QUALITATIVE PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT AND *IN-VITRO* ANTIOXIDANT ACTIVITY IN METHANOLIC EXTRACTS OF *MORCHELLA ESCULENTA Fr.*

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ABSTRACT

Among wild edible species of mushrooms, morels rank first in choice and delicacy and have been one of the highly prized wild edible mushrooms in the world. The bioactive components present in *Morchella esculenta* are responsible for the nutraceutical potential of the mushroom. The present study was carried out to assess the phytochemical screening, total phenolics and antioxidant activities of methanolic extracts of *M. esculenta*. Qualitative phytochemical analysis showed the presence of alkaloids, anthraquinone, anthocyanins, tannins, saponins, glycosides, flavonoids, terpenoids, phenols, carbohydrates as well as proteins and amino acids, only steroids are absent. The total phenolic content of methanolic extracts of morel was 238.52±0.021 (mg gallic acid equivalents per gram weight). The *in-vitro* antioxidant potential was analyzed by DPPH and hydrogen peroxide method. The DPPH scavenging activity was 85.2±0.371 % and peroxide was 84.1±0.281 % at 500 µg/ml concentration, comparable to that of ascorbic acid.

Keywords: morels, phytochemical screening, DPPH, hydrogen peroxide, antioxidant potential

INTRODUCTION

Human beings have been constantly searching for food sources that can improve biological functions and make people healthier, fitter, and to live longer. Mushrooms are the fungi that have been used as food since times immemorial [1].Wild edible mushrooms fall under the category of non- timber forest products (NTFP) which have been untapped resources because a wide variety of wild mushrooms are still unexplored [2].Wild edible mushrooms have long history of medicinal usages in addition to their nutritional value and have a valuable source of biologically active compounds [3, 4]. From India, many species of edible fungi have been reported to be traditionally and regularly consumed by the local inhabitants without causality and fatality. Wild edible mushrooms having very good commercial value are species of *Morchella, Helvella, Hericium, Sparassis, Hydnum, Trapeinda, Clavaria, Ramaria, Boletus, Albatrellus, Cordyceps, Lactarius* and *Rusulla* etc. [5]. All these wild edible mushrooms are consumed as well as sold fresh, collected and dried for sale [6,7].

In the North-West Himalayan region of India many species of mushrooms are collected and consumed. Among the wild edible varieties of mushrooms, the morels (*Morchella* spp.) have been consumed the most because of the indigenous nutritional and health benefits. Morels are among the most highly prized of all the wild harvested mushrooms, and commonly called as 'Guchhi' in the Indian market. This mushroom is collected from the wild and is exported to many countries for its excellent culinary properties [9-12]. In North West Himalayas, seven different species of *Morchella* have been analyzed for their nutritional and nutraceutical potential [7].

In the recent years, much attention has been paid to the investigation of nutraceuticals from various edible mushrooms [13]. Although the nutritional facts, culinary and indigenous medicinal uses of morels are well accepted all over the world, but the medicinal qualities have yet to make into the mainstream. *M. esculenta* is one of the dominantly found morel species in North –West Himalayas, and the ethnobotanical data gathered also reveals that this mushroom species has great nutritional and indigenous health benefits. The present study evaluates the quantitative phytochemicals, total phenolic content, *in-vitro* antioxidant activity in methanolic extracts of *M. esculenta;* so that it's unexplored nutraceutical potential can further be exploited.

MATERIAL AND METHODS

Sample collection

The fresh mushroom species were collected from the Northwest Himalayan region of Shimla, India (31º 6' 12" N; 77º 10' 20" E). The fruiting bodies were thoroughly cleaned of extraneous matter and dried completely and coarsely grounded.

Preparation of extracts

Grounded mushroom was extracted with solvent - methanol at room temperature prior to removal of solvent. 10 grams of the ground sample was mixed with six times of 99.6% methanol and kept for 24 hours. This process was repeated thrice and filtrates were collected. The filtrates obtained were concentrated under vacuum on a rotary evaporator (Buchi Rotary Evaporator, Model R-124) and stored at 4 °C for further use [14].

