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Research Article

IN VITRO ANTIOXIDANT ACTIVITY AND ELEMENTAL ANALYSIS OF EDIBLE MUSHROOM *AGARICUS CAMPESTRIS*

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ABSTRACT

Agaricus campestris is a widely eaten gilled mushroom closely related to the cultivated button mushroom *Agaricus bisporus*. It is commonly known as the field mushroom or meadow mushroom. The aim of the study is to examine *in-vitro* antioxidant activity and elemental analysis of the wild edible mushroom *Agaricus campestris*. Antioxidant activity was evaluated by using DPPH free radical scavenging activity. Total phenolic content was estimated as Gallic acid equivalents/g spectrophotometrically according to the Folin-Ciocalteu method. The results showed that the ethanolic extract *A. campestris* showed the most potent radical scavenging activity; 41.32±7.22 at 80 µg/ml. The IC_{50}value of *A. campestris* and standarad (Vit C) were 98.86±4.39 µg/ml and 36.36±3.45 µg/ml, respectively. The total phenolic content in *A. campestris* mushroom was 34.42 mg GAE g^{-1} (dry weight). Several elements like calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na) and zinc (Zn) in dry mushroom were also evaluated using inductively coupled plasma-optical emission spectrometry (ICP-OES).

Keywords: Mushroom, Total phenolic compounds, Antioxidant activity, *Agaricus campestris* , ICP-OES

1. INTRODUCTION

Some common edible mushrooms have currently been found to possess antioxidant activity, which is well correlated with their total phenolic content [1]. Moreover, in the last few years, an increasing interest in the consumption of mushrooms has arisen, due to their elevated polyphenol concentration, which correlates with an elevated antioxidant activity. Several studies analyzing the total phenols and antioxidant activity of fresh and cooked wild and commercial mushrooms have been published [2-5]. Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, *e.g.* antioxidant, antimicrobial, anticancer, cholesterol lowering and immune stimulatory effects, have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities [6-9].

Thus, the aim of this study is to examine *in-vitro* antioxidant and antimicrobial activity of the ethanolic extract of the *Agaricus campestris* mushroom. The *in vitro* antioxidant activity was evaluated through the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Bioactive compounds such as total phenolic content were also determined.

2. MATERIAL AND METHODS

2.1. Collection of mushroom species

Fruiting bodies of *Agaricus campestris* used in this experiment collected from the local area of Madura district, TN, India, during the month of September-November, 2019. The sample was washed with distilled water for several times and cleaved into small pieces. Fruiting bodies and gills of *Agaricus campestris* are shown in Fig.1.

2.2. Estimation of Elements

One g of dried mushroom powder was digested in 10ml of ultrapure metal free nitric acid in a microwave digester. After digestion, the content is diluted to 25 ml with distilled water. Estimation of elements is performed using inductively coupled plasma with optical Emission

Spectroscopy (ICP-OES). The microwave digested samples are aspirated into ICP-OES to estimate elements viz. Ca, Co, Cr, Cu, Fe, K, Mg,Mn, Na and Zn. The calibration standards are prepared by diluting the stock multi elemental standard solution (1000 mg of lit) in HNO_3.

Fig.1: *Agaricus campestris*(a) fruiting body (b) Gills

2.3. ICP-OES equipment

The determination of several trace elements [cobalt (Co), chromium (Cr), copper (Cu)] and major elements[calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na) and zinc (Zn)] in *Agaricus campestris* using inductively coupled plasma–optical emission spectrometry (ICP-OES) was evaluated. ICP-OES (Inductively coupled plasma - optical emission spectrometry) is a technique in which the composition of elements in (mostly water-dissolved) samples can be determined using plasma and a spectrometer. Here PERKIN ELMER OPTIMA 5300 DV ICP-OES is used for elemental analysis.

2.4. Extraction

Finely ground mushroom (50 g) was extracted using ethanol for 24 h. The extract was filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extract was stored at -18°C until used in the tests. The extract was dissolved in 5% dimethyl sulphoxide (DMSO).

2.5. Total Phenols Determination

The amounts of total phenolic contents of the mushroom was determined by the spectrophotometric method of Kim *et al.*, (2003) [10] with slight modification. Briefly, 1 mL of the extract (1 mg/mL) in a volumetric flask diluted with distilled water (46 mL). 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (20 to 100 µg/ ml). The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of Gallic acid (GAE) equivalents per gram of dried sample.

