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ANTIOXIDANT ACTIVITY OF PAPAIN HYDROLYSATES OF OYSTER MUSHROOM (PLEUROTUS OSTREATUS) PROTEIN

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ABSTRACT

In the present study Oyster mushroom *(Pleurotus ostreatus)* protein has been isolated and concentration of protein was found to be 12.4mg/ml by biuret method. The total protein was hydrolysed by papain. 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and superoxide free radical scavenging activity of total protein and papain hydrolysate were analysed. Papain hydrolysate was found to be having highest 2,2-diphenyl-1-picrylhydrazyl and superoxide free radical scavenging activity and was purified by using HPLC. Out of four fractions obtained, fraction 2 was found to be having highest 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity of 93.69% and 93.44% of superoxide free radical scavenging activity at 1mg/ml concentration.

INTRODUCTION

The reactive unpaired electrons present in free radicals can damage DNA, protein and other biological substances and can cause various diseases [1]. Antioxidants can scavenge free radicals and there are many synthetic antioxidants and disadvantage of the synthetic antioxidant is that they may cause cancer formation[2]

Antioxidants prevent damage to cells and play a major role in disease prevention[3] and there is an increasing use of synthetic antioxidants and also preference for natural products by consumers [4]. Recently bioactive peptides and proteins obtained from animal, plant, microbial and food source have gained lot of interest as they have potential to cure human and animal diseases. [5].

Pleurotus species is an edible mushroom that can be cultivated in temperate and subtropical countries. All over the world Pleurotus have been used for their medicinal and nutritional properties[6].Mushrooms are good source of proteins. Many studies have been reported on antibacterial, antifungal, antioxidant properties of different solvent extracts of mushrooms. The antioxidant activity of hydrolysed Oyster mushroom proteins has not been reported so far. Hence the antioxidant activity of Oyster mushroom proteins hydrolysed by Papain enzyme has been analysed in the present study.

MATERIALS AND METHOD

Sample collection

The mushroom *Pleurotus ostreatus* has been purchased from EFGC biological farms, Selayur, Chennai.

Isolation of total protein and Hydrolysis of protein

Total protein was isolated by using phosphate buffer method[7]. About 4 grams of Oyster mushroom was taken in mortar and pestle and were cut in to pieces and grinded in mortar with10ml of phosphate buffer. The contents were transferred to centrifuge tubes and centrifuged for 20 minutes at 5000rpm. The supernatant was collected and taken for further studies. Biurets method was used to estimate the total Protein content in the sample and Bovine serum albumin was used to prepare standard curve. Papain was used to hydrolyse Oyster mushroom protein for 4 hours at 37^0C[8]. Enzyme and substrate was added in the ratio of 1:5. Hydrolysed protein samples were lyophilized for 8 hours and stored until use.

Purification of papain hydrolysates by RP-HPLC

Purification of the Papain hydrolysates of Oyster mushroom protein was achieved as per the method of Mc Cann et al[9] using reverse phase chromatography (RP-HPLC). C-18 column was used with UV array detector. All eluates were monitored at 254 nm.

Antioxidant activity analysis

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of total protein and crude papain hydrolysates of total protein and HPLC fractions of Papain hydrolysates of Oyster mushroom protein were measured as per the method of Thaipong et al [10] with slight modifications. About 2 ml of 0.16mM DPPH in ethanol was mixed with 2ml of total mushroom protein and papain

hydrolysates of Oyster mushroom protein. Incubated at room temp in dark. After 30 minutes incubation, absorbance was read at 517nm. Ascorbic acid was used as positive control.

The following formula was used for calculating DPPH radical scavenging activity

$$\text{Radical scavenging effect in \%} = \frac{\text{Absorbance of blank-Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Super oxide free radical scavenging activity

Super oxide free radical scavenging activity of total protein, crude papain hydrolysates of total protein and HPLC fractions of Papain hydrolysates of Oyster mushroom protein were measured by method of Patel Rajesh and Patel Natvar[11].

$$\text{\% scavenging activity} = \frac{\text{Absorbance of control- absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Total protein isolation

The total protein concentration isolated from Oyster mushroom (Fig.1) was estimated by Biuret's method. From the standard curve plotted, the protein content present was found to be 12.4 mg/ml.

Fig.1: Oyster mushroom(*Pleurotus ostreatus*)

Antioxidant activity analysis

DPPH free radical scavenging and superoxide free radical scavenging activities of total protein and papain hydrolysates and fraction 2 of the HPLC eluate of papain hydrolysate along with ascorbic acid are given in Table.1 and 2, Fig 2 and 3, respectively. Four fractions obtained by HPLC were tested for antioxidant activity . Out of four fractions, only fraction 2 was found to be having DPPH radical scavenging activity and superoxide free radical

scavenging activity. Fraction 2 of the HPLC eluate of papain hydrolysate showed highest free radical scavenging activity when compared to Oyster mushroom protein and crude papain hydrolysate as such. Free radical scavenging activity of HPLC fraction 2 was comparable with ascorbic acid standard.

