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Antioxidant level ofwild edible mushroom: *Pleurotus djamor* (Fr.) Boedijn.

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Analyses from different stages of white ecological variant of pan tropical mushroom, *Pleurotus djamor* (Fr.) Boedijn. procured from northern tropical moist deciduous forests of Tripura (India) revealed that it contains 1.2-2.7 mg/g of total phenols on the fresh weight basis. Quantitative estimation of total phenols and antioxidants (viz., FRAP, SOD and POX) revealed that the species showed highest amount of phenols and antioxidants in juvenile bud stage (one day stage) and gradually decreased, but at post-mature (four days old) condition, amount of total phenol again increased. This present study also revealed that *Pleurotus djamor* is also a good source of antioxidants such as FRAP, SOD and POX in fresh condition.

Key words: *Pleurotus djamor*, antioxidants, total phenol.

Introduction

Mushrooms represent one of the world's greatest untapped resources of nutritious and palatable foods. Mushrooms have been used as traditional foods and medicines in different parts of the world, including Asia, Africa and America (Alvarez-Parrilla *et al*., 2007). Mushrooms have become attractive as functional foods and as a source of physiologically beneficial medicine. Some advantages of using mushrooms over plants as sources of bioactive compounds are that often the fruiting body can be produced in much less time, the mycelium may also be rapidly produced in liquid culture and the culture medium can be manipulated to produce optimal quantities of active products (Ferreira *et al*., 2007). Different wild mushroom species were reported to have antioxidant activity, which was mainly related to their phenolic content (Lee *et al*., 2008; Mau *et al*., 2002 and 2002; Murcia *et al*., 2002; Yang *et al*., 2002; Cheung *et al*., 2003; Lee *et al*., 2003; Song *et al*., 2003). Valentão *et al*. (2005) described that conservation procedures seem to affect the qualitative and

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quantitative amount of phenolics of edible mushrooms. The mushrooms growth stage also influences their antioxidant contents. In fact, fruiting bodies of edible mushrooms in the mature stage with mature spores, revealed lower content in antioxidants such as phenolics, ascorbic acid and β-carotene. This was explained with the involvement of those compounds in defence mechanisms inherent to the aging process (presence of mature spores), resulting in the lowering of their contents in the most advanced stage. The antioxidants found in mushrooms are mainly phenolic compounds (phenolic acids and flavonoids), followed by tocopherols, ascorbic acid and carotenoids (Ferreira *et al*., 2007).

The highest antioxidant contents were measured by Lilian Barros *et al*., 2007 in stage II (opened caps in between 4.5-7cm broad with immature spore) of *Lacarius piperatu*.. The best condition of extraction of antioxidants from Agaricus brasiliensis basidiocarp is with methanol solvent for 60 minutes and strains with closed basidiocarp have higher antioxidant than with opened basidiocarp (F. Mourao et al 20011).

Pleurotus djamor (Fr.) Boedijn. is a white ecological variety mushroom found in the northern tropical moist deciduous forests of Tripura ($20^{o}51'$-$24^{o}32'$ N latitude and $90^{o}10'$-$92^{o}21'$ E longitude). It is common vegetable to tribal of Tripura due to much palatability and other culinary purposes. Often, it is sold at the vicinity of Assam-Agartala national highway or rural markets and used as substitute of soybean or egg (Figures 1 and 2). Relevant to this, the objective of the present study was to analyze the differences of antioxidant level among various stages of wild edible mushroom *Pleurotus djamor*in accordance with their level of total phenols, collected from the mixed type of forest of Tripura, one of the seven North Eastern states of India.

