Research Article

Chemical Composition and Antioxidant Activity of *Schizophyllum commune*

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ABSTRACT

This study was conducted with ethanolic extract of edible mushroom, *Schizophyllum commune*, which was tested for total phenol, flavonoid, β-carotene, lycopene and ascorbic acid and *in vitro* antioxidant activity in terms of DPPH radical scavenging activity and reducing power and total antioxidant capacity were done with varying concentrations. 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 *S. commune* can be a potential source of natural antioxidants which may be used as a food supplement to treat various oxidative stress related diseases.

Keywords: Antioxidant, DPPH, Flavonoid, *Schizophyllum commune*, Phenol, Reducing power.

INTRODUCTION

Different biochemical reactions in our body generate free radicals and under different patho-physiological conditions, the balance between the generation and elimination of reactive oxygen species (ROS) is broken, as a result a wide range of essential biomolecules are damaged by this ROS mediated oxidative stress, leading to various diseases[1]. Every organism is equipped with defence system against oxidative damage but they often fall short, thus making the search for external antioxidants increasingly important. Antioxidants can protect human body from damages caused by reactive oxygen species. Although synthetic antioxidants are available in the market, their use is being restricted due to reported carcinogenicity and hepatotoxicity. Thus, in recent times anti-oxidants from natural sources are being sought extensively[2].

Among different natural sources mushrooms are now becoming more attractive because of its strong nutritional value and therapeutic potentiality. Mushrooms are rich in different biologically active compounds like phenolics, tocopherol, lycopene, β-carotene etc.[3]. Thus, mushrooms in recent times are gradually rising to an elite position as a source of bioactive compounds. Because of the geoclimatic variation India becomes a harbour for a large number of edible mushrooms, many of which have been evaluated for their therapeutic potentiality and established to have effective anticancer[4,5], antidiabetic[6], antiulcer[7], hepatoprotective[8-11], cardioprotective[12], immune modulatory[13-15], antimicrobial[16-19] and apoptogenic[20,21] activities. In the present investigation, we report the antioxidant properties of *Schizophyllum commune* based on *in vitro* antioxidant assay systems.

MATERIALS AND METHODS

Preparation of extract

Ethanolic extract of the sample was prepared following Dasgupta et al (2013)[22]. The sample was dried, powdered and extracted with ethanol at 25°C for 2 days. After filtration, the residue was then re-extracted with ethanol, as described above. The supernatant was concentrated under reduced pressure in a rotary evaporator. Then, this ethanolic extract of *Schizophyllum commune* was stored at 4°C for further analysis. The percentage yield extract was calculated based on dry weight as follows:-

$$\text{Yield (\%)} = (W1 \times 100) / W2$$

Where W1 = weight of extract after solvent evaporation; W2 = Weight of the minced mushroom.

Determination of total phenolic content

Total phenols were estimated following Singleton and Rossi (1965)[23]. To the extract sample (100 µl), 1 ml of 1N Folin-Ciocalteu reagent was added. 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.

Determination of flavonoids

100 µl of the sample extract was added to 80% ethanol containing 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1M Potassium acetate. The mixture was incubated at room temperature for 40 min and its absorbance was measured at 415 nm[24]. Quercetin was used as standard.

Determination of β-carotene and lycopene

For β-carotene and lycopene determination, 100 µl 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[25]. β-carotene and lycopene content were calculated according to the following equations:-

Lycopene (mg /100 ml) = -0.0458×A663 + 0.372×A505 - 0.0806×A453

β-carotene (mg /100 ml) = 0.216×A663 0.304×A505 + 0.452×A453

The results are expressed as mg of carotenoid/g of extract.

