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Research Article

Phytochemical Screening and Free Radical Scavenging Activity of Seven wild Mushrooms Growing in University of Chittagong campus

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ABSTRACT

Objective: This study was undertaken to evaluate the phytochemical constituent and antioxidant activity of seven wild mushrooms of University of Chittagong campus.

Methods: Phytochemical screening was performed using standard methods while DPPH radical scavenging assay was used to elucidate the antioxidant effect of eight wild mushrooms of University of Chittagong campus.

Results: Results found from the quantitative analysis revealed the presence of alkaloids, glycosides, carbohydrates, steroids, tannins, flavonoids and saponins in the methanol extracts of different mushrooms. Both *Ganoderma* lucidum and *Ganoderma* applanatum showed significant ($P<0.001$) increase in the percentage of scavenging activity at 400 µg/ml concentration when compared with ascorbic acid. An increase in the scavenging activity of DPPH radical was found with the increasing concentration of the mushrooms extracts. The methanol extract of *Ganoderma* lucidum, *Ganoderma* applanatum and *Fomitopsis cajanderi* showed strong antioxidant activity with an IC50 value of 35.33, 38.73 and 39.44 µg/ml respectively in comparison with the IC50 value (49.19 µg/ml) of ascorbic acid. *Daedaleopsis confragosa* (IC50: 51.21 µg/ml) showed almost similar antioxidant capacity as the positive control.

Conclusions: The mushroom species investigated have been shown to be great sources of antioxidants and phytochemical constituents. So, these mushrooms can be used in the management of oxidative stress induced diseases.

Keywords: Mushrooms; Phytochemical screening; Antioxidants; Oxidative stress; DPPH

Graphical Abstract

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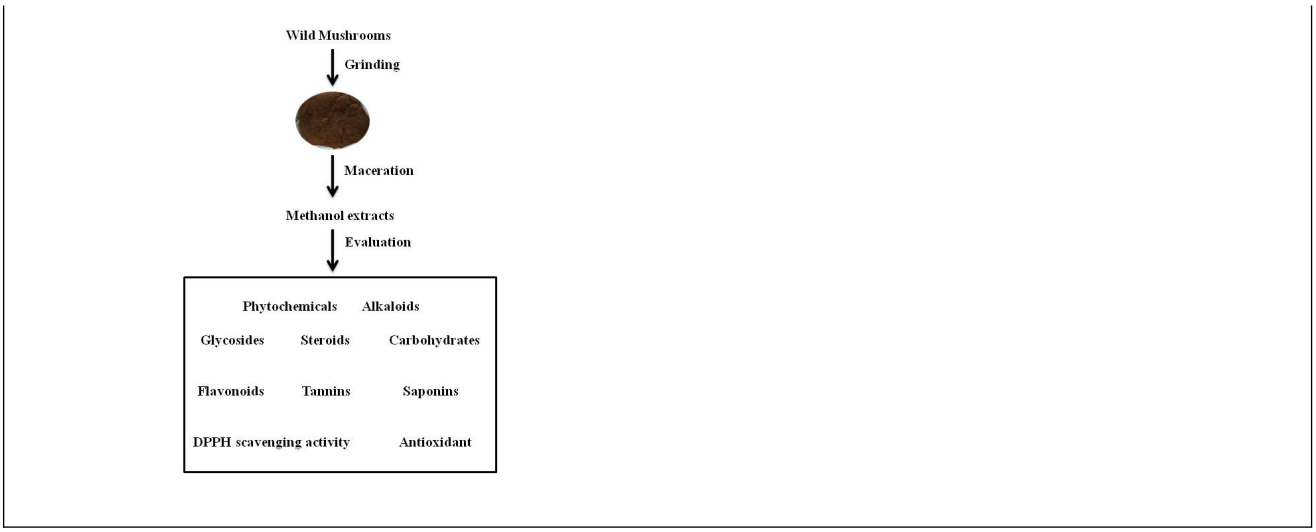

