



RESEARCH ARTICLE

Free radical scavenging activity of some edible macrofungi from Gorakhpur district

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Abstract

Macrofungi are heterotrophic life form which are very specific in their growth requirements. They can grow in variety of habitat. In present work six wild edible macrofungi viz., *Cantharellus subalbidus* Smith & Morse, *Lentinus connatus* Berk, *Macrolepiota rhacodes* (Vittad.) Vellinga, *Termitomyces heimii* K. Natarajan, *Volvariella bombycina* (Schaeff.) Singer and *Volvariella volvacea* (Bull.) Singer were evaluated for their antioxidant activity. Antioxidant activity was found to be higher in all macrofungi tested. It has direct relation with phenol content. *C. subalbidus* contains the highest phenol (41.41 ± 0.12 mg/g) and lowest EC_{50} value for antioxidant. DPPH scavenging activity was found to be 3.251 ± 0.09 mg/ml while β carotene bleaching assay for it was 1.532 ± 0.017 mg/ml. All the tested macrofungi possesses good free radical scavenging activity hence validating its importance as strong antioxidant supplement.

Keywords: antioxidant, β carotene, DPPH, *In-vitro*, macrofungi

Introduction

Macrofungi are untapped resource of various important nutrients. It contains high protein content, vitamins, fibers, minerals, trace elements. Macrofungi possesses various therapeutic and pharmacological properties viz., antioxidant, anticancer and anti-inflammatory activities (Fernando *et al.* 2015). Many Asian countries of the world use various wild edible macrofungi as source of delicious food (Vishwakarma *et al.* 2017). The use of macrofungi as functional foods, dietary supplements, and traditional medicines derived has been increasing day by day as they have possesses numerous health benefits as well as abundant nutrients (Lu *et al.* 2020). Macrofungi generally contains most of the qualities of nutritious food as they contain numerous essential nutrients in high quantity (Vishwakarma *et al.* 2014).

Antioxidants are substances that neutralize free radicals and their actions. There are natural antioxidant enzymes

such as superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin thiols and disulphides bonding which form the buffering system in every cell (Ansari *et al.* 2013). Different bioactive compounds of edible mushrooms are responsible for their antioxidants properties. From many reasons, mushrooms are considered to be a good source of natural antioxidants and seem useful as a natural source of potential antioxidant additives (Boonsong *et al.* 2016).

The current study aims to evaluate antioxidant activity of some wild edible macrofungi viz., *C. subalbidus*, *L. connatus*, *M. rhacodes*, *T. heimii*, *V. bombycina* and *V. volvacea* from Gorakhpur district.

Materials and Methods

Sample collection and processing

Six macrofungi viz., *C. subalbidus*, *L. connatus*, *M. rhacodes*, *T. heimii*, *V. bombycina* and *V. volvacea* were collected from different localities of Gorakhpur district between years 2011 to 2014 (Figure 1). Specimens were identified using the relevant literatures (Castellano *et al.* 2003, Bolhassan *et al.* 2012, Hedawoo 2010, Wei *et al.* 2006, Seok *et al.* 2002) and confirmed by mycokeys (www.mushroomexpert.com and www.mycokeys.com). Collected samples were cleaned for dust particles and foreign materials, dried in shadow for 14 days at room temperature. Samples were then made into fine powder with help of grinder (0.5 mm sieve) for their antioxidant analysis.

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Figure 1: External morphology of macrofungi (a). *Cantharellus subalbidus*, (b). *Lentinus connatus*, (c). *Macrolepiota rhacodes*, (d). *Termitomyces heimii*, (e). *Volvariella bombycina*, (f). *Volvariella volvacea*

Total bioactive compounds

β-Carotene and Lycopene

β-Carotene and lycopene were determined by the process described by Loganathan *et al.* (2009). The absorbance of the filtrate was measured at $\lambda=453, 505$ and 663 nm. *β*-Carotene and lycopene content were calculated according to the following equations:

$$\text{Lycopene (mg/100ml)} = 0.0458 A_{663} + 0.372 A_{505} - 0.0806 A_{453}$$

$$\beta\text{-Carotene (mg/100ml)} = 0.216 A_{663} - 0.304 A_{505} + 0.452 A_{453}.$$

