.10^{c}	50.54 ± 0.50^{c}
Crude protein (g/100 g)	20.16 ± 1.03^{d}	19.54 ± 0.50^{d}	17.10 ± 0.18^{d}	20.50 ± 0.50^{d}
Total carbohydrate (g/100 g)	41.80 ± 2.30^{c}	44.6 ± 0.83^{b}	58.33 ± 1.52^{b}	59.16 ± 1.01^{b}
Reducing sugar (g/100 g)	4.51 ± 0.50^{f}	2.51 ± 0.50^{g}	3.93 ± 0.11^{f}	2.35 ± 0.31^{f}
Starch (g/100 g)	4.22 ± 0.25^{f}	6.08 ± 0.32^{f}	3.38 ± 0.37^{f}	3.04 ± 0.17^{f}
Total free amino acid (g/100 g)	3.82 ± 0.21^{f}	2.61 ± 0.53^{g}	4.45 ± 0.40^{f}	4.02 ± 1.04^{f}
Crude fat (g/100 g)	2.83 ± 0.20^{f}	3.0 ± 0.10^{g}	1.63 ± 0.15^{g}	1.2 ± 0.20^{g}
Ash (g/100 g)	15.2 ± 1.05^{e}	13.21 ± 0.70^{e}	11.96 ± 1.26^{e}	9.13 ± 0.80^{e}
Energy (kcal/100 g)	273.36 ± 3.19^{a}	284.6 ± 3.29^{a}	322.3 ± 2.40^{a}	329.46 ± 3.93^{a}

* Results of moisture content are expressed in fresh weight (fw) basis and other nutrients are expressed in dry weight (dw) basis with the average of triplicate samples with mean ± SD ($n = 3$)

Difference in the superscript letters across same column indicate significant difference at $p < 0.05$

Table 2 Micronutrient contents of four *Ganoderma* species

Micronutrients	*G. applanatum*	*G. lipsiense*	*G. chalceum*	*G. tsugae*
Thiamine (mg/g)	$0.06 \pm 0.00^{d,*}$	0.77 ± 0.06^{c}	0.76 ± 0.05^{b}	0.45 ± 0.05^{c}
Riboflavin (mg/g)	ND	ND	ND	ND
Ascorbic acid (mg/g)	37.38 ± 0.53^{a}	37.69 ± 1.27^{a}	128.08 ± 2.13^{a}	37.48 ± 0.61^{a}
β-carotene (μg/g)	1.2 ± 0.20^{b}	2.3 ± 0.51^{b}	0.75 ± 0.14^{b}	1.3 ± 0.3^{b}
Lycopene (μg/g)	0.78 ± 0.08^{c}	0.8 ± 0.10^{c}	0.02 ± 0.01^{c}	0.04 ± 0.04^{d}

* Results are expressed in dry weight (dw) basis with the average of triplicate samples with mean ± SD ($n = 3$)

Difference in the superscript letters across all columns and rows indicate significant difference at $p < 0.05$

ND not detected

Table 3 Qualitative screening of phytochemicals in four *Ganoderma* species

	G. applanatum			*G. lipsiense*			*G. chalceum*			*G. tsugae*		
	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract
Phenols	+++	+++	++	++	++	++	++	++	++	++	++	++
Flavonoids	+++	+++	++	+++	+++	++	+++	+++	−	+++	+++	−
Alkaloid	++	++	−	++	++	−	+++	++	++	++	++	++
Steroids	++	+++	++	++	+++	++	−	++	+	−	++	+
Terpenoids	++	+++	++	++	+++	++	++	++	+	++	++	+
Saponins	+	++	+	+	++	+	+	+	+++	+	+	+++
Carbohydrates	++	++	++	++	++	++	++	++	++	++	++	++
Glycosides	++	+++	++	++	+++	++	+	++	+	+	++	+
Tanins	++	++	+	++	++	+	−	+	−	−	+	−

+ low, ++ moderate, +++ high; and − absent

molecular weight compound such phenolic compounds and high molecular weight compounds such as polysaccharides. Both kinds of compounds play important role in mushrooms with the several medicinal functions [33, 34]. The extraction yields obtained for methanol fractions were lower than the yields for ethanol and aqueous fractions. However, the percentage yields of studied mushroom extracts (ethanol, methanol and aqueous) were found in moderate quantities ranging from 15.11 to 26.55 % of the dry weight (Table 4) whereas mushrooms were reported to contain more polar constituents [34].