Ascorbic acid content determination

Ascorbic acid content was determined following Rekha et al (2012)[26] 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

Reducing power

Reducing power of the sample was determined following Oyaizu (1986)[27]. Varied concentrations of the sample (0.5 - 2 mg /ml) were added to 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanate. 2.5 ml of 10% trichloro acetic acid was added to the mixture after an incubation of 20 min at 50˚C. It was then centrifuged for 10 min at 12000 rpm. 2.5 ml of the supernatant was mixed with distilled water and 0.5 ml of 0.1% ferric chloride. Its absorbance of the reaction mixture was interpreted as an increase in reducing power of the sample. Antioxidant has ability of donation of electron and causes conversion of the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). Resulting Perl`s Prussian blue is measured at 700 nm and higher absorbance indicates higher reducing power.

DPPH radical scavenging assay

The model of scavenging DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Effect of antioxidants on DPPH free radicals is due to their hydrogen donating ability. Upon reduction, solution of DPPH fades from purple to yellow. Thus, a lower absorbance at 517 nm indicates a higher radical scavenging activity of extract. The DPPH radical scavenging ability of the sample extract was measured according to Shimada et al (1992)[28]. 2 ml of reaction mixture was prepared using different concentrations of sample (1 - 2.5 mg /ml) and methanol solution of DPPH (0.004 %) (w /v). The absorbance was read against a methanol blank at 517 nm after 30 min incubation at room temperature in dark. The degree of scavenging was calculated by the following equation:-

Scavenging effect (%) = {(α0-α1) / α0} × 100

where α0 and α1 were the absorbance of control and in presence of sample.

Determination of total antioxidant capacity (TAC)

The TAC was determined on the basis of reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate /Mo (V) complex at acidic pH. Total antioxidant capacity of the sample was investigated and compared against ascorbic acid. The TAC of the sample was determined by the assay prescribed by Preito et al (1999)[29] with little modifications. A reaction mixture was prepared consisting of 0.3 ml of reagent solution (0.6 M H_2SO_4, 28 mM Na_2SO_4, 4 mM NH_4Mo). Absorbance was measured at 695 nm after heating tubes at 95°C for 90 min. Ascorbic Acid was used to draw a standard curve and TAC was expressed as the equivalent of ascorbic Acid.

Statistical analysis

Statistical analyses were done using MS Excel (Microsoft Office 2010 Professional).

RESULTS AND DISCUSSION

Extractive value and estimation of phytochemicals

The studied fraction had an extractive value of 5.58%. Phenol was present in the highest amount i.e. 12.5 ± 1.4 µg /mg of the sample. Flavonoid (1.78 ± 0.36 µg /mg) and ascorbic acid (2.18 + 0.12 µg /mg) were present in moderate amounts whereas β carotene (0.0136 ± 0.001µg/mg) and lycopene (0.011 ± 0.007µg /mg) were present in trace amounts.

Phenolic compounds possess scavenging ability due to their hydroxyl groups and are known to be powerful antioxidant. In the present study, the total phenolic content of *S. commune* was found higher than *Pleurotus ostreatus*[30] and *Pleurotus citrinopileatus*[31] which were reported to be 5.49 µg/mg and 8.62 µg/mg respectively. The phenolic content was also found to be comparatively higher than that of *Russula albonigra* (9.53 µg/mg) as reported ealier[32].

Reducing power

Reducing power of a compound indicates its potential antioxidant activity. The reducers (i.e., antioxidants) reduces Fe^{3+} /ferricyanide complex to ferrous form. The yellow colour of the test solution is changed to various shades of green and blue, depending on the reducing power of the sample. *S. commune* was found to be a potential reducing agent, having an EC_{50} value of 0.825 mg/ml (Fig 1). Compared to previously reported studies,

Hypsizigus marmoreus[33], *Calocybe gambosa*[34], *Tricholoma giganteum*[35], *Russula albonigra*[32] *Amanita vaginata*[36] had slightly better reducing ability than *S. commune.* Thus, the sample has excellent reducing ability.