2.6. *In vitro* antioxidant activity: DPPH radical-scavenging activity

Different concentrations of mushroom extract (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid (20, 40, 60 and 80 µg/ml) was used as the reference standard. DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992) [11]. Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical Scavenging Activity (\%)} = 100 - \left(\frac{\text{Ac-As}}{\text{Ac}}\right) \text{x}100$$

Where A_C= Absorbance of the control and A_S = Absorbance of reaction mixture (in the presence of sample).

2.7. Statistical Analysis

The results were presented as mean ± SD. Data was statistically analyzed using student "t" test. For the calculation of $IC_{50,}$ Linear regression analysis was done using Graph Pad prism statistical software.

3. RESULTS AND DISCUSSION

3.1. Elemental analysis

Mushrooms are enriched with minerals elements such as K, P, Zn, Fe, Cu, Mn, Mo, Se and Ni necessary for their growth and metabolism. Metals such as Fe, Cu, Zn, Cr, Se and Mn are considered as essential metals required in biological processes such as enzyme activators, whereas, As, Pb, Ni, Hg and

Cd are non-essential metals, toxic even in trace quantities [12]. The quantification values of various elements such as Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na and Zn are shown in Table 1. The tested mushroom *Agaricus campestris* was rich in K (22400 mg/L) followed by Mg (1230 mg/L), Ca (560.9 mg/L), Na(507.4 mg/L).

Table 1: Elemental analysis of *Agaricus campestris*

Elements Symbol	Concn. (mg/L)	Wavelength (nm)
Ca	317.933	560.9 mg/L
Co	228.616	3.560 mg/L
Cr	267.716	1.720 mg/L
Cu	327.393	60.90 mg/L
Fe	238.204	234.1 mg/L
K	766.490	22400 mg/L
Mg	285.213	1230 mg/L
Mn	257.610	53.70 mg/L
Na	589.592	507.4 mg/L
Zn	206.200	98.20 mg/L

3.2. Total phenolic compounds

Phenolic compounds are a large group of secondary plant metabolites which play a major role in the protection of oxidation processes [13]. Phenolic compounds have antioxidant properties and can act as free radical scavengers, hydrogen donators and singlet oxygen quenchers [13]. Numerous studies have conclusively demonstrated that mushrooms also contain many phenolics which are important plant constituents because of their scavenging ability [14]. In addition, phenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory and anti-hyperglycemic [15]. It is well known that phenols are one of the major contributors to the antioxidant activity of fruits, vegetables and mushrooms [9]. For this reason, in this study, total phenols concentration, and antioxidant activity of *A. campestris* mushroom was determined. The total phenolic content of the extract, calculated from the calibration curve (R^2 = 0.997), was 34.42±0.62 mg GAE g^{-1}(a Data are expressed as means ± S.D. of triplicate measurements). Standard curve for total phenols using Gallic acid (GAE) shown in Fig.2. Phenolic compounds have redox properties, which allow them to act as antioxidants. Numerous studies have showed the consumption of foods high in phenolics can reduce the risk of heart disease by slowing the progression of atherosclerosis due to their antioxidative properties [16, 17].

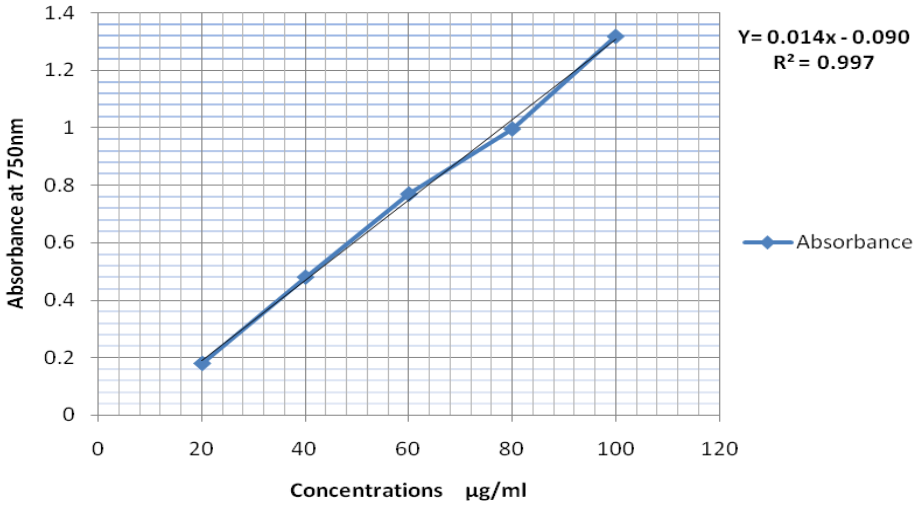

Fig. 2: Standard Curve for total phenols using Gallic acid (GAE)