Bioactive Components and Total Antioxidant Capacity

The constituents of bioactive components such as phenols and flavonoids of the studied wild mushrooms with their total antioxidant capacity are presented in Table 4. Ethanol extract of *G. applanatum* showed high phenolic content (220.41 mg catechol/g dw) whereas *G. lipsiense* showed high flavonoid content (171.44 mg quercetin/g dw) than other species of *Ganoderma*. The quantities found are in agreement with other studies on different mushroom species [35, 36]. Some authors have already reported a direct correlation between mushrooms antioxidant activity and total phenol content, although the antioxidant action is also raised by other substances such as vitamins and carotenes [37]. In fact, the bioactivity of phenolics might be related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [38]. Flavonoids can act as free radical scavengers and terminate the radical chain reactions that occured during the oxidation of triglycerides in food systems [39, 40]. Furthermore, the total antioxidant capacity in different solvent extracts of studied mushroom species ranged from 78.40 to 239.77 mg catechol/g dw (Table 4).

In Vitro Antioxidant Activity

In vitro antioxidant properties of different extracts (ethanol, methanol and aqueous) of the four studied wild mushrooms in different concentrations (25–100 μg/mL) were carried out in term of free radical scavenging activity (ABTS, DPPH and H_2O_2) and metal chelating effect and the results are shown in Fig. 1, 2, 3, 4. Free radical scavenging effect has been known as an established phenomenon in inhibiting lipid oxidation, which otherwise could be deleterious to the cellular components and cellular function [36]. Natural antioxidant can be used to replace the synthetic antioxidant

Table 4 Yield of extracts and bioactive components of four *Ganoderma* species

Mushrooms	Mushroom extracts	Extraction yield (%)	Total phenols (mg catechol/g of extract)	Flavonoids (mg quercetin/g of extract)	Total antioxidant capacity (mg catechol/g of extract)
G. applanatum	Ethanolic	$26.55 \pm 0.50^{a,*}$	220.41 ± 2.10^{a}	136.66 ± 1.52^{b}	239.77 ± 1.57^{b}
	Methanolic	15.11 ± 0.83^{f}	107.88 ± 1.82^{e}	46.58 ± 1.28^{f}	78.40 ± 1.50^{j}
	Aqueous	17.42 ± 0.51^{e}	80.23 ± 1.71^{h}	5.74 ± 0.65^{j}	95.29 ± 2.06^{h}
G. lipsiense	Ethanolic	23.86 ± 0.80^{c}	207.83 ± 1.25^{b}	171.44 ± 1.70^{a}	213.68 ± 1.53^{c}
	Methanolic	20.50 ± 0.50^{d}	106.61 ± 1.51^{e}	76.01 ± 1.83^{d}	154.98 ± 1.97^{e}
	Aqueous	22.50 ± 0.50^{c}	71.55 ± 0.77^{i}	11.11 ± 1.17^{i}	320.23 ± 1.36^{a}
G. chalceum	Ethanolic	23.16 ± 0.76^{c}	126.77 ± 1.07^{d}	61.74 ± 1.55^{e}	138.15 ± 2.01^{f}
	Methanolic	21.00 ± 1.00^{d}	91.22 ± 0.68^{g}	32.07 ± 2.00^{g}	90.42 ± 1.55^{i}
	Aqueous	25.93 ± 0.90^{ab}	40.95 ± 1.00^{j}	3.42 ± 0.51^{k}	89.05 ± 1.00^{i}
G. tsugae	Ethanolic	25.20 ± 1.05^{b}	151.20 ± 1.05^{c}	94.84 ± 1.61^{c}	176.68 ± 1.52^{d}
	Methanolic	22.93 ± 1.00^{c}	101.44 ± 0.74^{f}	24.24 ± 1.30^{h}	94.16 ± 1.03^{h}
	Aqueous	24.33 ± 0.57^{bc}	27.40 ± 2.21^{k}	4.55 ± 0.50^{j}	102.98 ± 2.63^{g}

