

ANTIOXIDANT ACTIVITY OF *MORCHELLA CONICA* COLLECTED FROM SWAT VALLEY

Abstract

The present study was conducted University of Agriculture Peshawar, KPK, during the year 2014- 2015. Mushroom phenolic considered as an excellent antioxidant and synergist. The main objective of this study was to determine the antioxidant activity of *Morchella conica* commonly called black morels at different concentration. For this purpose the *Morchella conica* extract was used to determining the free radical scavenging activity. The results showed that increase in the concentration increases the antioxidant activity. The antioxidant activity of *Morchella 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.

Keywords: Mushroom; antioxidant; synergist; *Morchella conica*; KPK

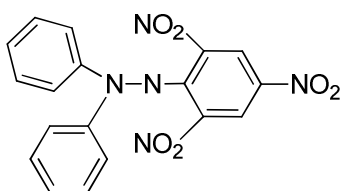
Introduction

Oxidative stress is the 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 productions occurs due to natural factors like extreme exercise or inflammation processes, or non-natural factors such as the presence of xenobiotics in the organism or situations causes several diseases. In turn, 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 to many living organism 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 oxidizing the biomolecules resulting the 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, deterioration of physiological functions. 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 highly 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 (C₁₈H₁₂N₅O₆) structure

Morchella conica is a fungal species belong to the genus *Morchella* and family *Morchelleceae*. It was first discovered [7] Morel is used by the people in every spring because of their supreme taste and highly antioxidant activity. Morels 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].

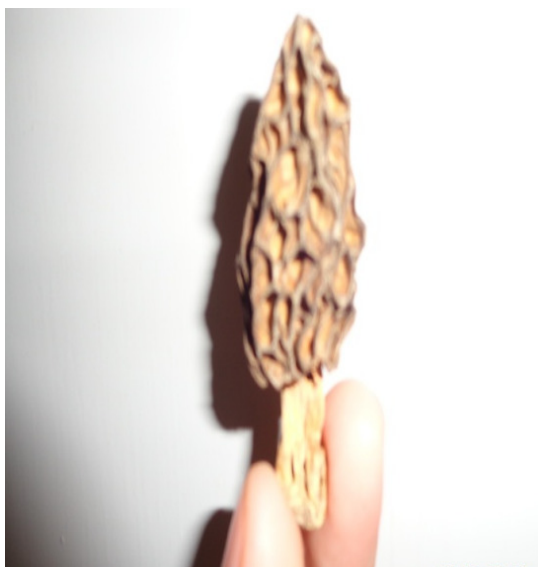


Fig. A *Morchella conica*

Therefore, there is a need for conducting research to screen native mycoflora of Pakistan for agricultural and pharmaceutical important compounds, Extraction of crude from *Morchella conica*, Antioxidant activity determination of *Morchella conica* extract at different concentration using DPPH method and Spectrophotometer absorbance comparison with different concentration of crude.

Material and method

Pre-dried mushrooms 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 2015. Pre- dried morels samples were weighed (1g) and then soaked in 100mL water at room temperature at 150 rpm for 24 h and then filtered. The residue was extracted under the same conditions 3 times. 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-diphenyl-2-picrylhydrazyl (DPPH) [9].

For this purpose DPPH solution (0.04%) in methanol was prepared. From this DPPH solution 01 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:

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

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 $\pm$ S.D).

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 antioxidants donate 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, measured by DPPH assay. All concentration 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 absorption at 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm concentration (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 was observed [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).

Table 1: Mean absorption 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

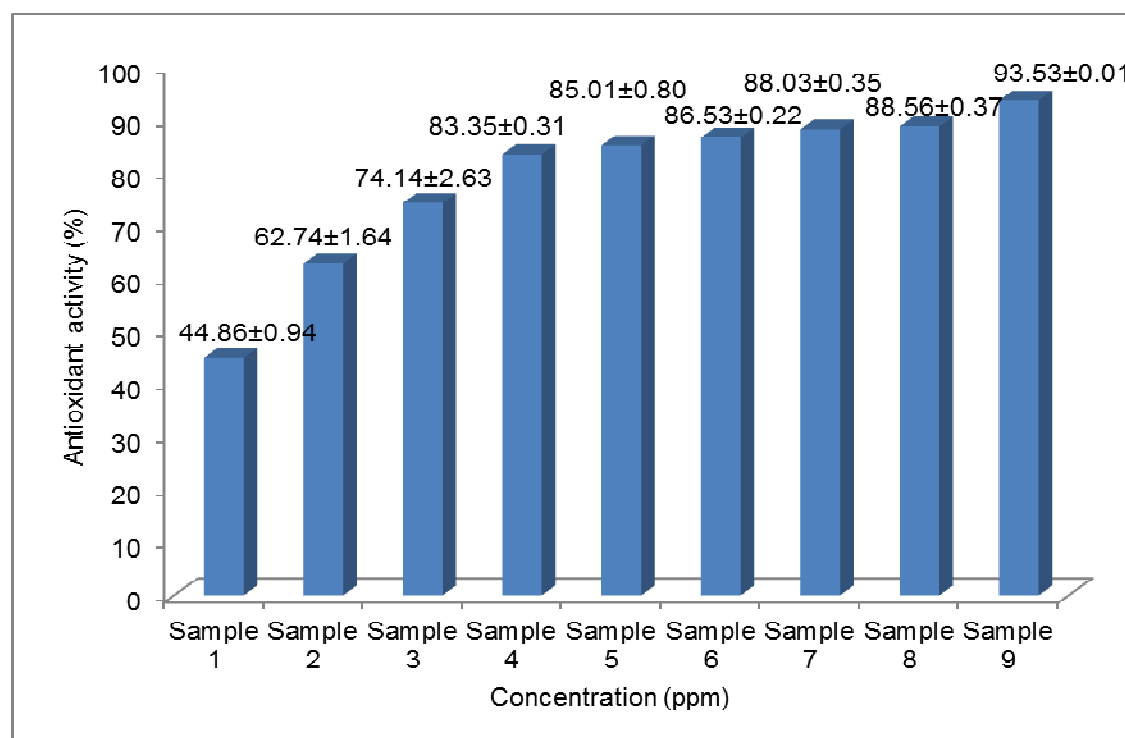


Fig 1: Water extracts concentration of *Morchella conica* and their antioxidant potential