Materials and methods

Collection of samples

Wild mushrooms of various stages were collected from the local vendor (Figures 1 and 2) and at the end of the rainy season from northern tropical moist deciduous forests of Tripura during July-August, 2010. Ten to twenty mushrooms of each stage (10g) were collected and kept on ice 4-6 h for transportation to the laboratory. Mushrooms were classified up to species level, using morphological and anatomical characteristics. Mushrooms samples were sent to Mycological Research Laboratory of ICAR, Lembucherra, for identification and were identified as *Pleurotus djamor* (Fr.) Boedijn. (White ecological variety). Wild mushrooms were cut, weighted and frozen at -10°C for 1 day, lyophilized for 48 h (Labconco Freeze dry/shell freeze system), milled and stored at -10°C. In order to minimize variability between individuals

from the same species, all mushrooms from the same specie were homogenized according to the modified method of Alvarez-Parrilla *et al*. (2007). Different stages of mushroom were expressed after emergence of fruit body in the following terms: Juvenile (one day old), Pre-mature (two days old), Mature (three days old) and Over-mature (four days old).

Estimation of total phenols

P. djamor mushroom extracts were obtained according to the methodology proposed by Kähkönen *et al.* (1999) and Alvarez-Parrilla *et al*. (2007). Total phenols were determined according to the method reported by Georgé *et al.* (2005), with the Folin-Ciocalteu reagent, using tannic acid in ethanol (80%) as standard. Absorbance at 650nm was determined by using Systronics UV-VIS Model-117 Spectrophotometer and results were expressed as mg of tannic acid (TNA) per 100 g fresh weight (FW) basis.

Estimation of antioxidant level

Antioxidant capacity in the present mushroom sample P. djamor was determined by the following methods:

Ferric reducing/antioxidant power assay (FRAP): FRAP was determined by the method of Benzie and Strain (1996). FRAP reagent was prepared at 37°C, by mixing 0.3 M acetate buffer, pH 3.6 with 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl plus 20 mM $FeCl_3$ (10:1:1 ratio). Assay solutions were prepared by mixing 180 µL of FRAP reagent with 24 µL of water: sample mixture (3:1) using 80% methanol (blank or as standard). Methanolic solutions of Fe^{2+} (80%) in the range of 100-3000 µM were prepared from a 3000 µM $FeSO_4 \cdot 7H_2O$ stock solution to obtain the standard calibration curves. All measurements were carried out at 37°C. Absorbance was measured at 595 nm, every 30 seconds, during 60 minutes monitoring period, using Systronics UV-VIS Model-117.

Super Oxide Dismutase (SOD): The mean activity of SOD (units/minutes/mg protein) was determined by the method of Marklund and Marklund (1974) in which one unit was considered to be the amount of enzyme that inhibited pyrogallol auto oxidation by 50%.

Glutathione Peroxidase (POX): The mean POX activity of the mushroom extracts was measured according to the method described by Sadasivam and Manickam (2004).

Statistical analysis

Values are presented as the mean ± SEM of five replicates. For standard error of the mean (SEM), the obtained results (data) were statistically analyzed according to Steel and Torrie (1980). Two-way ANOVA analyses were performed in order to determine differences between various biochemical parameters and various stages of mushrooms at 5% significant level, using the commercial software SPSS 13.0 (SPSS Inc. Headquarters Chicago, Illinois, USA).

Result

Mushrooms apart from being famous for their appetizing flavour, offer themselves as potential antioxidant source. The present mushroom under the investigation is a white ecological variety of *P.djamor* (Fr.) Boedijn.The results of total phenols and antioxidants property clearly indicated that among four different stages, the antioxidative property was highest in juvenile stage. The total phenol was found maximum (2.791 mg/g) in juvenile stage of the present studied mushroom followed by the over mature (2.085 mg/g) stage. The maximum amount of enzymatic antioxidants viz., FRAP, SOD and POX were recorded 3.841, 28.354 and 7.400 µmole respectively (Table 1) in juvenile stage.

The data of Table-1 revealed that the total phenol content was found highest (2.791 mg/g on fresh weight basis) at juvenile stage (1day) and gradually decreased in pre-mature and mature stages but recouped a little in the post mature stage. In the present study, the level of antioxidants (viz., FRAP, SOD and POX) were found to be highest in juvenile (1 day stage) and gradually decreased in pre-mature, mature and post-mature stages.