* Results are expressed in dry weight (dw) basis with the average of triplicate samples with mean ± SD ($n = 3$)

Difference in the superscript letters across each column indicate significant difference at $p < 0.05$

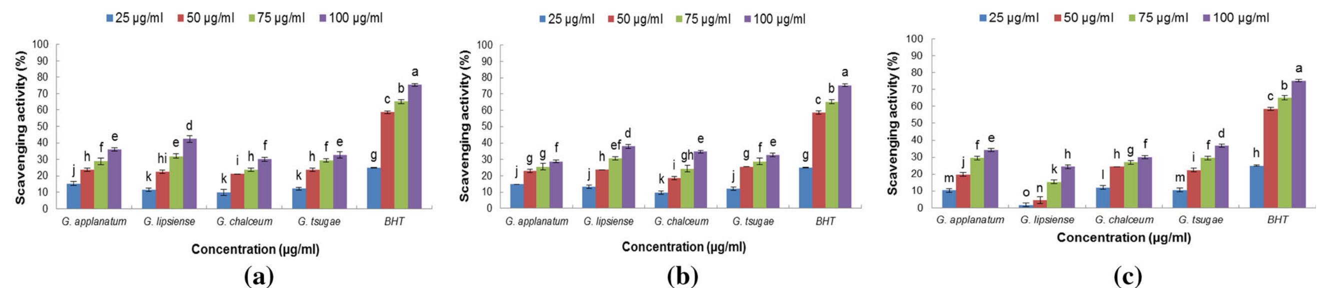

Fig. 1 ABTS scavenging activity of three different extracts, ethanolic (**a**), methanolic (**b**) and aqueous (**c**). The concentration of the mushroom extracts and standard BHT used are in μg/ml. *Different letters* in the same *bar* are significantly different ($p < 0.05$)

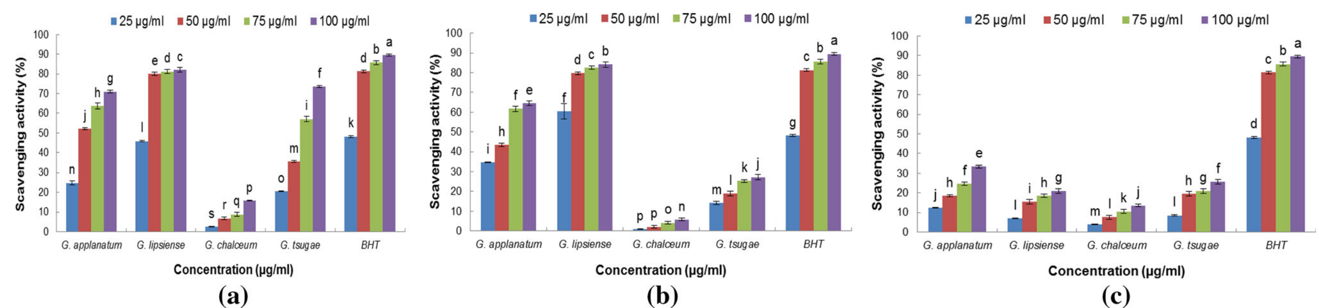

Fig. 2 DPPH scavenging activity of three different extracts, ethanolic (**a**), methanolic (**b**) and aqueous (**c**). The concentration of the mushroom extracts and standard BHT used are in μg/ml. *Different letters* in the same *bar* are significantly different ($p < 0.05$)

such as BHT, butylated hydroxyanisole (BHA) and TBHQ (*tert*-butylhydro quinone) in the food industry, which might possess mutagenic activity [41]. With the presence of radical scavenging activity, consumption of wild mushrooms might be beneficial to protect human body against oxidative damage, which could be developed into health related degenerative illnesses.

