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Antioxidant Activity of *Morchella conica* Collected from Swat Valley

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Authors' contributions

This work was carried out in collaboration between all authors. Author SF designed and practically conducted the study. Author ZI helped in designing the experiment and supervised all in-situ procedures. Author MI provided the chemicals facility and helped in data collection. Author SI critically analyze the data statistically. Author FSH provided lab equipment and facilitated throughout the study period both practically and help in write up. Author AW practically involved in experiment. Author Sohail Aslam made computer write up and initial setting. Authors MB and Seemab Ali wrote the protocol and write up of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study was conducted at the University of Agriculture Peshawar, KPK, during the year of 2014- 2015. Phenolic content of mushrooms is considered as an excellent antioxidant and synergist. The study was therefore conducted to determine the antioxidant activity of water extract of mushroom, *Morchella conica,* at different methanolic concentrations from 100-3000 ppm. Free radical scavenging activity was used for determination of antioxidant property of *M. conica*. Results of different methanolic concentrations of *Morchella* water extract showed increase in antioxidant capacity with increase in concentration. The antioxidant activity of *M. conica* was recorded 44.86 ± 0.94, 62.72± 1.64, 74.14 ± 2.63, 83.35 ± 0.313, 85.01 ± 0.80, 86.53 ± 0.22, 88.03 ± 0.35, 88.56 ± 0.37 and 93.53 ± 0.01 respectively at 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm.

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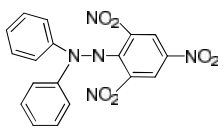
1. INTRODUCTION

Oxidative stress is a non-controlled production of free radicals and has been related to so many diseases including several kinds of cancer, diabetes, cirrhosis, cardiovascular diseases, and neurological disorders. Free radicals production occurs due to natural factors like inflammation processes or excessive exercise, or non-natural factors such as the presence of xenobiotics in the organism or situations causes several diseases. These radicals can start chain reactions in the body's cell and ultimately cause damage or death to the cell. Antioxidants are substances that terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions.

Free radicals are produced in normal cell metabolism and sometimes are very essential for the production of energy. However various exogenous and endogenous processes in human body or in food might produce highly reactive free radicals which have the ability to oxidize biomolecules resulting cell death [1]. Normally almost all organisms are well protected against free radical damage by oxidative enzymes like superoxide dismutase and catalase [2]. This mechanism of antioxidant protection becomes affected by some factors such as aging. However there are certain antioxidant supplements or food containing antioxidant compounds may reduce the oxidative damage [3]. Many species of fruit, vegetables, and cereals have high antioxidant activity [4]. Natural antioxidants are studied for their capacity to protect the organism's cells from damage [5].

1, 1-Diphenyl-2-2-picrylhydrazyl (DPPH) is a well-known scavenger for free radicals and is used as an indicator of the radical nature of that reaction. Because of a strong absorption at about 517 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 517 nm [6].



DPPH ($C_{18}H_{12}N_5O_6$) structure

Morchella conica is a macro fungal species that belongs to genus *Morchella* and family *Morchelleceae* [7]. Mushrooms or morels are used by the people because of their supreme taste and highly antioxidant activity. These are normally grown in temperate areas of the world. In Pakistan it is found in regions including Swat valley and Hindu Kush Mountains. *Morchella conica* mostly grows in Pine Hills under pine trees and makes 48% of the total morels collection of Swat [8].

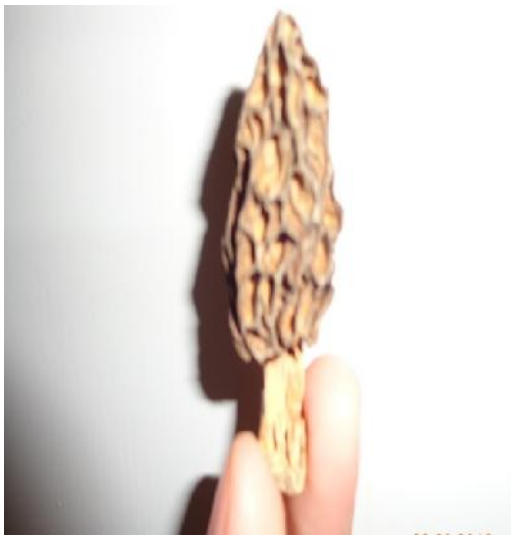


Fig. 1. A *Morchella conica*

Aim of this research was to determine *Morchella conica* for its total antioxidant capacity at different concentrations.

2. MATERIALS AND METHODS

Pre-identified and dried *Morchella conica* were obtained from Swat valley and were used for the determination of antioxidant activity. All work was performed in the laboratory of Agricultural Chemistry, The University of Agriculture Peshawar, Pakistan during the year of 2015. The experiment was laid out in a complete Randomized Design in Lab with three replication. Dried *Morchella* samples were powdered and weighed (1 g) and then soaked in 100 mL water on shaking condition at 150 rpm at room temperature for 24 h and then filtered. The residues were extracted 3 times under the same condition. The filtrate was dried using a rotary evaporator at 40°C. The extract was placed in a glass vial and then stored at 4°C to prevent

oxidative damage until analysis for a week. Free radical scavenging activity of the *Morchella conica* extracts were measured from bleaching of the purple-colored (0.04%) methanol solution of 1,1-diphenly-2-picrylhydrazyl (DPPH) [9].