3.3. Scavenging activity of DPPH radical

Free radicals produced by radiation, chemical reactions and several redox reactions of various compounds may contribute to protein oxidation, DNA damage, lipid peroxidation in living tissues and cells [18]. This oxidative stress may be related to many disorders, such as cancer, atherosclerosis, diabetes and liver cirrhosis [19]. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The radical scavenging activity of mushroom extract was tested against the DPPH. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extract. The method of scavenging DPPH free radicals can be used to evaluate

the antioxidant activity of specific compounds or extracts in a short time [20]. In Table 2**,** the scavenging activity of the DPPH radical due to its reduction by tested mushroom was illustrated.

The extract contained antioxidant components, which could react rapidly with DPPH radicals, and reduce most DPPH radicals. This result reveals that the extracts are a free radical inhibitor or scavenger, acting possibly as primary antioxidants. Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction [21-23]. Antioxidant activity of natural antioxidants has been shown to be involved in termination of free radical reaction [24]. The results indicated that ethanolic extract of *A.campestris* have a noticeable effect on scavenging free radical. The IC_{50} of *A. campestris* and standard were 98.86±4.39 and 36.36±3.45µg/ml, respectively, shown in Fig.3 and Fig.4.

Table 2: DPPH Radical scavenging activity of *Agaricus campestris* extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Standard (Ascorbic acid)	35.78±1.12	60.30 ±2.44	71.97±4.23	81.30±6.0	36.36±3.45
Agaricuscampestris extract	13.34±1.02	20.30±4.12	31.97±5.21	41.32±7.22	98.86±4.39

Values were expressed as Mean ± SD for triplicate

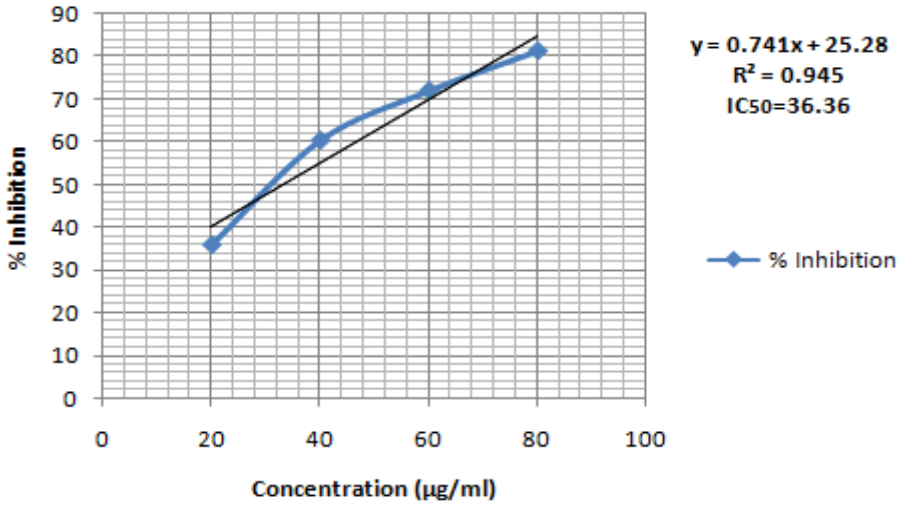

Fig.3: DPPH Radical scavenging activity of Standarad (Vitamin C) at different concentrations