ABTS Free Radical Scavenging Activity

The ABTS free radical scavenging assay is based on the inhibition of the absorbance of the radical cation $ABTS^{+}$ which has a characteristic long wave length absorbance spectrum [42]. Ethanol and methanol extracts of *G. lipsiense* showed highest ABTS free radical scavenging

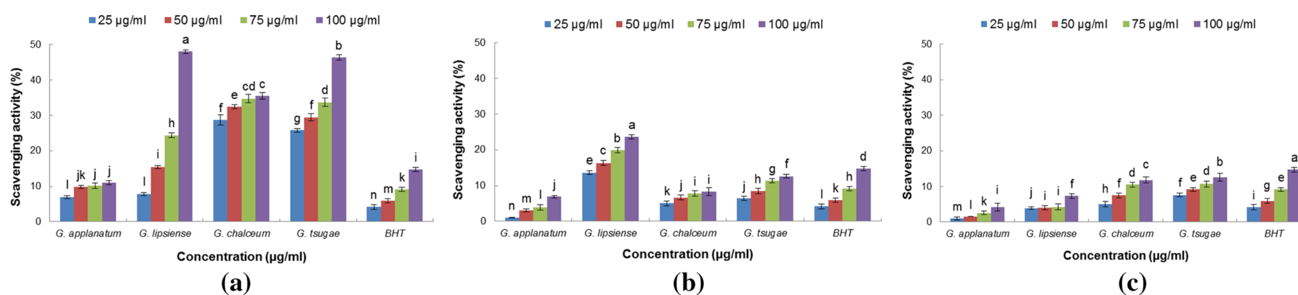

Fig. 3 H_2O_2 scavenging activity of three different extracts, ethanolic (**a**), methanolic (**b**) and aqueous (**c**). The concentration of the mushroom extracts and standard BHT used are in μg/ml. *Different letters* in the same *bar* are significantly different ($p < 0.05$)

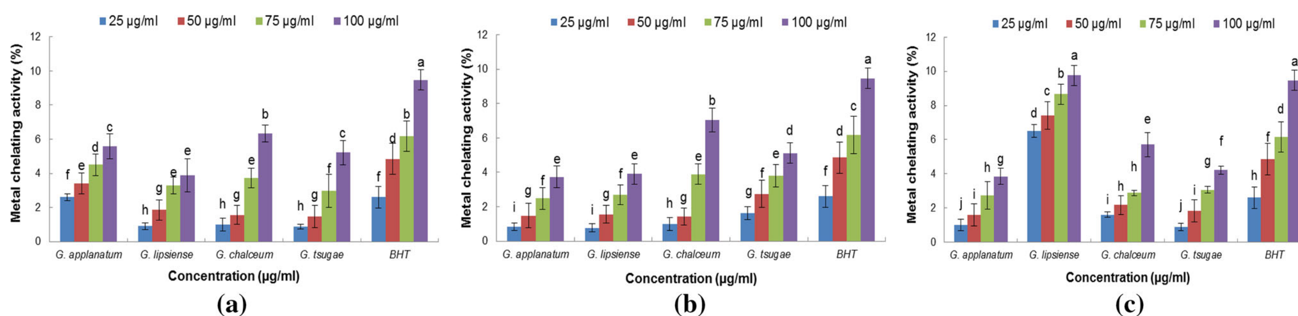

Fig. 4 Metal chelating activity of three different extracts, ethanolic (**a**), methanolic (**b**) and aqueous (**c**). The concentration of the mushroom extracts and standard BHT used are in μg/ml. *Different letters* in the same *bar* are significantly different ($p < 0.05$)

activity (42.30 and 37.82 % respectively) at 100 μg/mL concentration in comparison to other species and less comparable with standard BHT (Fig. 1). As shown in figure, all the three extracts (ethanol, methanol and aqueous) of the studied species inhibited or scavengeed the $ABTS^+$ radicals in increasing order of their concentration (25–100 μg/mL), which supported the earlier findings of several workers on both inhibition and scavenging properties of antioxidants towards $ABTS^+$ radicals [42, 43].