Ascorbic acid

The ascorbic acid content was determined titrimetrically according to Loganathan *et al.* (2009) by using 2, 6 Dichlorophenol Indophenol. The amount of ascorbic acid in each extract was calculated by the equation:

$$\text{mg of ascorbic acid per 100gm} = \frac{\text{titre} \times \text{Dye factor} \times \text{volume made}}{\text{Aliquot of extract} \times \text{weight}} \times 100$$

Determination of total phenolic compounds

Total phenolic compounds in the ethanol extracts were determined using Folin–Ciocalteu method (Loganathan *et al.* 2010). For the calibration curve Gallic acid was used as a standard. The phenol content was calibrated using the linear equation based on the calibration curve. The amount of the phenolic compound was expressed as mg Gallic acid equivalent/gm dry weight.

Antioxidant analysis: Antioxidant was evaluated by using following methods-

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging bioassay

It was evaluated according to Barros *et al.* (2007). For this stock solution of mushroom extract was prepared by taking 10 gm fresh sample of mushroom extract with 100 ml of 70%

ethanol. Different concentrations (1, 2, 3, 4, & 5 mg/ml) of 1 ml of prepared mushroom extracts were mixed with 1ml methanolic solution of 0.2 mM DPPH radicals. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm by spectrophotometer. The free radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the formula:

$$I (\%) = 100 \times (A_0 - A_s)/A_0$$

where A_0 represents the absorbance of control and A_s is the absorbance of the tested sample. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

Inhibition of *β*-Carotene bleaching

The antioxidant activity of fresh mushroom extracts was evaluated by the *β*-Carotene linoleate model system (Barros *et al.* 2007). A solution of *β*-Carotene was prepared by dissolving *β*-Carotene (2 mg) in chloroform (10 ml). Measure the absorbance at 470 nm using a spectrophotometer. Lipid peroxidation (LPO) inhibition activity was evaluated using the following equation:

$$\text{LPO inhibition} = \frac{\beta\text{-Carotene content after 2 hrs of assay}}{\text{initial } \beta\text{-Carotene content}} \times 100$$

The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration.

Statistical analysis

Experimental values are given as mean \pm standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Difference at $p < 0.05$ were considered to be significant.

Results and Discussion

Wild edible mushrooms are widely used in Asian and global cuisines as food and medicinal sources (Figure 2) Oxidative stress is generally caused by an imbalance metabolism. An excess of reactive oxygen species (ROS) generation leads to the generation of various disorders viz., metabolic diseases, heart disease, severe neural disorders like Alzheimer's and Parkinson's, premature aging and some type of cancers (Kozarski *et al.* 2015). In recent years edible macrofungi have attracted attention of consumers to be used as a commercial source of antioxidants. It can be used directly for the improvement of antioxidant defenses mechanism through dietary supplementation to decrease the level of oxidative stress in human (Ferreira *et al.* 2009). There is availability of various scientific evidences which supports the effectiveness of such strategy.

Bioactive compound of tested macrofungi

Phenolic compounds are widely present in macrofungi and have recently received considerable attention due to their antioxidant properties (Luo and Wu 2011). Phenol had a