Statistical analyses considering the amount of phenol and antioxidants with that of different stages of wild edible mushroom *P. djamor* in each experimental set (two-way ANOVA) revealed that calculated value of F (24.415) for different stages of wild edible mushroom *P. djamor* in methanol was much higher than tabulated value of F (4.355) and hence it can be concluded that phenol and antioxidants level among different stages of wild edible mushroom *P. djamor* was found significant with their properties (Table 2).

Fig. 1. Vendor displayed mushroom for selling in rural market

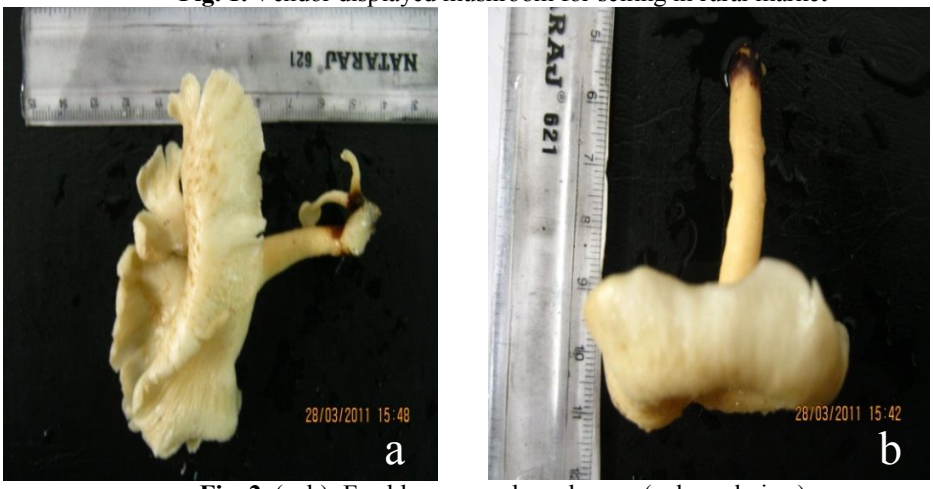


Fig. 2. (a, b): Freshly procured mushroom (enlarged view)

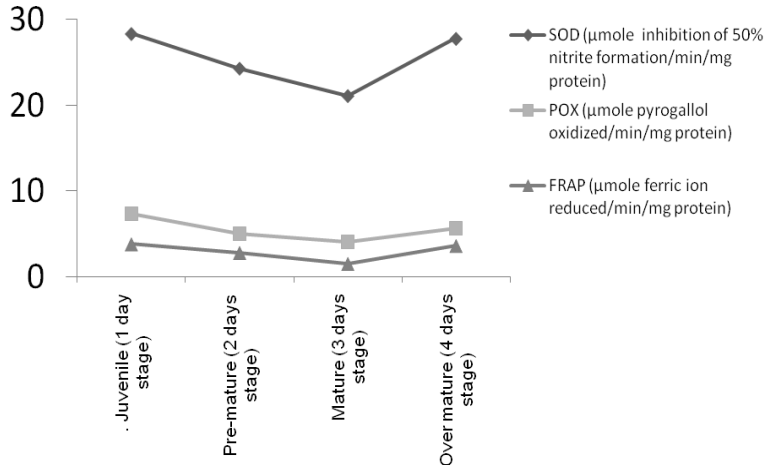
Table 1. Estimation of level of total phenols and antioxidants in *Pleurotus djamor*