Table.1: DPPH radical scavenging activity of Oyster mushroom Total protein, Papain hydrolysate, HPLC fraction 2 and Ascorbic acid.

Concentration in µg/ml	DPPH radical scavenging activity in %			
	Total protein	Papain hydrolysates	HPLC fraction 2	Ascorbic acid
100	33.84	46.45	54.55	66.17
200	37.63	50	59.6	69.7
300	41.67	53.03	64.15	77.53
400	45.46	56.57	68.69	80.81
500	48.99	59.85	78.26	83.84
600	52.02	62.63	81.82	86.87
700	55.81	66.17	85.6	89.15
800	59.35	69.45	89.4	91.17
900	62.38	72.73	90.66	94.7
1000	65.41	75.26	93.69	96.22

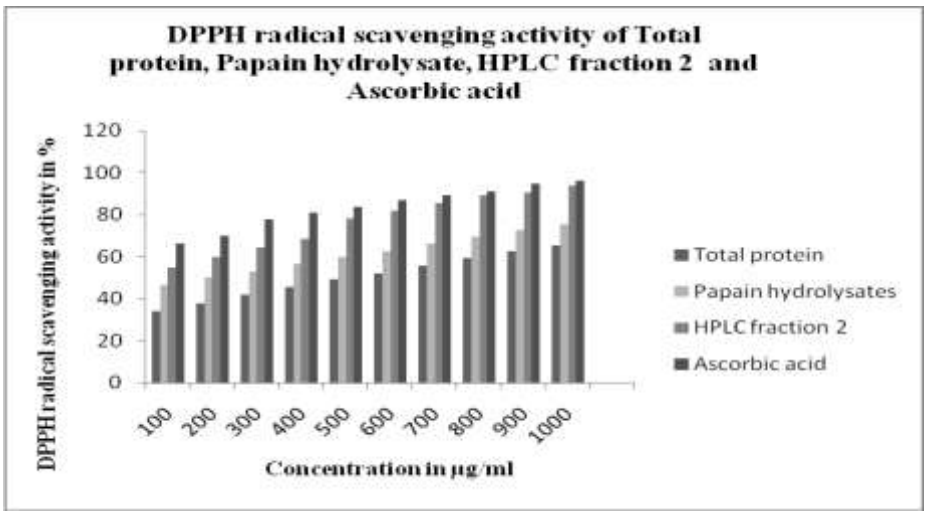

Fig. 2: DPPH radical scavenging activity of Oyster mushroom Total protein, Papain hydrolysate, HPLC fraction 2 and Ascorbic acid.

Table. 2: Superoxide free radical scavenging activity of Oyster mushroom Total protein, Papain hydrolysate, HPLC fraction 2 and Ascorbic acid

Concentration in µg/ml	Superoxide free radical scavenging activity in %			
	Total protein	Papain hydrolysates	HPLC fraction 2	Ascorbic acid
100	27.5	62.58	68.62	70.51
200	29.13	64.48	73.1	73.96
300	31.37	66.2	74.48	76.2
400	33.28	68	78.1	79.13
500	35.17	75.25	81.37	82.41
600	37.93	77.93	82.93	83.96
700	40.34	79.82	85	86.51
800	41.89	81.37	89.31	90.86
900	44.65	87.93	91.55	93.62
1000	47.93	89.22	93.44	95.36