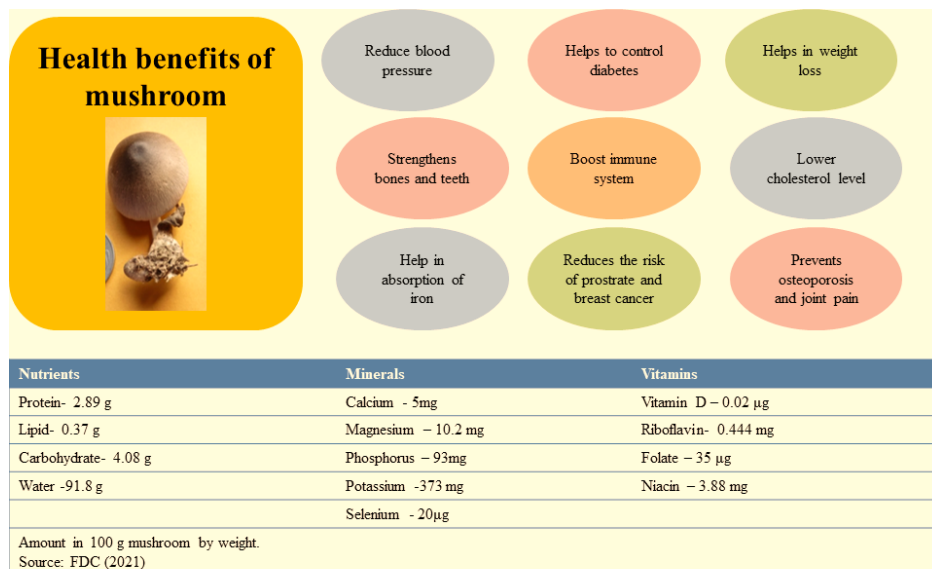


Figure 2: Health benefits of macrofungi

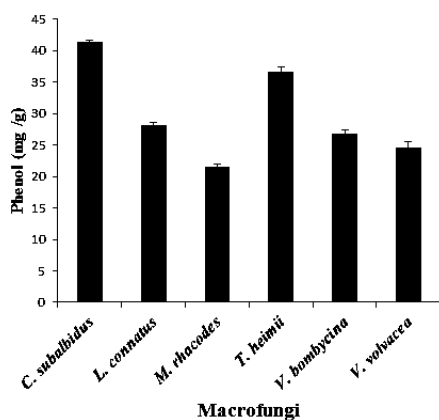


Figure 3: Total phenol content (mg/g) of mushrooms. Each value is the mean of three replicate determination ± standard deviation (p<0.05)

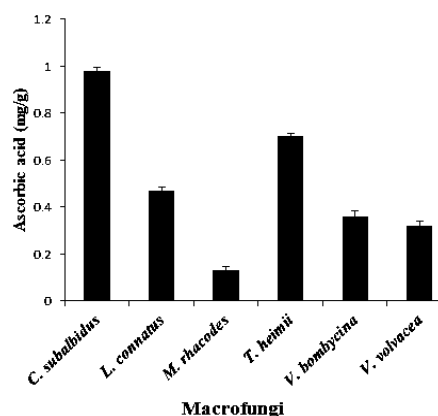


Figure 4: Ascorbic acid content (mg/g) of mushrooms. Each value is the mean of three replicate determination ± standard deviation (p<0.05)

direct relation with antioxidant activity. Higher the phenol, higher the antioxidant activity. It contains hydroxyl groups which act as good hydrogen donor agent. Hydrogen of phenolics can react with reactive oxygen and reactive nitrogen species and results in termination reaction, which breaks the cycle of generation of new radicals (Pereira *et al.* 2009). Phenolic compounds tend to accumulate in the dermal tissues of macrofungi body. Here it has a potential role in protection against ultraviolet radiations and as defense chemicals against pathogens and predators (Toor and Savage 2005).

In present study highest amount of phenol was reported from *C. subalbidus* (41.41±0.12 mg/g), followed by *T. heimii* (36.59±0.14 mg/g), *L. connatus* (28.22± 0.12 mg/g), *V. bombycina* (26.88±0.17 mg/g), *V. volvacea* (24.59±0.18 mg/g) and *M. rhacodes* (21.63±0.13 mg/g) (Figure 3). Similar result was obtained by Omar *et al.* (2011) for *Lentinus squarrosulus* (39.16 mg/100g). Sharma and Atri (2014) evaluated the