INTRODUCTION

Oxidation is a vital process in human which enables the transformation of nutrients such as carbohydrate, protein and fat into energy [1]. During this normal metabolic process, reactive oxygen species (ROS) are generated as a by-product. Highly reactive, unstable and partially reduced oxygen derivatives such as superoxide radicals (O2•–), hydroxyl radicals (•OH), hydrogen peroxide (H2O2), and singlet oxygen (1O2) are known as reactive oxygen species (ROS) [2]. At low-level ROS are crucial for various physiological processes and act as secondary messengers [3]. ROS at high concentrations may exert harmful effects cellular components such as DNA mutations, lipids peroxidation of membrane lipids and membrane protein damage [4]. The variation between the creation of ROS and antioxidant defence capacity of the body is known as oxidative stress [5]. Oxidative stress is responsible for causing several diseases such as cancer, atherosclerosis, cardiovascular disease, diabetes, metabolic disorders [6]. Antioxidants donate electrons to stabilize reactive oxygen species to prevent cell and tissue damage [7]. Endogenous antioxidants and exogenous antioxidants are two known types of antioxidants. The human body makes endogenous antioxidants which play an important role at low concentrations by scavenging the free radicals to keep maximum cellular functions. However, in case of oxidative stress, these endogenous antioxidants are found to be insufficient to protect the body from the harmful effect of reactive oxygen species. Diet or dietary supplements may be required as exogenous antioxidants to maintain optimal cellular function [8]. Nowadays, the industry which is responsible for producing food uses several synthetic antioxidants that have shown carcinogenicity. As a result, there is an urgency to search for antioxidants from natural sources [9]. Recently, consumption of mushrooms has increased greatly since they are high in carbohydrate, protein, fibre, essential amino acids and vitamins while low in fat, cholesterol, sodium and calories [10]. In Asian culture, the mushrooms have been used traditionally as a source of home remedy from long ago due to the presence of biologically active compounds to protect the body from various oxidative stress induced diseases [11]. Several scientific reports have reported the medicinal properties of mushrooms including free radical scavenging, antioxidant, immunomodulating, antitumor, antidiabetic, antihypercholesterolemia, antibacterial and antiviral effects [12]. Hence, the aim of this study was the evaluation of phytochemicals and the antioxidant potential of the wild mushrooms found in the campus of the University of Chittagong.

MATERIALS AND METHODS

Reagents

DPPH (1, 1-diphenyl, 2-picryl hydrazyl) was obtained from Sigma Chemical Co., USA. Ascorbic acid was purchased from SD Fine Chem. Ltd., Biosar, India. Other chemicals of analytical grade were supplied by the Department of Pharmacy, University of Chittagong.

Collection and identification of the mushroom

Seven naturally growing mushrooms including Lentinus Squarrosulus, Daldinia Concentrica, Trametes lactinea, Fomitopsis cajanderi, Daedaleopsis confragosa, Ganoderma applanatum and Ganoderma lucidum were collected from different areas of University of Chittagong campus. Specimens of the mushrooms were identified by Md. Owahidul Alom, Assistant Horticulture officer, Botanical garden, department of batany, university of Chittagong. The specimens accession number (Table 1) was given and preserved in department of pharmacy, University of Chittagong.

Table 1: Identified mushrooms with the accession number.

Mushroom	Family	Accession number
Ganoderma applanatum	Ganodermataceae	2018/004/ Fungi/CU/DP
Ganoderma lucidum	Ganodermataceae	2018/005/ Fungi/CU/DP
Lentinus squarrosulus	Polyporaceae	2018/007/ Fungi/CU/DP

Daldinia concentrica	Hypoxylaceae	2018/008/ Fungi/CU/DP
Trametes lactinea	Polyporaceae	2018/009/ Fungi/CU/DP
Fomitopsis cajanderi	Fomitopsidaceae	2018/010/ Fungi/CU/DP
Daedaleopsis confragosa	Polyporaceae	2018/011/ Fungi/CU/DP

Preparation of extract

After shade drying, the mushrooms were milled for efficient extraction. Exactly 100 gram of milled mushrooms powder was soaked in 500 ml methanol in a clean, sterilized and flat-bottomed glass container for seven days accompanying occasional stirring and agitation at room temperature. It was then filtered using filter papers (Whatman size no.1). The filtrate was allowed to evaporate the solvent by using a rotary evaporator. These extracts were kept in tightly closed glass containers and stored in the refrigerator for further use. The extracts of different mushrooms were named as follows: MELS = Methanol extract of *Lentinus squarrosulus*, MEDC1 = Methanol extract of *Daldinia concentrica*, METL= Methanol extract of *Trametes lactinea*, MEFC = Methanol extract of *Fomitopsis cajanderi*, MEDC2 = Methanol extract of *Daedaleopsis confragosa*, MEGL = Methanol extract of *Ganoderma lucidum*, METV = Methanol extract of *Trametes versicolor*, MEGA = Methanol extract of *Ganoderma applanatum*

Phytochemical screening

All of the methanol extracts of different mushrooms were qualitatively analyzed for the presence of different chemical groups, such as alkaloids, glycosides, steroids, carbohydrates, tannins, flavonoids and saponins [13, 14].