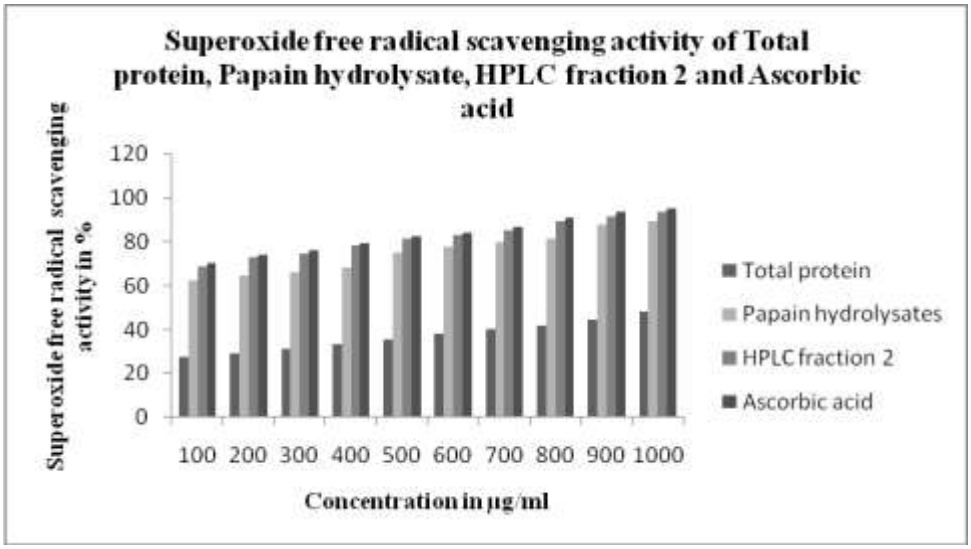

Fig.3: Superoxide free radical scavenging activity of Oyster mushroom Total protein, Papain hydrolysate, HPLC fraction 2 and Ascorbic acid

DPPH radical scavenging activity of total protein of Oyster mushroom was found to be 33.84% at 100 µg/ml concentration and 65.41% at 1mg/ml concentration whereas DPPH radical scavenging activity of papain hydrolysate was found to be 46.45% at 100 µg/ml concentration and 75.26% at 1mg/ml concentration respectively. Out of four fractions of papain hydrolysate purified by HPLC, fraction 2 was found to be having antioxidant activity and it was found to be having 54.55% at 100 µg/ml concentration and 93.69% at 1mg/ml

concentration respectively. Ascorbic acid standard was found to be having 66.17% at 100 µg/ml and 96.22% at 1mg/ml concentration respectively.

Superoxide free radical scavenging activity of total protein of Oyster mushroom was found to be 27.5% at 100 µg/ml concentration and 47.93% at 1mg/ml concentration whereas Superoxide free radical scavenging activity of papain hydrolysate was found to be 62.58% at 100 µg/ml concentration and 89.22% at 1mg/ml concentration respectively. Out of four fractions of papain hydrolysate purified by HPLC, fraction 2 was found to be having Superoxide free radical scavenging activity and it was found to be having 68.62 % at 100 µg/ml concentration and 93.44% at 1mg/ml concentration respectively. Ascorbic acid standard was found to be having 70.51% at 100 µg/ml and 95.34% at 1mg/ml concentration respectively.

Gezer et al [12] reported that 50% of inhibition value for ethanol extract of *Ramaria flava* mushroom at 270 µg/ml concentrations and reported that it is fairly significant when compared to commonly used synthetic antioxidant BHA and alphatocopherol. Keles et al [13] has reported antioxidant activity of methanol extract of Agaricus, Chlororhyllus, Macrolepiota and *Pleurotus ostreatus* as 67.86%, 80.64%, 90.07% and 86.35% respectively. Antioxidant properties of ethanol, cold and hot water extract of *Pleurotus citrinopileatus* has been studied by Yu-ling lee et al [14] and reported ethanol extracts were more effective in antioxidant properties.

Jayakumar et al [15] investigated antioxidant potential of ethanol extracts of Oyster mushroom (*Pleurotus ostreatus*) and reported 56.20% and 60.02% hydroxyl and superoxide radical scavenging effect at 10mg/ml concentrations. Joan Hua Yang et al [16] reported antioxidant activities of methanol extracts of Winter, Shitake and Oyster mushrooms and reported highest antioxidant activity of 54.3% for tree Oyster mushroom at 40mg/ml concentration.

Donatha Damian Tibuhwa [17], investigated the DPPH radical scavenging activity of methanol extract of Cantharellus and 2 Afrocantharellus species in both dry and fresh forms and reported that higher activity of fresh forms than dry forms. All the cited reports were about the antioxidant properties of ethanol, methanol, acetone and hot water extracts of mushroom.So far antioxidant activity of hydrolysed Oyster mushroom has not been reported. In the present study antioxidant activity of Oyster mushroom proteins hydrolysed by Papain enzyme and HPLC purified fractions of papain hydrolysate have been studied. Fraction 2 of

papain hydrolysate of Oyster mushroom protein has been found to be having higher antioxidant activity when compared to ethanol and methanol extracts of mushrooms of cited reports and the activity is comparable with ascorbic acid.

CONCLUSION

Results of the present study showed that HPLC fraction 2 of papain hydrolysate of Oyster mushroom protein was having highest antioxidant activity than Oyster mushroom protein and crude papain hydrolysate as such and it was found to be having 93.69% DPPH radical scavenging activity and 93.44% superoxide free radical scavenging activity at 1mg/ml concentration. Hydrolysis of Oyster mushroom protein by papain resulted in fraction with good antioxidant activity. These protein fraction can be used as antioxidants after *invivo* studies.

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