phenol content of five *Lentinus* species viz., *L. sajor-caju*, *L. connatus*, *L. torulosus*, *L. cladopus* and *L. squarrosulus* and found it to be in range of 6.39–20.11 mg/100g of gallic acid which is much smaller as evaluated in present study. Amount of phenol for *L. connatus* and *Volvariella* are found to be same as reported by Boonsong *et al.* (2016) for *Lentinus edodes* and *V. volvacea* (36.19±0.59 and 22.97±0.29 mg/g respectively) while phenol content is lower than as evaluated by Punitha and Rajasekaran (2014) (36.67 mg/g). Phenol content of *C. subalbidus* is found to be 41.41±0.12 mg/g which is same as reported by Ebrahimzadeh *et al.* (2015) for *Cantharellus cibarius* which was 40.97 ± 0.99 mg gallic acid equivalent g⁻¹ of extract.

Ascorbic acid also known as Vitamin C is a water soluble vitamin. It helps to prevent damage of RBC membranes which occurs because of free radicals such as superoxide and hydroxyl radical in plasma (Devi *et al.* 2014). Vitamin C quickly debugs the reactive oxygen and nitrogen species

just as superoxide, hydroperoxide radicals, aqueous peroxy radicals, singlet oxygen, ozone, peroxy nitrite, nitrogendioxide, nitroxide radicals and hypochlorous acid thereby protecting other substrate of the oxidative damage (José and María 2013). Ascorbic acid also had a direct association with antioxidant property. Among the six macrofungi tested highest amount of ascorbic acid was found in *C. subalbidus* (0.98 ± 0.05 mg/g), while lowest in *M. rhacodes* (0.13 ± 0.07 mg/g) (Figure 4). In present study *L. connatus* contains 0.47 ± 0.02 mg/g of ascorbic acid which is same as reported by Sharma and Atri (2014) for different species of *Lentinus* (0.42–0.49 mg/100g). Ascorbic acid content of both *Volvariella* spp. (0.36 ± 0.01 , 0.32 ± 0.08 mg/g) was found to be same as evaluated by Sudha *et al.* (2008) for paddy straw mushroom (*V. volvacea*) (0.35–0.54 mg/g) while it is found to be much lower than as evaluated by Punitha and Rajasekaran (2014) (1.52 mg/g).

Carotenoids are fat soluble colorants which are widespread in nature. More than 700 naturally occurring carotenoids are identified till now but approx. 50 carotenoids are present in the human diet and can be absorbed and metabolized by the human body (Maiani *et al.* 2009).

β -Carotene has potential antioxidant properties due to its chemical structure and interaction with biological membranes. It quenches singlet oxygen with a multiple higher efficiency than α -tocopherol and in addition to it (Z)-isomers of β -Carotene possess antioxidant activity *in vitro* (Muller and Bohm 2011). In present study β -Carotene ranges from 0.16 ± 0.024 μ g/mg - 0.32 ± 0.015 μ g/mg (lowest in *C. subalbidus* while highest in *L. connatus*) (Figure 5). Similar result was also obtained by Sharma and Atri (2014) for different species of *Lentinus*. Amount of β -Carotene for *T. heimii* in present study is same as reported by Loganathan *et al.* (2010) for *T. reticulates* (0.18 ± 0.019 vs 0.026 ± 0.015 - 0.115 ± 0.007 μ g/mg).

Lycopene, a type of carotenoid is a powerful antioxidant agent with a singlet-oxygen-quenching capacity 47 to

100 times more than that of β -Carotene and vitamin E respectively (Liu *et al.* 2005). The chemo-preventive potential of lycopene can partially be explained by its strong singlet oxygen quenching activity. It is an open chain of unsaturated carotenoid pigment, which imparts red color to fruits and vegetables (Singh *et al.* 2012). Lycopene consists of a tetraterpene hydrocarbon polyene chain with 11 conjugated and two unconjugated double bonds. This bond can easily be attacked by electrophilic reagents, resulting in development of extreme reactivity toward oxygen and free radicals (Kelkel *et al.* 2011). In present study lycopene ranges from 0.10 ± 0.012 - 0.85 ± 0.010 (*C. subalbidus* and *V. volvacea* respectively) (Figure 6). Amount of lycopene in *T. heimii* (0.22 ± 0.012 μ g/ml) is same as reported by Loganathan *et al.* (2010) for *T. reticulates* (0.052 ± 0.007 - 0.206 ± 0.040 0.32 μ g/ml).