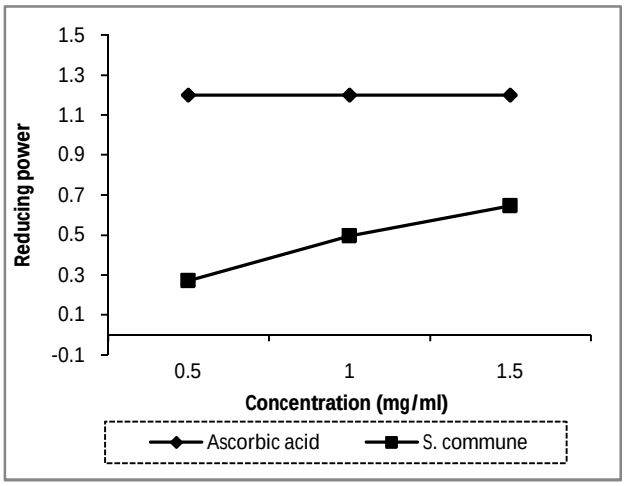

Figure 1: Reducing ability of ethanolic extract of *S. commune.*

(Ascorbic acid was used as the standard. Results are mean ± standard deviation of three separate experiments each in triplicate).

DPPH radical scavenging assay

DPPH is a stable free radical that shows a characteristic absorbance at 517 nm, which decreases significantly when exposed to radical scavengers by providing hydrogen atom or electron to be a stable diamagnetic molecule. Ethanolic fraction of *S. commune* had an EC_{50} value of 0.883 mg /ml (Fig 2) which indicates the potent DPPH radical scavenging potential of the extract. In comparison, the DPPH radical scavenging activities of *Russula albonigra*, *Pleurotus flabellatus* and *Amanita vaginata* were much lower than that of S. Commune. Expressed in the descending order, *S. commune* >*P. flabellatus*[22]> *A. vaginata*[37]>*R. albonigra*[33]. So, it is clear that the ethanolic extract of the sample has significant DPPH radical scavenging activity.

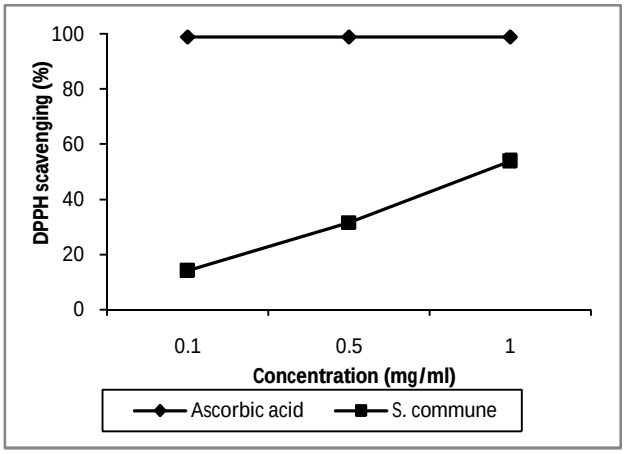

Figure 2: Reducing ability of ethanolic extract of *S. commune.*

(Ascorbic acid was used as the standard. Results are mean ± standard deviation of three separate experiments each in triplicate).

Total antioxidant capacity

Total antioxidant capacity of ethanolic fraction of *S. commune* was determined by the formation of green phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate/Mo (V) complex with the maximal absorption at 695 nm. Total antioxidant activity of the extract was estimated, using ascorbic acid as standard. On analysis, it was found that 0.1 mg of *S. commune* is as functional as approximately 67 ± 8 µg of ascorbic acid, expressed as 67 µg ascorbic acid equivalent (AAE).

CONCLUSION

The data recorded in the above experiments showed that the ethanolic fraction of *Schizophyllum commune* has good antioxidant property and free radical scavenging power in different *in vitro* assays including Ferrous iron chelating, ferric iron reducing, DPPH free radical scavenging and total antioxidant activity, having a good amount of phenolics, flavonoids and ascorbic acid. Thus, it can be a good source of natural antioxidants for use as food additives.

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