Quantitative Phytochemical screening

Methanolic extracts of *M. esculenta were* used for qualitative screening of phytochemicals as per standard biochemical procedures. The preliminary tests for methanol extracts were performed to confirm the presence of alkaloids, anthraquinones, anthocyanins, carbohydrates, flavonoids, glycosides, phenols, proteins and amino acids, saponins, steroids, tannins and terpenoids [15].

Estimation of Total Phenolic content

The total phenolic content in methanolic extracts of grounded sample was estimated by Folin-Ciocalteu reagent, as described by Singleton and Rossi [16]. 100 mg of gallic acid was dissolved in 100 ml ethanol to prepare Gallic acid stock solution (1000 µg/ml). Various dilutions of standard gallic acid were prepared from this stock solution. 1 ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 and 100 µg/ml of gallic acid solution were mixed with 5.0 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4.0 ml of sodium carbonate solution (75 g/l) and calibration curve was plotted. The absorbance was measured after 30 min at 20 °C at 765 nm. 1 ml extract was mixed separately with the same reagents and absorbance was measured at 765 nm after 1 hour. The total phenolic compound in the extract was determined using the formula:

$$C = C_1 \times {}^{V}/_{m}$$

C= Total content of phenolic compounds in mg/g in GAE (Gallic acid equivalent); C_1= The concentration of gallic acid established from the standard curve in mg/ml; V=The volume of extract in ml, m =Weight of extract in grams.

In-vitro evaluation of antioxidant activity

In-vitro antioxidant activity of the extract of *M. esculenta* was determined by using two different methods: DPPH and hydrogen peroxide radical smethods.

Free radical scavenging activity using DPPH method: The free radical scavenging activity of extracts were measured by 1, 1-dipheny l-2-picrylhydrazyl (DPPH) [17]. 0.1 mM solution of DPPH in ethanol was prepared and 1.5 ml of this solution was added to 0.5 ml of extract solution in ethanol at different concentrations (50-300 µl/ml). The mixture was shaken and allowed to stand at room temperature for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer (UV- VIS, Systronics). A blank without DPPH was used to remove the influence of the color of the extracts and an ethanolic solution of DPPH was used as a negative control. Ascorbic acid was used as a reference. All of the measures were carried out in triplicates. The DPPH radical scavenging activity was calculated using the following equation:

$$DPPH\ scavenging\ effect\ (\%) = \frac{Ao - As}{Ao} \times 100$$

where, A_0 is the absorbance of negative control, A_s is the absorbance of sample respectively.

Free radical scavenging activity using hydrogen peroxide: The free radical scavenging activity of different extracts was determined by using hydrogen peroxide radical [18]. An aliquot of 0.6 ml of hydrogen peroxide (43Mm) and 1.0 ml of various concentrations of extracts prepared using phosphate buffer (200-400 μg/ml) were mixed followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4). The resulting solution was kept for 10 minutes and the absorbance was recorded at 230 nm. All measures were taken in triplicates. For each concentration, mixture without sample was taken as a control and a mixture without hydrogen peroxide was taken as a blank. Ascorbic acid was used as a standard compound. The percentage scavenging activity of hydrogen peroxide was calculated as:

$$Scavenging\ activity\ (\%) = \frac{Ao - As}{As} \times 100$$

where, A_o is the absorbance of negative control, A_s is the absorbance of sample respectively.

Statistical Analysis

All of the measurements were performed in triplicates. The data is given either as ±SD or mean ± S.E.M.

