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Research Article

In Vitro Free Radical Scavenging Activity of a Wild Edible Mushroom, *Sparassis crispa* (Wulf.) Fr., from North Western Himalayas, India

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Bioactive compounds and antioxidant activity of methanolic extract of *Sparassis crispa* collected from North Western Himalayan region of India were analyzed. Phenolic content 11.14 ± 0.08 mg tannic acid equivalent per g of the extract and flavonoids 1.96 ± 04 mg catechin equivalent per g of the extract were recorded to be the major antioxidant components in this wild edible mushroom. Significant antioxidant efficiency on inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was observed when compared to standard antioxidant like L-ascorbic acid. IC_{50} value of the extract was 2.11 mg/mL. The findings suggest *S. crispa* as an easily accessible source of natural antioxidants.

1. Introduction

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, uncontrolled production of oxygen derived free radicals results in the onset of many diseases, such as cancer, rheumatoid arthritis, and atherosclerosis, as well as in degenerative processes associated with ageing [1]. Almost all organisms are well protected against free radical damage by antioxidant enzymes such as superoxide dismutase (SOD) and catalase or chemical compounds such as ascorbic acid, α-tocopherols, carotenoids, polyphenol compounds, and glutathione [2]. As improved antioxidant status helps to minimize the oxidative damage and thus delay or prevent pathological changes. Potential antioxidant therapy should be, therefore, included either as natural free radical scavenging antioxidant enzymes or as an agent which is capable of augmenting the activity of antioxidant enzymes [3].

Traditionally, wild edible mushrooms are used by most of the Asian and other countries worldwide as food and medicinal sources [4, 5]. They are also a good source of antioxidants. In the past few years, the suspected toxicity of some of the synthetic compounds used in food has raised interest in natural products; therefore, compounds from natural sources that possess antioxidant activity are being sought. Naturally occurring substances having antioxidant property are becoming one of the most appealing modes of modern therapy. Among them, mushrooms or their derivatives or extracts occupy an elite position to perform this function [6–11].

Mushrooms are gaining importance both as nutrient supplement and disease curing medicine. Mushrooms are unlimited source of therapeutically useful and biologically active agents. Compounds of mushrooms have been reported to have antifungal, anti-inflammatory, antibacterial, antiviral, antitumor, hepatoprotective, antidiabetic, antithrombotic, hypotensive, and antioxidant capacity [12–14].

North Western Himalayan region of India has been known as a belt containing rich varieties of wild mushrooms including *Sparassis crispa*, a well-known edible mushroom. *S. crispa* has been collected, worked out, and identified by earlier workers [15]. However, antioxidant properties of fruiting bodies of this mushroom from North Western Himalayan region have not been reported. In the present study, antioxidant properties of *Sparassis crispa* have been reported. The contents of potential antioxidant components like phenols, flavonoids, ascorbic acid, β-carotene, and lycopene have also been estimated.

2. Materials and Methods

2.1. Standards and Reagents. DPPH (2,2-diphenyl-1-picryl-hydrazyl), L-ascorbic acid, tannic acid, (+)catechin, and folin ciocalteu reagent were obtained from Sigma (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade.

2.2. Sample Collection and Extract Preparation. Fruiting bodies of *S. crispa*, an edible wild mushroom, were collected from Potter's Hill forest of North Western Himalayas, India, and identified at Department of Biosciences, H.P.U., Shimla (Figure 1). Fresh fruiting bodies of mushrooms were cut into small pieces and sun-dried. A coarse powder was obtained using a mill. For extraction, 10 g sample was extracted by stirring at 100 rpm with 100 mL methanol at 30°C for 24 hours and filtered through Whatman number 1 filter paper. The residue was then extracted with two additional 100 mL portions of methanol, in a similar manner. The combined methanolic extracts were evaporated by rotary evaporator at 40°C to dryness, redissolved in methanol to a concentration of 20 mg/mL, and stored in the dark at 4°C for further use [16].

