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Phytochemical analysis and antioxidative behaviour of ethanolic extract of *Pleurotus cornucopiae* from Eastern Himalayan Region

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Abstract: Different biochemical reactions in our body generate free radicals and these are capable of damaging crucial biomolecules. Mushrooms have the capacity to destroy such free radicals formed in our body as they possess antioxidant properties. The purpose of this study was to determine the antioxidant activities of mushroom, *Pleurotus cornucopiae* collected from Eastern Himalayan region. Antioxidant activity was determined using reducing power assay, DPPH radical scavenging assay, chelating effect of ferrous ion and total antioxidant capacity assay. Ethanolic fraction from the above mentioned edible mushroom was also tested for total phenol, flavonoid, β-carotene, lycopene and ascorbic acid content. Findings showed that most of the EC_{50} values were more than 2 mg /ml except DPPH radical scavenging test. The extract exhibited 50% DPPH radical scavenging activity at only 1.5 mg /ml concentration. Estimated putative antioxidant components was in order of phenol > ascorbic acid > flavonoids > β-carotene > lycopene. Result implies that *P. cornucopiae* can be a potential source of natural antioxidants which may be used as a food supplement to treat various oxidative stress related diseases.
Keywords: Free radical scavenging, Eastern Himalayan, Mushroom, *Pleurotus cornucopiae*, phenol.

Introduction

The role of free radicals is believed to be a primary factor in various degenerative diseases including cancer and heart diseases[1]. Reactive oxygen species like superoxide anion, hydroxyl radical and hydrogen peroxide are generated by normal metabolic processes or from exogenous factors and these are capable of damaging crucial biomolecules[2].

The harmful action of free radicals can be blocked by antioxidant substances which scavenge the free radicals and detoxify the organisms. In recent years, there has been increasing interest in finding natural antioxidants since they can protect the human body from free radicals and retard the progress of many chronic diseases[3].

The search for newer and natural sources has ever since increased. Among the natural sources, mushrooms in recent times are gradually reaching to the elite position and has established themselves as a

potential source of bioactive compounds. Mushrooms are rich in phenolics, tocopherols, ascorbic acid, carotenes and polysaccharides[4].

A number of mushrooms from India were evaluated and have shown their therapeutic potentiality for the treatment of cancer[5,6], cardiovascular problems[7], diabetes[8], hepatic damage[9-12], ulcer[13], microbial pathogens[14-16], parasitic infections[15, 17] and can improve immunity[18-20]. In this study, the antioxidant activity of ethanolic extract of wild edible mushroom, *Pleurotus cornucopiae* found in Manipur was investigated.

Materials and methods

Preparation of extract

The dried fruiting bodies of *P. cornucopiae* were grinded and the powdered material (10 gm) was used for extraction[21]. Then, the mushroom powder was soaked in 200 ml ethanol and stirred at 25ºC for 1 day at 150 rpm. Subsequently, the solvent was separated through Whatman No. 1 filter paper and the entire above mentioned procedure was repeated. After filtration, the combined solvent was rotary evaporated at 40ºC under vacuum to acquire ethanolic fraction of *P. cornucopiae* and stored in amber coated bottle at 4ºC for further analysis.

DPPH radical scavenging assay

The DPPH scavenging activity was measured following Shimada et al (1992)[22]. Various concentrations of the sample were added to 2ml of 0.004% methanol solution of DPPH (w/v). After 30 min. incubation at room temperature in dark, the absorbance was read against a methanol blank at 517 nm. EC_{50} value is the effective concentration at which DPPH radicals were scavenged by 50%. Ascorbic acid was used as a standard. The degree of scavenging was calculated by the following equation:

$$\textbf{Scavenging \%} = \frac{\text{Absorbance of control (A0)-Absorbance of test sample (A1)}}{\text{Absorbance of control (A0)}} \times 100$$

Determination of reducing power

Reducing power was determined by the method prescribed by Oyaizu et al (1986)[23]. Various concentrations of ethanolic extract of the wild edible mushroom (0.5-2.5 mg/ml) were mixed with 80% ethanol and the volume was made up to 0.5ml. After that, Sodium Phosphate buffer (0.2 M, pH 6.6) was freshly prepared. 1% potassium ferricyanide was added to the reaction mixtures with an equal volume of 1.25 ml. The mixtures were incubated at 50°C for 20min. During this incubation period, ferricyanide is reduced to ferrocyanide. Then, an equal volume of 10% trichloroacetic acid was added to each of the reaction mixtures to stop the reaction. The reaction mixtures were centrifuged at 5,000 g for 10 min. The upper layer of each of reaction mixtures (2.5 ml) were then mixed with an equal volume of 80% ethanol and 0.25 ml of freshly prepared 0.1% ferric chloride solution. The reaction mixtures were incubated for 15 min at room temperature. During this incubation period, ferric chloride reacts with ferrocyanide to form a prussian blue colored complex ferric ferrocyanide. The absorbance was measured at 700 nm against a blank in a spectrophotometer to determine the amount of ferric ferrocyanide formed in the reaction mixtures. A stronger absorbance will indicate an increased reducing power. The assay was carried out in replicates for each of the concentrations. The EC_{50} value was calculated from the graph of absorbance at 700 nm.