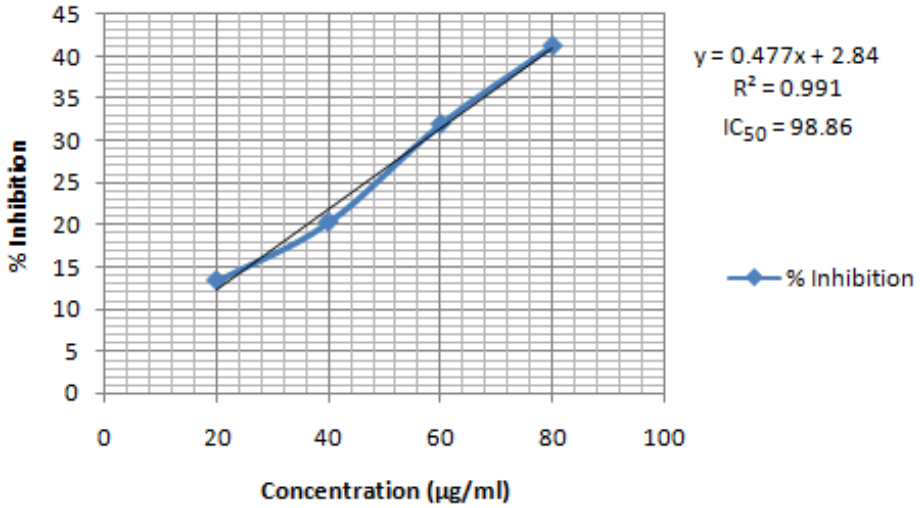

Fig.4: DPPH Radical scavenging activity of *Agaricus campestris* extract at different concentrations

The inhibition concentration at 50% inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. A lower IC_{50} meant better radical scavenging activity. Ethanol extract of the tested mushroom showed a good scavenging activity on DPPH radical.

4. CONCLUSIONS

Phenols are important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to the antioxidative action and play an important role in stabilizing lipid peroxidation. In conclusion, it can be stated that tested mushroom, *A. campestris* have a rich source of phenolic compounds and thereby might serve as possible nutraceutical food in human diet, and could help in the reducing the oxidative damage. Based on these results, mushrooms appear to be good and safe natural sources of antioxidants. Further studies should be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant activity.

5. ACKNOWLEDGEMENT

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6. REFERENCES

1. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, et al. *Food Chem*, 2007; **103**:413-419.
2. Lakshmi B, Tilak JC, Adhikari S, Decasagayam TPA, et al. *Pharmaceutical Biol*, 2004; **42**:179-185.
3. Lo KM, Cheung PCK. *Alba Food Chem*, 2005; **89**:533-539.
4. Choi Y, Lee SM, Chun J, Lee HB, et al. *Food Chem*. 2006; **99**:381-387.
5. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. *Food Chem*, 2007; **100**:1511-1516.
6. Oyetayo VO, Dong CH, Yao YJ. *Open Mycology J*, 2009; **3**:20-26.
7. Mau JL, Chang CN, Huang SJ, Chen CC. *Food Chem*, 2004; **87**:111-118.
8. Barros L, Calhelha RC, Vaz JA, Ferreira ICFR, et al. *Eur Food Res Technol*, 2007; **225**:151-156.
9. Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. *Food Chem*,2007; **100**:1511-1516.
10. Kim DO, Jeong SW, Lee CY. *Food Chem*, 2003; **81**:321-326.
11. Shimada K, Fujikawa K, Yahara K, Nakamura T. *J Agri Food Chem*, 1992; **40**:945-948.
12. George PL, Ranatunga TD, Reddy SS, Sharma GC. *Am J Food Tech*, 2014; **9**:360-369.
13. Croft KC. Antioxidant effects of plant phenolic compounds. In: Basu, T.K., Temple, N.J., Garg, M.L. (Eds.), Antioxidants in Human Health and Disease. CAB International Publishing Inc, Oxford, UK, 1999; p. 109-121.
14. Boonsong S, Klaypradit W, Wilaipun P. *Agri Nat Resources*, 2016; **50**:89-97.
15. Liu Y, Sun J, Luo Z., Rao S, Su Y, Xu R, Yang Y. *Food Chem Toxicol*, 2012; **50**:1238-1244.
16. Visioli F, BorsaniL, Galli C. *Cardovas Res*., 2000; **47**:419-425.
17. *Meng CQ, Somers PK, Rachita CL, Holt LA, et al. Bioorganic Med Chem*, 2002; **12**:2545-2548.
18. Morrissey PA, O'Brien NM. *Int Dairy J*, 1998; **8**:463-472.
19. *Muramatsu H, Kogawa K, Tanaka M, Okumura K*, et al. *Cancer Res*, 1995; **55**:6210-6214.
20. Keles A, Koca I, Gençcelep H. *J Food Process Technol*, 2011; **2**:130.
21. Frankel EN. *J Sci Food Agric*, 1991; **54**:495-511.
22. Shahidi F, Wanasundara PKJPD. *Critical Rev Food SciNutr*. 1992; **32**: 67-103.
23. Brand-Williams W, Cuvelier ME, Berset C. *LWT-Food Sci Technol*, 1995; **28**:25-30.
24. Tsai SY, Tsai HL, Mau JL. *LWT-Food Sci Technol*, 2007; **40**:1392-1402.