Mushroom sample	Total phenol (mg /g)	SOD (µmole inhibition of 50% nitrite formation/minutes/ mg protein)	POX (µmole pyrogallol oxidized/minutes/mg protein)	FRAP (µmole ferric ion reduced/minutes/mg protein)
1.Juvenile (1 day stage)	2.791 ± 0.042*	28.354 ± 0.049	7.400 ± 0.060	3.841 ± 0.023
2. Pre-mature (2 days stage)	1.447 ± 0.065	24.311 ± 0.065	5.112 ± 0.042	2.832 ± 0.014
3. Mature (3 days stage)	1.271 ± 0.018	21.110 ± 0.088	4.117± 0.052	1.543 ± 0.015
4. Over mature (4 days stage)	2.085± 0.021	27.785 ± 0.047	5.689 ± 0.057	3.654 ± 0.025

*All the readings are based on 5 replicates ± SEM

Table 2. Two-Way ANOVA analyses of the data

Sources of variation	Df	SS	MS	F	F (0.05)
Row	4	0.04425	0.00712	4.131	3.174
Column	4	0.94111	0.04233	24.415	4.355
Error	16	0.02135	0.00411		
Total	24	1.00671			



Discussion

This quantitative amount of phenol in the present studied mushroom was less than the earlier reports of Puttaraju*et al*. (2006) where they found 13.22 mg/g phenol in *P. djamor* in alluvial soil. Yang *et al*. (2002) and Ramkumar *et al*. (2010) reported only the methanol extraction of mushroom strains had the highest antioxidant activity. Thus this present report conveys similarities with the earlier literature survey.

Edible mushrooms are widely consumed in Asian countries and have currently been found to possess antioxidant activity which would well correlated with their total phenolic content (Cheung and Cheung, 2005; Cheung *et al*., 2003; Mau *et al*., 2004; Yang *et al*., 2002; Yen and Hung, 2000). High phenolic compounds might account for good antioxidant properties found in all species of *Leucopaxillus giganteus* (6.29 mg/g), *Sarcodon imbricatus* (3.76mg/g) and *Agaricus arvensis* (2.83 mg/g) (Barros *et al*., 2007a and 2007b).

Recently, Ramkumar *et al*. (2010) estimated the level of SOD and POX in dried specimen of *P.djamor* and found to be 20.29 µmole and 4.00 µmole

respectively. Our present study also depicted similar trends in the results but variations remained due to the freshly harvesting, preservation conditions and soil factors.

Among edible mushrooms, *Helvella crispa* revealed the highest content of phenolic compounds expressed per gram of freshly harvested sample extract (34.65mg/g), while *Sparassis crispa* from Korea revealed the highest value expressed in a dry weight basis (0.76 mg/g)(Ferreira *et al*., 2007). The total phenols content in different growing stages of fruit body of *P.djamor* was found to vary from 0.12-0.27 percent on fresh weight basis (Acharya and Saha, 2010). The methanol extract of tested wild edible mushroom species (white variety of *P.djamor*) presented significant antioxidant activity which included scavenging of free radicals and excellent reducing power of ferric ions (Ramkumar *et al*., 2010). The antioxidant activity of *Lactarius piperatus* was recorded by Barros *et al*. (2007) in order of Stage II (mature cap opened with immature spore) > Stage I (immature cap closed) > Stage III (mature with mature spore) > Stage IV (degraded). The present study also bears similarly with the findings of Barros *et al*. (2007) in the way that the juvenile stage of *P. djamor* (open fruit body with immature spore) contain highest antioxidant capacity. But in *Agaricus brasiliensis* higher phenolic content and chelating ability for ferrous ions was observed in mature fruit body (Soares *et al.,* 2009) The reports of F. Mourao *et al.* (2011) on *Agaricus brasiliensis* shows similarity having higher antioxidant activity in closed than opened caps condition of fruit body. Total soluble proteins and nitrogen contents analyses from different stages of fruit body of *P. djamor* was revealed that it contained 1.88-2.96 percent and 0.10-1.44 percent on fresh weight basis. Thus, the present studied mushroom can be utilized as a source of natural antioxidants in food and neutraceutical industries in addition to as an antimicrobial agent and a protein source.

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