Antioxidant activity of tested macrofungi

Reactive oxygen species-mediated tissue injury is a final common pathway for a myriad of disease processes. The body is in continuous expose of free radicals and ROS, both from external sources (sunlight, other forms of radiation, pollution) and endogenously generated sources (Luo and Wu 2011). The antioxidant activity of macrofungi increased with the increased in the concentration of samples, higher the antioxidant property lower the EC_{50} values. A lower EC_{50} value means better radical scavenging activity.

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. DPPH is stable free radical at room temperature and accepts an electron / hydrogen radical to become a stable diamagnetic molecule and reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution.

The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses,

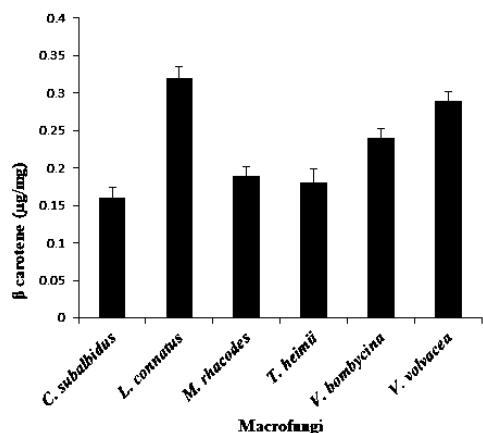


Figure 5: β carotene content (μ g/mg) of mushrooms. Each value is the mean of three replicate determination \pm standard deviation ($p < 0.05$)

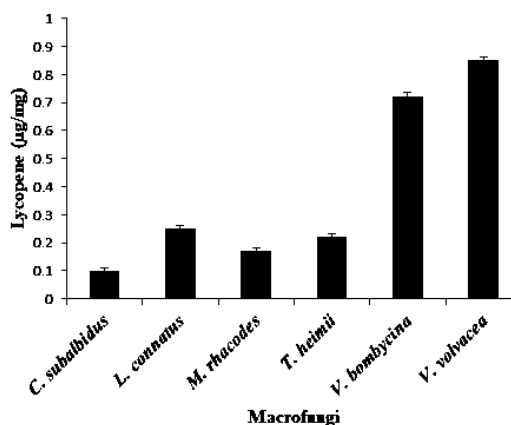


Figure 6: Lycopene content (μ g/mg) of mushrooms. Each value is the mean of three replicate determination \pm standard deviation ($p < 0.05$)

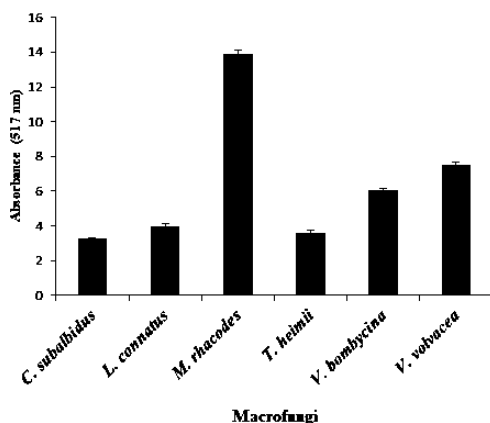


Figure 7: DPPH Radical scavenging activity (EC_{50}) of mushrooms. Each value is the mean of three replicate determination \pm standard deviation ($p < 0.05$)