Antioxidant activity

Antioxidant activity of different mushrooms extracts was carried out using the method of Brand-Williams et al. [15]. 2 ml of each mushroom extract with different concentrations (12.5, 25, 50, 100, 200 and 400 µg/ml) were mixed with 3 ml of a 0.004% w/v methanol solution of DPPH. Then the tubes containing the mixture were kept at room temperature for 30 min in a dark place to complete the reaction. The absorbance was taken at 517 nm against a blank using a UV-visible spectrophotometer (Halo SB-10 single-beam spectrophotometer, Dynamica Scientific Ltd., UK). Ascorbic acid was used as a positive control. The capability to scavenge the DPPH radical was calculated from [(A0-A1)/A0] ×100, where A0 is the absorbance of the control reaction (DPPH + Methanol) and A1 is the absorbance of the sample.

Statistical analysis

All experiments were carried out in triplicate. The data are presented as mean ± standard deviation (SD). Significance of percentage scavenging effect of the extracts of mushrooms was determined by using the one-way analysis of variance (ANOVA) test, followed by Dunnet's t-test (2-sided) compared with the positive control. Values of $P < 0.001$ were considered significant. The data were analyzed using SPSS (Statistical Package for the Social Sciences) program (version 16.0 SPSS Inc., Chicago, IL, USA). The half-maximal inhibitory concentration (IC50) values were calculated by nonlinear regression analysis [log (inhibitor) vs. response ~ Variable slope (four parameters)] with the use of GraphPad Prism software version 6.01 (GraphPad Software, San Diego, CA, USA) and the chart was also drawn using the same software.

RESULTS

Phytochemical screening

(Table 2) shows the phytochemical components present in the methanol extract of mushrooms. This phytochemical screening revealed the presence of flavonoids and steroids in methanol extract of every mushroom. Alkaloids were absent in only Trametes lactinea and *Ganoderma* lucidum while glycosides were present in all except Lentinus Squarrosulus. This phytochemical analysis also showed that tannins were present in Lentinus Squarrosulus, Trametes lactinea, *Daedaleopsis confragosa* and *Ganoderma* lucidum. The presence of carbohydrates was not detected only in Trametes lactinea and *Daedaleopsis confragosa* among all other mushrooms. Saponins were present in all mushrooms except *Fomitopsis cajanderi* and *Ganoderma* lucidum.

Table 2: Phytochemical constituents present in the methanol extracts of the mushrooms.

Secondary Metabolite	MELS	MEDC1	METL	MEFC	MEDC2	MEGL	MEGA
Alkaloids	+	+	-	+	+	-	+
Glycosides	-	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+
Carbohydrates	+	+	-	+	-	+	+
Flavonoids	+	+	+	+	+	+	+
Tannins	+	-	+	-	+	+	-

Saponins	+	+	+	-	+	-	+

Antioxidant activity

Antioxidant activity of different mushrooms was determined by DPPH radical scavenging assay. At 400 µg/ml concentration both *Ganoderma* lucidum and *Ganoderma* applanatum showed significant (P<0.001) increase in the percentage of scavenging activity when compared with ascorbic acid. An increase in the scavenging activity of DPPH radical was found with the increasing concentration of the mushrooms extracts (Figure 1). The results indicated that methanol extract of *Ganoderma* lucidum, *Ganoderma* applanatum and *Fomitopsis cajanderi* showed strong antioxidant activity with an IC50 value of 35.33, 38.73 and 39.44 µg/ml respectively in comparison with the IC50 value (49.19 µg/ml) of ascorbic acid. *Daedaleopsis confragosa* (51.21 µg/ml) showed almost similar antioxidant capacity as the ascorbic acid. The IC50 value of the remaining mushrooms was less than 100 µg/ml except for Trametes lactinea. The IC50 values of all the mushroom extracts have been depicted in (Table 3).

Figure 1: DPPH scavenging activity of wild mushroom species of University of Chittagong campus.