2.3. Scavenging Ability on 2,2-Diphenyl-1-picrylhydrazyl Radicals. The hydrogen atoms or electron donation ability of the extracts was measured from the bleaching of the purple coloured DPPH methanolic solution with little modification. 3 mL of various concentrations of the extracts in methanol was added to 1 mL of methanolic DPPH (final concentration of DPPH was 200 μM). The mixture was shaken vigorously and allowed to stand for 30 min at room temperature and absorbance of the resulting solution was measured at 517 nm using spectrophotometer (Merck Spectroquant Pharo 100) [17]. Inhibition of the DPPH free radicals in (%) was calculated as

$$\text{Inhibition (\%)} = \frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100, \quad (1)$$

where, absorbance control is the absorbance of DPPH radical + methanol; absorbance sample is the absorbance of DPPH radical + sample extract/standard.

2.4. Determination of Antioxidant Components

2.4.1. Total Phenolic Contents of the Extract Was Determined following Makkar et al. [18]. The reaction mixture was prepared by mixing 0.1 mL of the extract and 0.9 mL of double distilled water. 2.5 mL of sodium carbonate solution (20%) was added to it, followed by 0.5 mL of FCR (1 N). After 40 minutes at room temperature, absorbance was read at 725 nm. Tannic acid (0.5 mg/mL) was used to prepare standard curve. The results were expressed as mg of TAEs per g of the extracts.

2.4.2. The Amounts of Total Flavonoids (TFC) Were Determined Colorimetrically. A suitable aliquot 250 μL taken for estimation [19] was mixed with 1 mL of water in a test tube. At the start, 75 μL of 5% aqueous $NaNO_2$ was added to the test tube; then after 5 min 150 μL of 10% $AlCl_3$ and after 6 min 500 μL of 1.0 M NaOH were added sequentially. Finally, 275 μL distilled water was added. The reaction mixture was mixed thoroughly. The absorbance was noted at 510 nm using a spectrophotometer. TFC, calculated using a standard calibration curve, were reported as (+)catechin equivalents (mg CE/g of the extract).

Figure 1: *S. crispa* in its natural habitat.

2.4.3. β-Carotene and Lycopene Were Determined according to the Method of Nagata and Yamashita [20]. The dried extract (100 mg) was vigorously shaken with 10 mL of acetone-hexane mixture (4 : 6) for 1 minute and filtered through Whatman number 1 filter paper. The absorbance of the filtrate was measured at 453, 505, and 663 nm. The contents of β-carotene and lycopene were calculated according to the following:

$$\begin{aligned} \text{Lycopene}\left(\frac{\text{mg}}{100\,\text{mL}}\right) &= -0.0458A_{663} \\ &\quad + 0.372A_{505} + 0.0806A_{453} \\ \beta\text{-carotene}\left(\frac{\text{mg}}{100\,\text{mL}}\right) &= 0.216A_{663} \\ &\quad - 0.304A_{505} + 0.452A_{453}. \end{aligned} \quad (2)$$

2.4.4. The Ascorbic Acid Content Was Determined from Dried Methanolic Extract. A 100 mg of the extract was mixed with 1% metaphosphoric acid (10 mL) and incubated at room temperature for 45 min and filtered. 1 mL of filtrate was mixed with 9 mL of 2,6-dichloroindophenol and absorbance was recorded at 515 nm in 30 min against a blank. The ascorbic acid content was calculated using calibration curve of L-ascorbic acid. The results were expressed in terms of mg of ascorbic acid per g of extract.

2.5. Statistical Analysis. All the analyses were performed in triplicates and results were reported as means ± standard deviation (SD).

3. Results and Discussion

3.1. The Macrofungi Extract Showed Positive Antioxidant Activity by Fading the Violet Colour of DPPH Solution to Yellow and Pale Violet. The scavenging activities of radical

were in direct proportion with the concentrations of the extract. As the concentration of extract was increased, the scavenging activity towards DPPH radicals was also elevated. The results showing in Figure 2 clearly indicates the increase in % inhibition of DPPH free radical on increasing concentration. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity [21].