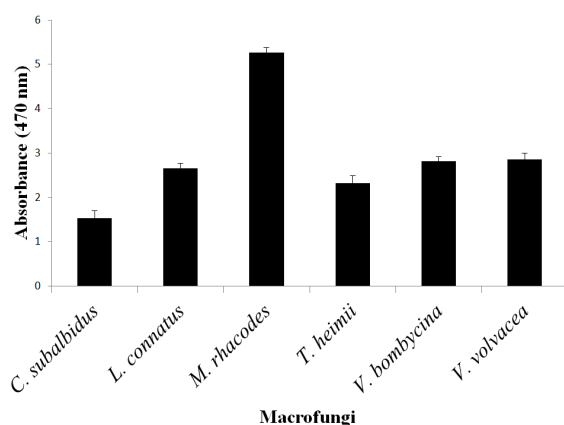


Figure 8: β -Carotene bleaching assay (EC_{50}) of mushrooms. Each value is the mean of three replicate determination \pm standard deviation ($p < 0.05$)

which results in the scavenging of the radical by hydrogen donation (Ansari *et al.* 2013; Garcia *et al.* 2012). In present study antioxidant activity with respect to DPPH radical scavenging activity was found to be in increasing order of: *M. rhacodes* (13.88 ± 0.12), *V. volvacea* (7.548 ± 0.15), *V. bombycina* (6.054 ± 0.11), *L. connatus* (3.975 ± 0.17), *T. heimii* (3.605 ± 0.12) and *C. subalbidus* (3.251 ± 0.09) (Figure 7). The EC_{50} value against DPPH radicals was found to be 3.605 ± 0.12 mg/ml for *T. heimii* which is very near to the findings of Loganathan *et al.* (2010) for *T. reticulates* (4.92 ± 0.3 mg/ml) while Mitra *et al.* (2016) found it to be 0.49 mg/ml for *T. heimii*.

β -Carotene bleaching process measured the ability of an antioxidant to inhibit the lipid peroxidation. In the β -Carotene bleaching method, a model system made of β -Carotene and linoleic acid undergoes a rapid discoloration in the absence of an antioxidant. The oxidation of linoleic acid produces free radicals due to the removing of hydrogen atom from diallylic methylene groups of linoleic acid which lost the double bonds and therefore imparts its characteristic orange color. The highly unsaturated β -carotene then will be oxidized by the generated free radical. The β -Carotene

bleaching technique is generally based up on the loss of the yellow color of β -Carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β -Carotene bleaching can be slowed down in the presence of antioxidants. The rate of bleaching of the β -Carotene solution was measured by the difference between the initial reading in spectral absorbance at 470 nm at time 0 min and after 60 min. The antioxidant activity was expressed as per cent inhibition relative to the control (Lai and Lim, 2011; Lu *et al.* 2014). Lower EC_{50} value means higher antioxidant activity. In present study, β -Carotene bleaching assay, *C. subalbidus* (1.532 ± 0.017) contains highest antioxidant activity followed by *T. heimii* (2.326 ± 0.017), *L. connatus* (2.656 ± 0.011), *V. bombycina* (2.815 ± 0.010), *V. volvacea* (2.854 ± 0.014), *M. rhacodes* (5.266 ± 0.013) (Figure 8).

The EC_{50} value for β -Carotene bleaching assay in present study was same as reported by Loganathan *et al.* (2010) for *T. reticulates* (2.326 ± 0.017 vs. 2.587 ± 0.25 mg/ml respectively).

Conclusion

Understanding the diversity of an area helps the people to identify new alternative nutraceutical and pharmaceuticals which are safe to use as well as cost effective. Protein content of macrofungi is very high which can help to overcome malnutrition. Calorie content is very low which can be used by various persons having medical complications like diabetes, cardiac problem and also helps to manage weight. Macrofungi are rich source of various important metabolites such as polyphenols, saponin, alkaloids and steroids that make them rich source of antioxidant compounds which helps to prevent degenerative diseases viz., cardiovascular illnesses, neurodegenerative disorders, rheumatoid arthritis and cancer. It helps the body to fight against numerous free radicals and helps to prevent aging and also various complications which occur because these free radicals in body.

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