DPPH Free Radical Scavenging Activity

The DPPH free radical possesses a characteristic absorption at 517 nm (purple in color), which decreases significantly on exposure to radical scavengers (by providing hydrogen atoms or by electron donation). A lower absorbance at 517 nm indicated the higher radical scavenging activity of the mushroom extract. Free radical scavenging is one of the well-known mechanisms by which antioxidants inhibit lipid oxidation. This antioxidant assay offers a rapid technique for screening the radical scavenging activity of specific compound or extracts [44]. Scavenging effects of all the three extracts (ethanol, methanol and aqueous) from the *Ganoderma* mushrooms on DPPH radical increased with the increased concentration (Fig. 2). Scavenging effect of *G. lipsiense* was high in case of ethanol and methanol extract (82.01 and 84.12 % respectively) at a concentration of 100 μg/mL in comparison with other mushroom species, whereas *G. tsugae* showed high scavenging activity in case of aqueous extract (25.67 %). The results of the present finding were comparable with standard BHT (Fig. 2). The DPPH scavenging activity of studied mushrooms were comparable with other species of mushrooms reported by several authors [2, 35].

H_2O_2 Free Radical Scavenging Activity

Hydrogen peroxide (H_2O_2) itself is not very reactive, but it can give highly reactive species OH^- through Fenton reaction [41]. It was suggested that H_2O_2 could induce DNA break in the intact cell and purified DNA [45]. Thus, removal of H_2O_2 is crucial for medicinal importance. The H_2O_2 reducing capacity of compound or extract might serve as indicators of its potential antioxidant capacity [46]. Results of H_2O_2 reducing capacity are presented in Fig. 3. From the analysis, one can conclude that the scavenging effect of mushroom extracts on H_2O_2 radicals increased with the concentration increase and were excellent for *G. lipsiense* (48.05 and 23.57 % with ethanol and methanol extract respectively), even higher than the scavenging effects of BHT (14.76 %) at 100 μg/mL concentration.

Metal Chelating Activity

All the three extracts of four *Ganoderma* species displayed very low metal chelating activity (Fig. 4). The result indicated that scavenging ability of mushroom extracts was very closely similar to standard BHT (9.47 % at 100 μg/mL). Metal ions can initiate lipid peroxidation and start a chain reaction that leads to the deterioration of food [47]. The catalysis of metal ions also correlates with incidence of cancer and arthritis [48]. Ferrous ions are the most effective pro-oxidants which are commonly found in food systems [49]. However, Iron stimulates lipid peroxidation by the Fenton reaction and accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that could abstract hydrogen and perpetuate the chain reaction of lipid peroxidation [49]. In the present metal chelating assay, extracts of the mushroom species interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and capture ferrous ions before ferrozine. Mau et al. [35] reported that *Ganoderma* mushrooms were good chelators for ferrous ions. Accordingly, it is suggested that