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *M. esculenta* extracts showed the presence of alkaloids, anthraquinone, glycosides, tannins, saponins, flavonoids, terpenoids, phenolics, carbohydrates, anthocyanins as well as proteins and amino acids and steroids were found (Table 1). The results revealed that *M. esculenta* may be used as potential sources of phytochemicals and thus can be used for designing drugs that can prove to be of keen interest in the treatment and prevention of diseases like cancer, tumor, heart diseases, etc.Very less work has been done on the phytochemical screening of morels. Duyilemi and Lawal [19] investigated the antibacterial activity and phytochemical screening of *Chrysophyllum albidum* leaves. Egwim *et al.* [20] studied the proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms. Johnsy and Kaviyarasan [21] studied the preliminary phytochemical screening, antimicrobial and antioxidant activity of methanolic and aqueous extracts of *Lentinus sajor- caju*.

Table 1: Qualitative phytochemical screening of *M. esculenta* methanolic extracts

S. No.	Phytochemical	*M. esculenta* extracts
1	Alkaloids	+
2	Anthraquinone	+
3	Anthocyanins	+
4	Proteins and amino acids	+
5	Carbohydrates	+
6	Phenols	+
7	Terpenoids	+
8	Sterols	-
9	Saponins	+
10	Glycosides	+
11	Flavonoids	+
12	Tannins	+

NOTE: (+): Shows the presence of phytochemicals; **(-)**: Shows the absence of phytochemical

The amount of total phenols was determined with Folin- Ciocalteu reagent. Gallic acid was used as standard compound. The standard curve of gallic acid concentrations and absorbance is shown in Fig. 1. The absorbance for various dilutions of gallic acid with Folin- Ciocalteu reagent and sodium carbonate were found.The total phenolic content of methanol extracts of *M. esculenta* was 238.52±0.0012 with absorbance at 765 nm was 2.59±0.0012 (mg gallic acid equivalents per gram weight). Data expressed as mean ± standard error of three samples analyzed separately.