It is clearly indicated that with the increase in concentration of the water extract of the selected morel (*Morchella conica*) an increase in the antioxidant potential occurs (fig-1). It is observed that water extract of *Morchella conica* might **has** a highly antioxidant properties. Among all the solution 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.

The current findings are according to the finding of [11] showed that with the increase of phenolic acids contents in mushroom increase in antioxidant activity occurs. The current study showed that *Morchella conica* water extract might contain more phenolic acids contents 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 that phenols are important components of plants. It is reported that to eliminate radicals due to their hydroxyl groups and they effect directly to antioxidant effect of system. Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity. The phenolic compounds may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables.

The current findings are in collaboration with the findings of [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. Chelating capacity of the extracts was also increased with the concentration. DPPH used as a standard and showed highly antioxidant activity by increasing the concentration. The current findings are just like [13] studied that the scavenging effects of mushrooms methanolic extracts on DPPH radicals increase with the concentration increase and were excellent for *L. giganteus* (100% at 5 mg/ml), even higher than the scavenging effects of BHA (96% at 3.6 mg/ml) and α -tocopherol (95% at 8.6 mg/ml). The results were good for *S. imbricatus* (80% at 5 mg/ml) and moderate for *A. arvensis* (68.3% at 5 mg/ml). The current study indicated that DPPH standard showed highly antioxidant activity at 517nm in the water extract of *Morchella conica* and might be highly reactive against mutagenic and carcinogenic diseases.

The present study showed that 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. The current findings are just like the result found by Isabel *et al.* the wild mushrooms contain antioxidant properties Wild mushrooms might be used directly in diet and promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present.

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]. The present study found that *Morchella conica* contains very high antioxidant activity. The water extract was found to possess better antioxidant activities and a general association between higher antioxidant activity and a larger amount of total phenolics was found in mushroom extracts.

159 4. Conclusion

160 It is concluded from the study that water extraction of *Morchella conica* possesses highly
161 antioxidant activity that with increase in extracts concentration.

162 References

- 163 1. Halliwell, B. and J. Gutteridge, Free radicals in biology and medicine. 2007.
- 164 2. Niki, E., H. Shimaski, and M. Mino, Antioxidantism-free radical and biological defense.
165 Gakkai Syuppn Center, Tokyo,.1994; 3
- 166 3. Halliwell, B. and J.M. Gutteridge, *Free radicals in biology and medicine*. Oxford university
167 press Oxford.1999; 3
- 168 4. Elmastas, M., Investigation of antioxidant properties of spearmint (*Mentha spicata* L.). Asian
169 Journal of Chem, 2005;17(1): 137
- 170 5. Cozzi, R., Ascorbic acid and beta-carotene as modulators of oxidative damage.
171 carcinogenesis,. 1997;18(1): 223-228.
- 172 6. Alger, M.S., Polymer science dictionary. Springer Science & Business Media. 1997
- 173 7. Persoon, C.H., Traite sur les champignons comestibles, contenant l'indication des especes
174 nuisibles; precede d'une introduction a l'histoire des champignons. Avec quatre planches
175 coloriees. Par CH Persoon. 1818: chez Belin-Leprieur, libraire, quai des Augustins.
- 176 8. Hamayun, M., M.A. Khan, and S. Begum, Marketing of med plants of Utror-Gabral Valleys,
177 Swat, Pakistan. Ethnobotanical Leaflets.2003;1: 13.
- 178 9. Barros, L., *Effects of conservation treatment and cooking on the chemical composition and*
179 *antioxidant activity of Portuguese wild edible mushrooms*. Journal of Agricultural and
180 Food Chemistry,. 2007;55(12): 4781-4788.
- 181 10. Elmastaş, M., *Antioxidant Activity of S-Carvone Isolated from Spearmint (Mentha Spicata L.*
182 *Fam Lamiaceae)*. Journal of liquid chromatography & related technologies,. 2006;
183 29(10): 1465-1475.
- 184 11. Turkoglu, A., Antioxidant and antimicrobial activities of *Morchella conica* Pers. African
185 Journal of Biotechnology,. 2006;5(11).

- 186 12. Gursoy, N., *Antioxidant activities, metal contents, total phenolics and flavonoids of seven*
187 *Morchella species*. Food and Chemical Toxicology, 2009; 47(9):2381-2388
- 188 13. Li, L., T. Ng, and L. Zhao, Antioxidant activity with content of phenolics in extracts from the
189 culinary-medicinal abalone mushroom *Pleurotus abalones*, Chen et Chang
190 (*Agaricomycetideae*). Int. J. Med. Mushrooms, 2005;7: 237-242.
- 191 14. Cheung, L. and P.C. Cheung, *Mushroom extracts with antioxidant activity against lipid*
192 *peroxidation*. Food Chemistry, 2005;89(3) :403-409