For this purpose DPPH solution (0.04%) in methanol was prepared. From this DPPH (0.04%) solution, 1 ml was added to the 100, 150, 200, 250, 300, 500, 1000, 2000, 3000 ppm methanol solution of the water extract of the selected morel. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 517nm in a spectrophotometer. The capability to scavenge the DPPH radical was calculated as:

$$I\ (\%) = [(A\ blank - A\ sample)/A\ blank] \times 100$$

Inhibition (I) of free radical by DPPH in percent (I (%)) was calculated as follows:

Where A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. Experiment was repeated three times. Data values were represented as mean of three replicates with standard deviation (mean ± S.D).

3. RESULTS AND DISCUSSION

The water extract of selected morel was subjected to screening for their antioxidant activity. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As the antioxidant donates protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts can be measure by DPPH assay. All concentrations (100-3000 ppm) studied, showed free radical scavenging activity.

3.1 Antioxidant Activity of Water Extracts

Methanolic solution of the water extract of *M. conica* showed 1.301, 0.852, 0.551, 0.392, 0.357, 0.330, 0.287, 0.265 and 0.155 Au absorbance at 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm concentration as shown in Table 1.

The antioxidant activity (%) of the 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm methanolic solution of the water extract of *Morchella conica* was recorded *as* 44.86 ± 0.94, 62.72± 1.64, 74.14 ± 2.63, 83.35 ± 0.313, 85.01 ± 0.80, 86.53 ± 0.22, 88.03 ± 0.35, 88.56 ± 0.37 and 93.53 ± 0.01 respectively. The maximum antioxidant activity (93.53 ± 0.01) of the water extract was observed with the concentration 3000 ppm whereas minimum antioxidant activity (44.86 ± 0.94) was observed with the concentration 100 ppm (Table 1). Similarly the maximum concentration showed [10] that increase in concentration also increases the antioxidant activity. The present study indicated that higher concentration of the water extract of *Morchella conica* showed higher % antioxidant activity (Table 1).

From Table 1 it is clearly indicated that antioxidant potential of *M. conica* increases with increase in concentration of its water extract. Therefore it can be concluded that water extract of *Morchella conica* has a highly antioxidant property. Among all the methanolic concentrations of *Morchella conica* water extract, 3000 ppm showed higher antioxidant activity while 100 showed minimum activity. This increase in activity might be due to the presence of more antioxidant metabolites in the water extract of *M. conica*. Literature reported many such types of studies where with increase in concentration of mushroom extract, increase in antioxidant potential occurs.

Table 1. Mean absorption and percent antioxidant activity of the water extract of *Morchella conica* detected at 517 nm using spectrophotometer

S. no	Concentration (ppm)	Absorption	Antioxidant potential (%)
1	100	1.301	44.86 ± 0.94
2	150	0.852	62.72 ± 1.64
3	200	0.551	74.14 ± 2.63
4	250	0.392	83.35 ± 0.313
5	300	0.357	85.01 ± 0.80
6	500	0.330	86.53 ± 0.22
7	1000	0.287	88.03 ± 0.35
8	2000	0.265	88.56 ± 0.37
9	3000	0.155	93.53 ±0.01

The current findings are according to the finding of Turkoglu [11] which showed that with the increase of phenolic acid content in mushroom, increase in antioxidant activity occurs. The current study showed that *Morchella conica* water extract might contain more phenolic acid content so having high antioxidant activity. According to this, it is possible that the high inhibition value of water extract is due to the high concentration of phenolic compounds. The important role of phenolic compounds as scavengers of free radicals is emphasized in several reports.

The current findings are in collaboration with the findings of Gursoy [12]. In the case of DPPH, methanol extract of *Morchella. conica* showed high antioxidant activity. The reducing power of the methanol extracts of mushrooms increased with concentration. The current study indicated that DPPH standard showed highly antioxidant activity at 517 nm in the water extract of *Morchella conica* and might be highly reactive against mutagenic and carcinogenic diseases.

Maintenance of free radical production and antioxidant defenses is an essential condition for normal organism functioning. When this balance has a tendency for the production of free radicals it is considered that the organism is in oxidative stress. In this condition, the excess of free radicals may damage cellular lipids, proteins and DNA, affecting normal function and leading to various diseases. In aerobic organisms, the free radicals are constantly produced during the normal cellular metabolism, mainly in the form of Reactive Oxygen Species and Reactive Nitrogen Species.

Exposition of the organism to free radicals has led to the development of endogenous defense mechanisms to eliminate them. These defenses were the response of evolution to the inevitability of Reactive Oxygen Species production in aerobic conditions. Natural products in *Morchella conica* with antioxidant activity may help the endogenous defense system. The antioxidants present in the diet assume a major importance as possible protector agents reducing oxidative damage. Mushrooms that possess high antioxidant activity like *M. conica* can be use directly in diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present.

The present study found that *Morchella conica* has a very high antioxidant activity in its water extract which may contain a larger amount of total phenolic content in the extract [13].

The antioxidant activity of mushroom extracts with stronger inhibition of lipid peroxidation occurring at high concentrations of the extracts. The possible mechanism of antioxidant activity of mushroom extracts includes scavenging of free radicals possibly through hydrogen-holding capacity and oxidation by peroxy radicals [14].

4. CONCLUSION

Screening of different methanolic concentrations of water extract of *M. conica* showed increase in antioxidant activity with increase in concentration. It is therefore concluded that water extract of *Morchella* possesses a strong antioxidant activity with highest potential in 3000 ppm water extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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