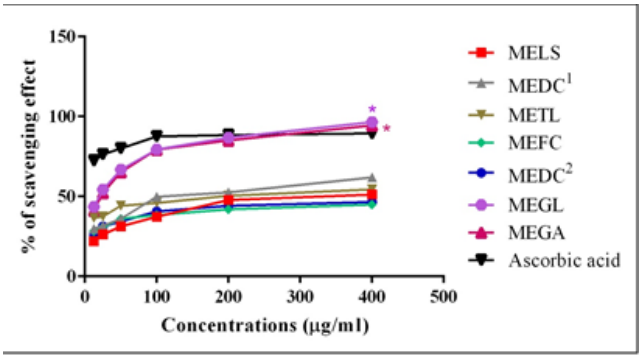

Table 3: Scavenging activity and IC50 values of mushrooms extracts.

Concentration	12.5 (µg/ml)	25 (µg/ml)	50 (µg/ml)	100 (µg/ml)	200 (µg/ml)	400 (µg/ml)	IC50 (µg/ml)
Samples	% Scavenging Activity						
MELS	21.88± 0.28	26.23± 0.26	31.25± 0.42	37.36± 0.54	47.75± 0.80	51.16± 0.08	96.63
MEDC1	29.84± 0.49	31.69± 0.17	36.02± 0.24	49.77± 0.17	52.50± 0.14	61.97± 0.08	97.13
METL	36.62± 0.04	37.43± 0.21	44.21± 0.14	45.88± 0.11	50.44± 0.11	54.44± 0.12	112.40
MEFC	28.26± 0.14	30.88± 0.04	36.06± 0.04	38.40± 0.07	41.94± 0.14	44.93± 0.14	39.44
MEDC2	26.41± 0.29	31.30± 0.24	34.35± 0.04	40.63± 0.12	44.14± 0.08	46.57± 0.11	51.21
MEGL	43.50± 0.04	54.26± 0.04	66.83± 0.04	79.40± 0.04	86.85± 0.04	96.53± 0.07*	35.33
MEGA	41.23± 0.04	52.20± 0.08	65.16± 0.04	79.35± 0.04	85.12± 0.04	94.47± 0.04*	38.73
Ascorbic acid	72.41± 0.04	76.25± 0.00	80.02± 0.04	87.59± 0.04	88.38± 0.08	89.38± 0.00	49.19

DISCUSSION

Mushrooms show different medicinal properties due to the presence of several phytoconstituents. Alkaloids are phytometabolites which contain various group of nitrogen. Alkaloids exhibit strong pharmacological actions that include analgesic, anti-inflammatory, antimalarial, antimicrobial, antiviral, anti-cancer, anti-ageing, cerebro-protective, muscle relaxant, sedatives and stomatics effects. Except for Trametes lactinea and *Ganoderma* lucidum, alkaloids were present in all mushrooms of this experiment. Glycosides are organic compounds formed of a sugar group (glycon) and non-sugar group (aglycon) linked together by a glycosidic bond. Glycosides are used as analgesic, anti-rheumatic, antibiotic, cardiotonic, demulcent and purgative agent. This study showed that glycosides were absent in only Lentinus Squarrosulus among all mushrooms. The presence of steroids was determined in every mushroom. Steroids are widely used for the treatment of inflammation and several autoimmune diseases. Anaesthesia can be induced by using steroids. Carbohydrate based therapeutics are widely used in the treatment of cardiovascular and haematological problems. Carbohydrates were not detected only in T. lactinea and *Daedaleopsis confragosa* among all other mushrooms. Saponins were present in Lentinus Squarrosulus, Daldinia Concentrica, Trametes lactinea, *Daedaleopsis confragosa* and *Ganoderma* applanatum mushroom. Saponins are one kind of plant glycosides which possess various pharmacological properties such as anti-viral, anti-inflammatory and anti-carcinogenic activities. Different pharmacological activities have shown phenolic compounds among which antioxidant and antimicrobial effects are more prominent. Several reports have also been suggested the utilization of flavonoids and many other phenolic compounds as free radical scavenging, anticancer, anti-inflammatory, cardioprotective and immune system promoting agents. Flavonoids were present in every mushroom while tannins were absent in Daldinia Concentrica, *Fomitopsis cajanderi*, and *Ganoderma* applanatum. Due to the presence of phenolic compounds like flavonoids and tannins, every mushroom has shown free radical scavenging activity to some extent. DPPH is a free radical which is stable and synthetic. A stable diamagnetic molecule is created when DPPH accepts an electron or hydrogen atom. The DPPH radical gives absorbance at 515-517 nm due to the presence of an odd electron in it. Because of this odd electron, it also produces a purple color solution in methanol. When this purple color solution is mixed with a substance with antioxidant molecules that can donate an electron or hydrogen atom, it becomes decolorized. As a result,

