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ABSTRACT

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Keywords

Catalase, Peroxidase, Ascorbic acid and Tocopherol. In order to assess the antioxidant activity of the basidiocarp of mushrooms, *viz., Boletus edulis, Ganodrema tsugae* and *Micoporus xanthopus.*, an *invitro* investigation was carried out. The result of the study revealed that the enzymatic antioxidants like catalase and peroxidase were recorded maximally in the basidiocarp of the fungus *M. xanthopus* (34.190 U mg⁻¹ and 116.53 U mg⁻¹ enzyme protein) and minimally in *G. tsugae* and *B. edulis* (9.602 U mg⁻¹ and 66.59 U mg⁻¹ enzyme protein). The non-enzymatic antioxidants like ascorbic acid (vitamin C) and tocopherol (vitamin E) were much pronounced in *B. edulis* basidiocarp (17.15 μ g⁻¹ and 56.13 μ g g⁻¹) compared to *M. xanthopus* and *G. tsugae* (17.15 μ g⁻¹ and 26.07 μ g g⁻¹) respectively.

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Introduction

Mushrooms have been widely used as functional food and as a source of physiologically beneficial and non toxic medicines. They have been reported to have significant pharmacological effects or physiological properties such as bioregulation, maintenance of homeostasis, and regulation of biorhythm, cure of various diseases, and prevention and improvement of life threatening diseases such as cancer, cerebral stroke and heart diseases. They have been also demonstrated to have effective substances for decreasing blood cholesterol, hypolipidemic, antithrombotic, hypotensive and other applications (Wasser and Weis, 1999 and Fan *et al.*, 2008).

The basidiomycetous fungi *Ganoderma* species, belongs to the order Polyporales (Chang, 1995 and Wasser and Weis, 1999a). *Ganoderma tsugae*, also known as "Hemlock varnish shelf", is a flat polypore mushroom. On the white underside of the pileus, there are numerous tiny pores. It is recognized by its varnished, reddish cap and stem. *Microporus xanthopus* also belongs to the order polyporales is found on rotting wood. The mature fruiting bodies have thin, funnel shaped caps that are concentrically zoned in various shades of brown and are supported by a yellow footed stem. *Boletus edulis*, commonly known as *porcini* or *cep*, a basidiomycete fungus belongs to the order Boletales. The fruit body consists of a large and imposing brown cap which on occasion can reach at least 35 cm in diameter and 3 kg in weight.

Antioxidant compounds can scavenge free radicals and increase shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage (Halliwell and Gutteridge, 1999). Mushrooms are rich sources of antioxidant compounds. The antioxidants contained in foods, especially vegetables are phenolic compounds (phenolic acid and flavonoids), carotenoids, tocopherol and ascorbic acid. Oxidation is also one of the most important processes of food deterioration since it may affect food safety, colour flavor and texture. Cells are equipped with several defence systems against free radical damage, including oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or chemical

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compounds such as α -tocopherol, ascorbic acid, carotenoids, polyphenol compounds and glutathione (Barros *et al.*, 2008b).

The aim of present investigation was to estimate the production of enzymatic and non-enzymatic antioxidants in the basidiocarp powder of *Boletus edulis*, *Ganoderma tsugae* and *Microporus xanthopus*.

Materials and methods

Collection and preparation of samples

The basidiocarp of *Boletus edulis*, *Ganoderma tsugae* and *Microporus xanthopus* collected from wooden logs in Kannur district, Kerala State were identified based on the morphology of the pileus and stipe of the basidiocarp. The basidiocarp of the mushroom sample was shade dried and powdered. The powdered sample was used for the study.

Preparation of enzyme assay from fungal basidiocarp (Boletus edulis, Ganoderma tsugae and Microporus xanthopus)

1.0g of powdered basidiocarp was macerated in 5 ml of 0.1 M phosphate buffer (pH 6.5 for peroxidase and 6.8 for catalase) in a pre-chilled porcelain mortar and pestle with a pinch of acid washed sand. The homogenate was centrifuged in a refrigerated centrifuge (Plasto crafts-superspin R.Mumbai) at 10,000 x g for 15 minutes. The supernatant served as enzyme source.

Estimation of antioxidants

Catalase activity was determined according to the method of Luck 1974 by monitoring the decomposition of H_2O_2 and Peroxidase activity was determined using pyrogallol oxidation method by Malik and Singh (1980).Total ascorbic acid was quantitatively determined according to Roe and Kuether (1953) and Tocopherol was determined on the reduction of ferric ions by tocopherol (Rosenberg,1992).