IC_{50} value of the selected mushroom extract was 2.11 mg/mL compared with L-ascorbic acid IC_{50} = 0.062 mg/mL. The results of DPPH scavenging effect of methanolic extracts were higher than what was reported by Puttaraju et al. [22]. DPPH assay is a widely used method to evaluate antioxidant activities in a relatively short time compared to other methods [23]. It has the advantage of being unaffected by certain side reactions which is common in laboratory-generated free radicals such as the hydroxyl and superoxide anion [24].

3.2. Bioactive Components. The present studies also concentrate on five different bioactive components like phenols, flavonoids, ascorbic acid, β-carotene, and lycopene (Table 1). Phenolic compounds form a major class of phytochemicals, which are responsible for inhibiting the oxidative damage caused by free radicals generated inside of our body [25]. Total phenolic content in the methanolic extract was found to be 11.14 mg tannic acid equivalent per g of the extract. The total phenolic content in methanolic extracts of *S. crispa* clearly demonstrates that it can be considered as a better source of polyphenols. The key role of phenolic compounds as scavengers of free radicals is emphasised in several reports [26–28], and these compounds seem to be associated with antioxidant activity [29, 30].

Flavonoids are well-known dietary biochemical agents, which show pH dependent antioxidant behaviour in human body. These molecules are also effective for cardiovascular system and work as cardioprotective agents [31]. The total flavonoid contents in methanolic extracts of wild *S. crispa* were found to be 1.96 mg catechin equivalent per g of the extract.

Ascorbic acid was found in small amounts (2.12 mg/100 g) and β-carotene and lycopene were only found in vestigial amounts. Several references are available on insignificant quantities of ascorbic acid, lycopene, and β-carotene in methanolic extracts of fruiting bodies as naturally occurring antioxidant components [32, 33]. Though other antioxidants were probably present in this mushroom extract, the amount of ascorbic acid, lycopene, and β-carotene found was very low, which emphasised the idea that phenolic compounds could make a significant contribution to the mushrooms antioxidant activity.

4. Conclusion

The findings from the study can be used to disseminate the information regarding antioxidant properties to the public. The results suggest that the consumption of *S. crispa* may enhance the antioxidant protection system of human body. Comprehensive studies on the antioxidant properties of this mushroom are not available, so the findings from this study can act as a baseline reference for further research.

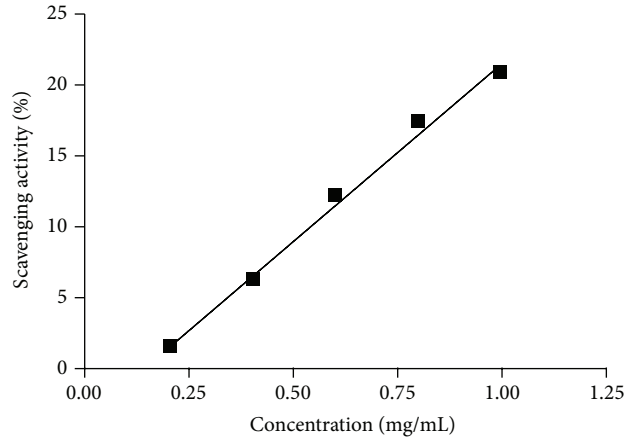

Figure 2: Antioxidant activity of *S. crispa* extract using DPPH test. Slope = 25.22 ± 1.180, Y-Intercept = −3.423 ± 0.7825, r^2 = 0.9935.

Table 1: Contents of total phenols, flavonoids, ascorbic acid, β-carotene, and lycopene in the mushroom extract.

Mushroom extract of *S. crispa*	
Total phenols (mg/g)	11.14 ± 0.08
Total flavonoids (mg/g)	1.96 ± 0.040
Ascorbic acid (mg/100 g)	2.12 ± 0.00
β-carotene (mg/g)	0.121 ± 0.011
Lycopene (mg/g)	0.066 ± 0.003

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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