there is a change in the absorbance will be observed. A lower absorbance at 517 nm indicates a higher radical-scavenging activity of the extract [23, 24]. A dose dependent increase in DPPH scavenging activity of all mushrooms extracts is observed. A significant (P<0.001) increase in the scavenging of DPPH radical is observed at 400 µg/ml concentration of both *Ganoderma* lucidum and *Ganoderma* applanatum with the comparison of positive control. Half inhibitory concentration, IC50 is used to express antioxidant activity. It refers to the dose of antioxidant necessary to reduce by half of the initial DPPH radical concentration. So, a lower IC50 represents higher antioxidant activity. The results obtained from this study reported that a strong antioxidant activity with an IC50 value of 35.33, 38.73 and 39.44 µg/ml showed by *Ganoderma* lucidum, *Ganoderma* applanatum and *Fomitopsis cajanderi* mushroom respectively when compared with the IC50 value (49.19 µg/ml) of positive control. *Daedaleopsis confragosa* showed an IC50 value of 51.21 µg/ml which is almost similar to the IC50 value (49.19 µg/ml) of ascorbic acid. So, *Daedaleopsis confragosa has shown antioxidant capacity as like as ascorbic acid.* The IC50 value of the remaining mushrooms was less than 100 µg/ml except for Trametes lactinea suggesting mild antioxidant activity compared to ascorbic acid. The major source of exogenous antioxidants is the phytochemicals of the plants. Antioxidants can scavenge of the reactive species which are responsible for causing oxidative stress. At low concentrations, antioxidants can prevent the oxidation of substrate. Antioxidant is a great choice in the treatment of oxidative stress induced diseases such as cardiovascular diseases, cancer and diabetes. One of the main contributing factors in causing atherosclerosis is the oxidation of low density lipoprotein. Antioxidants block the oxidation of low density lipoprotein to prevent atherosclerosis. Reactive oxygen species are responsible for the promotion of cell migration and invasion in metastatic cancer cells. Reactive oxygen species scavenging potentials of antioxidants helps to prevent the development of cancer. Not only diabetes but also diabetic complications such diabetic neuropathy is induced by oxidative stress. Superoxide a reactive oxygen species contributes to the dysfunction of endothelial in diabetes mellitus. Antioxidants fight with the oxidative stress in diabetes to reduce the hyperglycemic stage. So, the investigated mushrooms with antioxidants can be used to prevent diseases.

CONCLUSION

The results of the present study exhibited that *Ganoderma* lucidum, *Ganoderma* applanatum and *Fomitopsis cajanderi* mushroom exerts strong antioxidant effects. This could be due to the presence of phenolic compounds like flavonoids and tannins in these mushrooms. Therefore, these mushrooms can be used in the management of oxidative stress induced diseases.

REFERENCES

1. Sánchez C. Reactive oxygen species and antioxidant properties from mushrooms. Synth Syst Biotechnol. 2016; 2(1):13-22.
2. `Chio IIC, Tuveson DA. ROS in Cancer: the burning question. Trends Mol Med. 2017;23:411–29.
3. Rajendran P., Nandakumar N., Rengarajan T., Palaniswami R., Gnanadhas E.N., Lakshminarasaiah U. Antioxidants and human diseases. Clin Chim Acta. 2014;436:332–347.
4. Karim, M.A., et al., Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (Bruguiera gymnorrhiza and Heritiera littoralis) in Bangladesh. Clinical Phytoscience, 2020. 6: 8.
5. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, Gargiulo G, Testa G, Cacciatore F, Bonaduce D, Abete P. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018 26;13:757-772.
6. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. Oxidative Stress: Harms and Benefits for Human Health. Oxid Med Cell Longev. 2017;2017:8416763.
7. S. Khatua, S. Paul, K. Acharya Mushroom as the potential source of new generation of antioxidant: a review Res. J. Pharm. Technol. 2013; 6 (5) 496-505.
8. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutr J. 2016;15(1):71.
9. Abdullah, N., et al., Evaluation of Selected Culinary-Medicinal Mushrooms for Antioxidant and ACE Inhibitory Activities. Evidence-based complementary and alternative medicine : eCAM, 2012. 2012: 464238.
10. Rashidi, A. and T. Yang, Nutritional and Antioxidant Values of Oyster Mushroom (P. sajor-caju) Cultivated on Rubber Sawdust. International Journal on Advanced Science, Engineering and Information Technology, 2016. 6: 162.