Results and discussion

Enzymatic antioxidants (Table I) catalase

The basidiocarp of the fungus *M. xanthopus* recorded maximum catalase activity of 34.190 U mg⁻¹ enzyme protein and minimum activity was registered in *G. tsugae* 9.602 U mg⁻¹ enzyme protein (P < 0.01 = 0.22).

Similar result was reported by Keyhani *et al.* (2006). They have obtained catalase enzyme activity of 11.7 units during their

study on antioxidants stress enzymes in Pleurotus ostreatus. Radhika (2010) also reported catalase enzyme activity of 8.63 U mg^{-1} enzyme protein in *Pleurotus estreatus*.

Peroxidase

The peroxidase enzyme activity was expressed maximally in *M. xanthopus* basidiocarp 116.53 U mg^{-1} enzyme protein and its expression was slightly at a lower level in *B. edulis* (66.59 U mg^{-1} enzyme protein).

The present finding is in accordance with the finding of Keyhani et al. (2006). They have obtained 9.4 U mg⁻¹of peroxidase activity in P. ostreatus. Radhika (2010) reported peroxidase activity of 69.88 U mg⁻¹ enzyme protein in P.cornucopiae.

Non-enzymatic antioxidants

Ascorbic acid (Vitamin C)

The highest ascorbic acid content of 39.08 μg^{-1} was registered by *B.edulis* basidiocarp and 17.15 μg^{-1} by *M*. *xanthopus* (P < 0.01 = 1.96).

The present result is in agreement with the result of Selvi *et* al. (2007). They reported that the ascorbic acid content in the basidiocarp of fresh and powdered milky mushroom (Calocybe indica) and in oyster mushroom (Pleurotus florida) were 1.033 and 0.4 mg g^{-1} and 0.38 and 0.27 mg g^{-1} respectively.

Similar result was noted by Kumari and Achal (2008) during their investigation on the production of non-enzymatic antioxidant activity of Pleurotus ostreatus on different substrates. The ascorbic acid (Vitamin C) content was found to be 0.277 mg g⁻¹ in fresh basidiocarp. Radhika (2010) also obtained 33.07 μ g g⁻¹ of ascorbic acid in *Pleurotus cornucopiae*. **Tocopherol** (Vitamin E)

The tocopherol content (Vitamin E) was very much pronounced in the basidiocarp of *B.edulis*, 56.13 μ g g⁻¹ compared to G. tsugae which was 26.07 $\mu g g^{-1}$.

Selvi et al. (2007) reported that the level of vitamin E in the fresh and powdered basidiocarp of milky mushroom (Calocybe *indica*) were found to be 2.933 and 0.80 μ g g⁻¹ and in oyster mushroom (*Pleurotus florida*), 7.28 and 5.15 μ g g⁻¹.

The present result is in accordance with the result of Kumari and Achal (2008). The tocopherol content of fresh and dried basidiocarp (fruit body) was found to be 7.23 mg g^{-1} and 5.93 mg g⁻¹ in *Pleurotus ostreatus*. Radhika (2010) also obtained tocopherol activity of 49.03 μ g g⁻¹ in *P. cornucopiae*.

Conclusion

Maintenance of equilibrium between free radical production and antioxidant defences (enzymatic and non enzymatic) is an essential condition for normal organism functioning. Natural products with antioxidant activity may help the endogenous defence system. In this perspective, the antioxidants present in the diet assume a major importance as possible protector agents reducing oxidative damage. Thus, it can be inferred from the present investigation that the mushrooms might be used directly in diet to promote health, taking advantage of the additive and synergistic effects of all the bioactive compounds present. Mushrooms with their content in antioxidant compounds such as tocopherols, can detoxify potentially damaging forms of activated oxygen.

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Table – I Enzymatic antioxidants and non-enzymatic antioxidants

S.No.	Mushroom	Enzymatic antioxidants		Non-enzymatic antioxidants	
		Catalase	Peroxidase	Ascorbic acid (Vitamin C)	Tocopherol (Vitamin E) (µg
		U mg ⁻¹	U mg ⁻¹	$(\mu g g^{-1})$	g^{-1})
1.	Ganoderma tsugae	9.602	74.90	20.83	26.07
2.	Microporus xanthopus	34.19	116.53	17.15	37.39
3.	Boletus edulis	18.63	66.59	39.08	56.13
SED		0.060	0.11	0.53	0.58
CD (P < 0.01)		0.224	0.41	1.96	2.14

Unit of enzyme activity is expressed as mg⁻¹ glucose released mg⁻¹ enzyme protein.