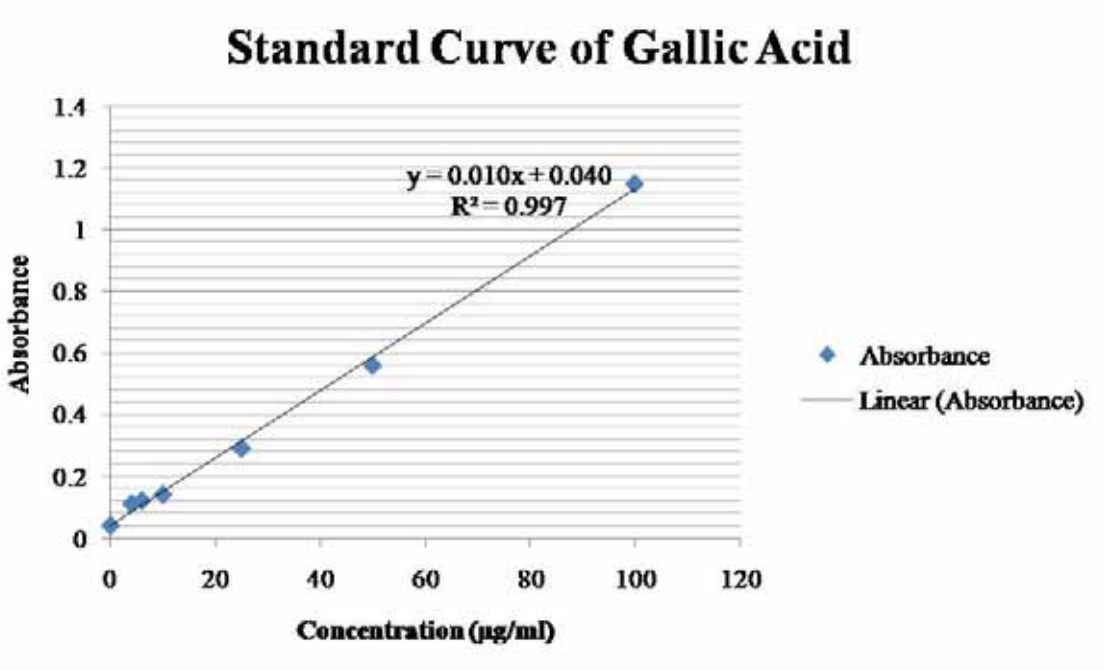

Figure 1. Calibration curve of gallic acid

Antioxidant activity of sample extract at different concentrations was determined using two different techniques viz. DPPH method and H_2O_2 method. The DPPH is a stable organic free radical with an absorption maximum band around 515-528 nm [22] and is widely used for evaluation of antioxidant potential of compounds. Results are presented in Table 2 and Table 3. The results obtained from both the methods revealed that *M. esculenta* exhibits high antioxidant activity. At the concentration of 500µg/ml, the extracts showed 85.20% and 84.12% scavenging activity by DPPH and hydrogen peroxide method respectively. The methanolic extracts of *M. esculenta* showed good antioxidant activity as evaluated by both the methods.

Table 2. Percentage scavenging activity of the *M. esculenta* methanolic extracts using 1,1-diphenyl-2-picrylhydrazyl radical

Concentration (µg/ml)	Percent scavenging activityof DPPH radical	
	Ascorbic acid	***Morchella* extract**
100	48.08 ± 0.136	32.33 ± 0.3142
200	58.59 ± 0.2423	32.95 ± 0.4307
300	75.45 ± 0.3153	56.88 ± 0.3142
400	76.89 ± 0.3579	76.89 ± 0.4307
500	87.89 ± 0.3724	85.20 ±0.3710

Values are the average of triplicate experiments and represented as mean ±S.E.M

Table 4. Percent scavenging activity of the *M. esculenta* methanolic extracts using hydrogen peroxide radical

Concentration of extract (µg/ml)	Percent scavenging activity of H_2O_2	
	Ascorbic acid	**Morchella extract**
100	51.56 ± 0.1453	55.95 ± 0.0233
200	61.19 ± 0.5008	60.23 ± 0.0726
300	77.15 ± 0.0145	74.42 ± 0.0176
400	78.80 ± 0.1152	77.72 ± 0.0240
500	87.46± 0.1152	84.12 ±0.2810

Values are the average of triplicate experiments and represented as mean ±S.E.M

Various other scientists have also worked on the antioxidant activity of morels. Mau *et al*. [23] and Ferreira *et al*. [24] investigated the antioxidant properties of several wild and medicinal mushrooms. Mau *et al*. [25] investigated the antioxidant properties of ethanolic extracts from *Grifola frondosa*, *M. esculenta* and *T. albuminosus* mycelia. Gursoy *et al*. [26] studied the Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. Wagay [27] studied the phenolic quantification and anti-oxidant activity of *M. esculenta*. Hamzah *et al.* [28] studied the phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms.

CONCLUSION

The present finding highlights that consumption of diets rich in wild edible mushrooms is associated with various health benefits. The mushroom species due to presence of phenolic compounds have antioxidant activities and delay the oxidative damage in the human body. Arising from the awareness of the relationship between diet and disease has evolved the concept of nutraceuticals. In essence, mushrooms nutraceuticals are foods that are eaten not only to satisfy functional dietary needs but also elicit additional health needs.

REFRENCES

[1] Rahi DK *et al*. (2004). Mushrooms and their sustainable utilization. *Everyman's Sci*. 38: 357-365.

[2] Devkota S. (2008). Distribution and status of highland mushrooms: a study from Dolpa, Nepal. *J. Nat. History Mushrooms.* 23: 51-59.

[3] Crisan EV and Sands A. (1978). Nutritional value.the biology and cultivation of edible mushrooms. Chang ST; Hayes, WA Eds.; Academic Press: London. pp. 137-168.

[4] Rajewska and Balasinka. (2004). Biologically active compounds of edible mushrooms and their beneficial impact on health. *Postepy Hig Med Dosw*. 58: 352-357.

[5] Lakhanpal TN. (2000). Wild and cultivated mushrooms. Natural Resources and Development in Himalaya. (ed. L.R. Verma). Malhotra Publishing House, New Delhi, pp. 402-417.