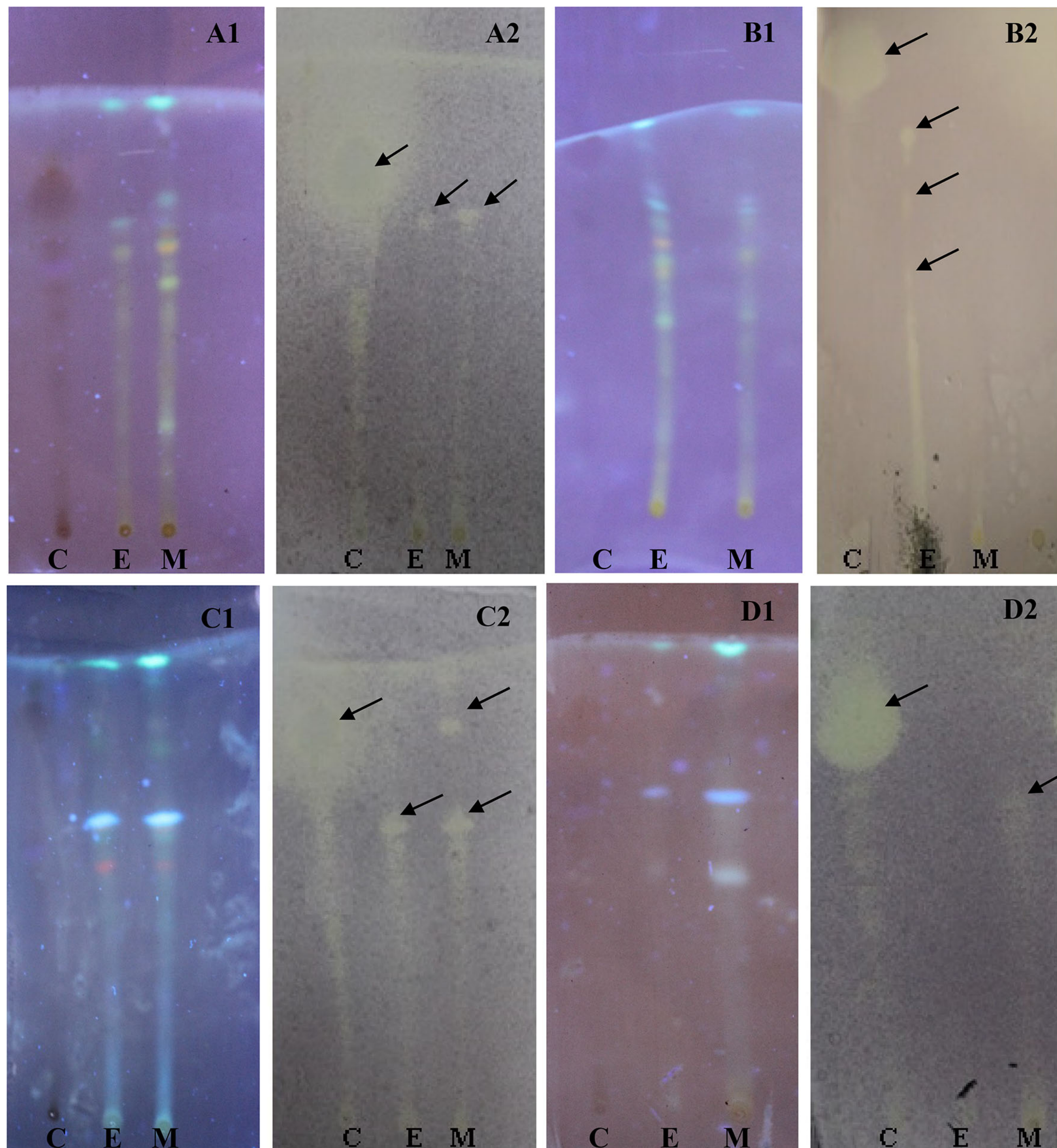

Fig. 5 Thin layer chromatography plates of *G. applanatum* (**a1** and **a2**), *G. lipsiense* (**b1** and **b2**), *G. chalceum* (**c1** and **c2**) and *G. tsugae* (**d1** and **d2**) under UV light and after DPPH spray respectively (*C* catechol, *E* ethanol extract and *M* methanol extract). *Arrow* shows different bands after DPPH spray on TLC plates

the ferrous ions chelating ability of these studied mushroom could be highly beneficial to protect against oxidative damage.

Thin Layer Chromatography (TLC) Analysis for Antioxidant Constituents

Antioxidant constituents in different extracts of the studied mushrooms have been determined on TLC plate. Based on previous literature, different solvent systems were tested for detection of phenolic rich compounds in the mushroom extracts and the best solvent system was selected for further analysis. The TLC plates were developed with standardized solvent system toluene: ethyl acetate: acetic acid (5:5:1) and sprayed with DPPH reagent which is specific to detect the antioxidant constituents such phenols, flavonoids, alkaloids etc. Ethanol and methanol extract of *G. applanatum* gave two antioxidant constituents each with Rf values 0.65 and 0.66 respectively. In case of *G. lipsiense*, only ethanol extract gave three antioxidant constituents with Rf values 0.51, 0.57 and 0.64. *G. chalceum* gave one antioxidant constituent with ethanol extract (Rf value 0.64) and two with methanol extract (Rf values 0.64 and 0.87) whereas *G. tsugae* gave only one antioxidant constituent with methanol extract (Rf value 0.66). All the extracts of the studied mushrooms were compared with standard catechol that displayed only one spot with Rf value 0.84. The eluted compounds showed yellow color corresponding with antioxidant behavior (Fig. 5). Under the UV light the eluted spots showed blue, blue green, orange and yellow fluorescence. Mobile phase of the present study is more specific to phenols, flavonoids, alkaloids and that can be detected with specific reagent (DPPH reagent). The antioxidant properties of the mushroom extracts might be due to the presence of various bioactive compounds such as phenol, flavonoid, alkaloid etc. that possess diverse biological properties such as anti-inflammatory, antitumor, antioxidant and anti-microbial activity [33, 34].