Total phenol content determination

Total phenol content of the extract was estimated following Oyaizu (1986) [24]. 1 ml of 1N Folin–Ciocalteu reagent was added to 100 µl of sample extract. After 4 min, a saturated sodium carbonate solution (approximately 35 g/100ml, 1 ml) was added to it. The absorbance of the reaction mixture was measured at 725 nm after incubation for 1 hr 30 min at room temperature. Gallic acid was used as a standard, and the results were expressed as milligram Gallic Acid equivalent (mg GAE)/g of extract.

Total flavonoid determination

Total flavonoid content of the extract was estimated following Adebayo et al (2012)[25]. A reagent mixture comprising of 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1 M Potassium acetate was made at first. Then 100 µl of sample extract was added to it. The reaction mixture was incubated at room temperature for 40 min and its absorbance was measured at 415 nm. Quercetin was used as a standard.

β-carotene and lycopene estimation

For β-carotene and lycopene determination, the following method was employed with a little modification[26]. 100 mg of the sample extract was vigorously shaken with 10 ml of an acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, and 663 nm. β-carotene and lycopene content were calculated accordingto the following equations:-

β-carotene (mg /100 ml): $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$

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

Ascorbic acid determination

Ascorbic acid content was determined by a method as described by Rekha et al (2012)[27] with a little modification. Standard ascorbic acid (100 µg /ml) was taken in a conical flask and made up to 10 ml by 0.6% oxalic acid. It was titrated with a dye, 2, 6-dichlorophenol indophenol. The amount of dye consumed (V1 ml) is equivalent to the amount of ascorbic acid. The sample (w µg /ml) was similarly titrated with the dye (V2 ml). The amount of ascorbic acid was calculated using the formula:-

Ascorbic acid (µg /mg) = [{(10 µg /V1ml) × V2 ml} × w µg] × 1000

Results and discussion

Extractive values and bioactive components

The extract of *P. cornucopiae* was brown in colour, sticky in nature and had an extractive value of 6.96%. Total phenols were the major naturally occurring antioxidant component as estimated at 8.6 ± 0.36 µg gallic acid equivalent /mg of extract. *P. cornucopiae* also contained flavonoid as 0.78 ± 0.16 µg quercetin equivalent /mg of extract. Very low amounts of β-carotene and lycopene were found (0.007 ± 0.002 µg /mg and 0.006 ± 0.004 µg /mg of the extract respectively). Ascorbic acid was estimated at 0.793 ± 0 µg /mg of extract.

DPPH radical scavenging assay

DPPH assay has been widely used to test free radical scavenging ability of various food samples. DPPH is a stable N_2-centered free radical which accepts an electron /hydrogen to gain stability. Antioxidant has ability to donate electron that can neutralize DPPH by transfer of an electron or hydrogen atom[28].

In methanol solution DPPH produces violet colour. Suitable reducing agent donates electron to DPPH and the solution loses colour depending upon the number of electrons taken up. Colour changes from purple to yellow and the reduction capacity of DPPH is determined by a decrease in its absorbance at 517 nm[29]. With regard to scavenging ability of DPPH radicals, *P. cornucopiae* performed well as evidenced by its low EC_{50} value (2.37 ± 0.02 mg /ml) (Fig 1). Ascorbic acid was established to be an excellent scavenger (EC_{50} 4.3 ± 0.3 µg /ml).

The EC_{50} value for the ethanolic extract of *R.albonigra*[30], *P.flabellatus*[21] showed more EC_{50} values. Therefore, the DPPH radical scavenging activities of the ethanolic extract from different mushrooms were in descending order: *P.flabellatus* > *R.albonigra* > *P.cornucopiae*.