[6] Lakhanpal TN and Rana M. (2005). Medicinal and Nutraceutical genetic resources of mushrooms. *P G Res – Charact & Utiliz.* 20: 1-17.

[7] Lakhanpal TN *et al*. (2010). Biology of Indian Morels. I K International Publishing House, pp. 266.

[8] Dermek A. (1982). Mushrooms and other fungi. Galley Press. England. pp. 223.

[9] Lakhanpal TN and Shad OS. (1986a). Studies on wild edible mushrooms of Himachal Pradesh (N.W. Himalayas)—I. Ethnomycology, production and trade of Morchella species. *Indian J. Mushroom* 12/13: 5–13.

[10] Lakhanpal TN and Shad OS. (1986b) Studies on wild edible mushrooms of Himachal Pradesh (N.W. Himalayas)—II. Ecological relationships of *Morchella* species. *Indian J. Mushroom.*12/13: 15–20.

[11] Shad OS. (1989) Biological studies on *Morchella* species (morels) of Himachal Himalayas. Ph D. Thesis, H.P. University, Shimla.

[12] Rana M. (2002) Ethnobotanical studies on the morels of district Kinnaur (H.P.). M.Phil. Dissertation, Himachal Pradesh University, Shimla.

[13] Ye M *et al*. (2011). Purification, characterization and hypoglycemic activity of extracellular polysaccharides from *Lachnum calyculiforme. Carbohyd. Polym*. 86: 285–290.

[14] Song WY *et al*. (2010). Effect of ethanol extracts from red pepper seeds on antioxidative defense system and oxidative stress in rats fed high-fat, high-cholesterol diet. *Nutr. Res. Pract*. 4: 11-15.

[15] Tiwari P *et al*. (2011). Phytochemical screening and extraction: a review. *Intern. Pharmaceu. Sci.* 1: 98-106.

[16] Singleton VL and Rossi JA. (1965). Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *Am. J. Enol. Vitic*. 6: 144-158.

[17] Kaur P *et al*. (2008). *In vitro* evaluation of free radical scavenging activity of *Rubia cordifolia* L. *J. Chinese Cli. Med*. 3: 278-284.

[18] Mohamad A *et al*. (2010). Antioxidant and free radical scavenging activity of *H. officinais* H, var. *Angustifolia*, *V. odorata*; *B. hyrcana*; *Speciosu*m. *Pak. J. Pharma. Sci*. 23: 29-34.

[19] Duyilemi OP and Lawal IO. (2009). Antibacterial activity and phytochemical screening of *Chrysophyllum albidum* leaves. *Asian J. Food Agro-Indus*. S75-S79.

[20] Egwim EC *et al*. (2011). Proximate composition, phytochemical screening and antioxidant activity of ten selected wild edible Nigerian mushrooms. *American J. Food Nutr.* 1: 29-94.

[21] Johnsy G and Kaviyarasan V. (2014). Preliminary phytochemical screening, antimicrobial and antioxidant activity of methanolic and aqueous extracts of *Lentinus sajor-caju. World J. Pharm. Pharmaceutical Sci.* 3: 1459 – 1472.

[22] Stankovic MS (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Svi*. 33: 63-72.

[23] Mau JL *et al*. (2002). Antioxidant properties of several medicinal mushrooms. *J. Agri. Food Chem.* 50: 6072-6077.

[24] Ferreira ICFR *et al*. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chem.* 16: 1543-1560.

[25] Mau JL *et al*. (2004). Antioxidant properties of ethanolic extracts from *Grifola frondosa, Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chem*. 87: 111-118.

[26] Gursoy N *et al*. (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. *Food and Chem. Toxicol*. 47: 2381-2388.

[27] Wagay JA. (2011). Phenolic quantification and anti-oxidant activity of *Morchella esculenta. International J. Pharma and Biosc*. 2: 188-197.

[28] Hamzah RU *et al*. (2014). Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. *Asian Pacific J. Trop. Disease* 4: S153-S157.