Antibacterial Activity

The results of antibacterial screening of the studied wild mushrooms against five human pathogenic bacterial strains and their comparison with standard antibiotic tetracycline (50 µg/mL) are presented in Table 5. Ethanol and methanol extracts of *G. applanatum* displayed potential antibacterial activity only against *S. aureus* and *E. coli* (14.0–18.83 mm inhibition zone). The ethanol and methanol extract of *G. lipsiense* showed antibacterial activity only against *V. cholerae* (13.7–15.0 mm inhibition zone). All the three extracts of *G. chalceum* showed selective antibacterial activity against *P. aeruginosa* and *E. coli* (except aqueous extract) with the zone of inhibition ranging between 11.76–14.4 mm

Table 5 Antibacterial activity (inhibition zone in millimeter) of four *Ganoderma* species

Pathogenic strains	*G. applanatum*			*G. lipsiense*			*G. chalceum*			*G. tsugae*			*Tetracycline* (50 µg/ml)
	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	Ethanol extract	Methanol extract	Aqueous extract	
S. aureus	18.83 ± 1.25[a]	16.33 ± 1.52	ND	ND	ND	ND	ND	ND	ND	14.53 ± 0.50	13.16 ± 0.47	15.43 ± 0.81	23.33 ± 1.15
V. cholerae	ND	ND	ND	15.0 ± 0.40	13.7 ± 0.72	ND	ND	ND	ND	ND	ND	ND	20.66 ± 1.15
P. aeruginosa	ND	ND	ND	ND	ND	ND	11.76 ± 0.75	13.03 ± 0.28	13.96 ± 0.45	ND	ND	ND	35.66 ± 0.57
B. subtilis	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.06 ± 0.30	15.16 ± 0.28	15.93 ± 0.93	27.33 ± 0.57
E. coli	17.0 ± 1.0	14.0 ± 2.0	ND	ND	ND	ND	12.86 ± 0.80	14.4 ± 0.43	ND	12.66 ± 0.57	13.16 ± 0.56	14.56 ± 0.51	32.00 ± 2.00

[a] Results were expressed as the average of triplicate samples with mean ± SD ($n = 3$)

ND not detected

whereas *G. tsugae* exhibited antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli* (12.66–16.06 mm inhibition zone) (Table 5). Comparatively, *G. tsugae* showed high antibacterial activity against the human pathogens than other mushroom species. Tetracycline which was taken as the positive control displayed higher activity (20.66–35.66 mm inhibition zone) against all the pathogens. The antibacterial effects of the studied wild mushrooms are comparable with the previous data reported by several authors [50, 51]. All the extracts were used to measure potential health benefits taking advantage of the additive and synergistic effects of all the bioactive compounds present in the extracts as it has been noted in other findings [52, 53]. Therefore, mushrooms might be used not only for their nutritional properties but also as a source for the development of drugs and nutraceuticals. Antibacterial activity of the studied mushrooms in most cases were not detected and were very low, that might be due to their lower content of bioactive compounds. The presence of phenolic compounds in the mushroom extracts might also be another reason for the different results obtained in the antimicrobial study. Usually, pure active compounds reveal more activity than crude extracts. Searching wild sources could bring new natural products with antimicrobial properties that provide good protection against the infectious diseases.

Future Perspective

The finding of the present study suggests that wild mushrooms are an excellent source of natural antioxidants and antibacterial agents that could be used potentially by the pharmaceutical, and cosmetic industries in the development of drugs against various bacterial and free radical related diseases.

Conclusion

Studied *Ganoderma* mushrooms are good sources of nutrients with high carbohydrate and protein content, though they are generally non-edible in nature. Based on their bioactive compounds (vitamins and phenolics) and antioxidant potential, they might find applications in the prevention of free radical related diseases and could be used as source of potent nutriceuticals. Furthermore, all these mushrooms showed limited antibacterial activity against few pathogenic bacteria. Such antibacterial activity could be beneficial as nutraceuticals in the food supplements.

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