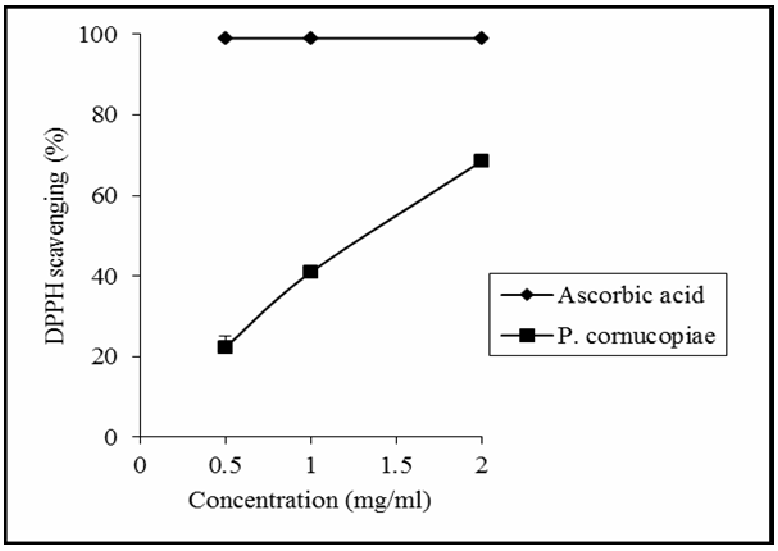

Fig.1: DPPH radical scavenging activity of *Pleurotus cornucopiae.*

(Values are the mean ± standard deviation of three separate experiments, each in triplicate).

Determination of reducing power

Reducing properties of antioxidants are associated with hydrogen atom donation abilities. Antioxidant can break free radical chains by donating hydrogen atoms and can also react with certain precursors of peroxide to prevent peroxide formation[31]. For the measurements of the reductive ability, $Fe^{3+} \rightarrow Fe^{2+}$ transformation, in the presence of *P. cornucopiae* was investigated (Fig. 2). Reducing power of *P. cornucopiae* was compared to ascorbic acid. The EC_{50} value for the ethanolic extract of *R.albonigra*[30], *P.flabellatus*[21] showed less EC_{50} values whereas *R. laurocerasi*[31] showed a higher EC_{50} value instead.

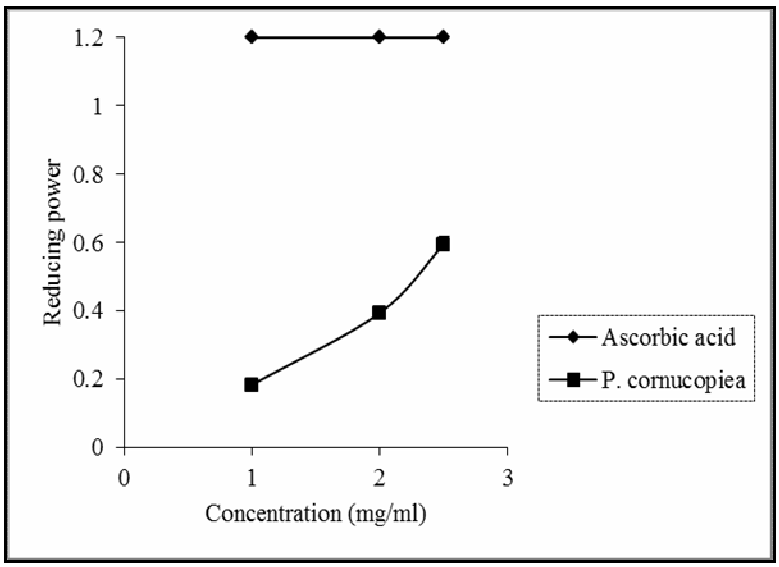

Fig. 2: Reducing activity of the mushroom *P. cornucopiae.*

(Values are the mean ± standard deviation of three separate experiments, each in triplicate).

Antioxidant components

In the present study, Table 1 demonstrates total phenol, flavonoid, ascorbic acid, β-carotene and lycopene content in *P. cornucopiae*. Data shows that phenol and flavonoid are the major antioxidant components. The extract was also found to be consisted of vestigial amount of β-carotene, lycopene and ascorbic acid.

Values are mean ± SD of three separate experiments each in triplicate. Total phenols are expressed in gallic acid equivalent (GAE), and flavonoids as quercetin equivalent (QAE).

Table 1: Total phenol, flavonoid, β-carotene, lycopene and ascorbic acid contents of ethanol fraction of *P. cornucopiae*

Phenol (μg /mg)	Flavonoid (μg /mg)	β-carotene (μg /mg))	Lycopene (μg /mg)	Ascorbic acid (μg /mg)
8.6 ± 0.36	0.78 ± 0.16	0.007 ± 0.002	0.006 ± 0.04	0.793 ± 0

Conclusion

The ethanolic extract of *Pleurotus cornucopiae* was found to be an effective antioxidant in different *in vitro* assays including ferrous iron chelating, DPPH free radical scavenging and total antioxidant activity and can be suggested as a natural additive in food and pharmaceutical industries.

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