Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(4); 493-497

ISSN: 0975-4873

Research Article

Radical Scavenging Activity of Edible Fungal Sporocarp Extracts from the Niger Delta Region of Nigeria

Ita B N[1*], Eduok S I[2]

[1]*Department of Chemistry, University of Uyo, Uyo, Akwa Ibom State, Nigeria*
[2]*Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria*

Received: 11th Feb, 17; Revised 19th March, 17, Accepted: 12th April, 17; Available Online:25th April, 2017

ABSTRACT

The radical scavenging activity of acetone, ethanol and aqueous extracts from *Agaricus placomyces, Cantharellus cibarius and Pleurotus ostreatus* from the Niger Delta region of Nigeria was determined with their metal chelating and reducing power. The extracts exhibited significant free radical scavenging activities in the DPPH assay (IC_{50} = 2.11 to 4.37 mg/mL) with the aqueous extract from *P. ostreatus* being the most potent. Also, the aqueous extracts exhibited better metal chelating abilities and reducing power than the ethanol and acetone extracts. In addition, higher content of total phenolics and flavonoids was found in the aqueous extract of *A. placomyces* (55.63mg GAE/g) and *P. ostreatus* (15.55 mgQE/g) respectively, whereas ascorbic acid ranged from 0.33 to 1.22 mgAAE/ in all the extracts. Overall, our results indicate that mushrooms particularly *Agaricus placomyces, and Pleurotus ostreatus* contain high amount of polyphenolic compounds with significant free radical scavenging abilities, notable metal chelating activities and promising reducing power. These properties indicate that the mushrooms are functional foods with the potential to attenuate oxidative stress associated with free radical damage in biological systems.

Keywords: Free radical scavenging activity, Niger Delta region, *Agaricus placomyces, Pleurotus ostreatus, Cantharellus cibarus.*

INTRODUCTION

Free radicals are reactive species produced during normal metabolic processes in cells and can exert dose-dependent adverse effects known as oxidative stress. The effect in cellular systems vary and includes disrupted and peroxidised lipids, denatured protein and damaged DNA which reduce their normal biological activities[1]. Oxidative stress is implicated in a variety of diseases such as cancer, diabetes, cardiovascular and neurodegenerative disorder amongst others. Consumption of natural foods that can scavenge these radicals is of paramount interest because of the health benefits associated with their use[2].

Mushrooms are appreciated by many cultures around the world as nutritional foods with enticing flavours. They are increasingly important in human diet because of low calories and fat content, high protein and polyunsaturated fatty acids with appreciable content of vitamins, dietary fibre, minerals, amino acid, alkaloids, phenolic compounds and terpenes[3]. In addition, their therapeutic properties such as antitumor, antifungal, antidiabetic, antiproliferative, antioxidant, antibacterial activities as well as cholesterol lowering and immune stimulation have been reported[4-6]. These beneficial effects are due to the phytoconstituents present in the mushroom and the composition is affected by strain, time of harvest, method of handling and habitat.

The Niger Delta region in Nigeria lies between longitude 50E to 80E and latitudes 40N to 60N. The Nigerian states of Rivers, Akwa Ibom, Ondo, Abia, Imo, Bayelsa, Delta, Edo and Cross River are within this region. With an equatorial mansoon climate, this ecosystem has extensive areas with forest cover suitable for the growth of mushrooms. It harbours a variety of mushrooms including *Agaricus placomyces, Cantharellus cibarius and Pleurotus ostreatus*. These mushrooms are harvested by rural dwellers within this region primarily for culinary purposes. Although there are reports on the radical scavenging abilities of mushrooms elsewhere[7], studies from this region is scanty despite the large quantity and variety of mushroom consumed. Thus, the present study evaluates the radical scavenging activity of acetone, ethanol and aqueous extracts from *Agaricus placomyces, Cantharellus cibarius and Pleurotus ostreatus* together with their metal chelating and reducing properties.

MATERIALS AND METHODS

Sample collection and treatment

Three mushroom species namely *Agaricus placomyces, Cantharellus cibarius and Pleurotus ostreatus* were harvested from decayed wood and soil in wet season month of June 2016 within Akwa Ibom State, Nigeria and identified by a mycologist. They were destalked, cleaned, air dried and pulverised. The pulverised sample (40 g) was extracted with acetone and ethanol respectively in a Soxhlet apparatus for 8 h. The samples were filtered and evaporated *in-vacuo* to obtain the crude acetone and

**Author for Correspondence: basil_ita@yahoo.com.*

ethanol extracts, respectively. For the aqueous extract, a modified method of Kozarski *et al*[2] was used. Briefly, 40 g of each sample was stirred in 300 mL of water at 120 rpm for 24 h and filtered through a Whatman No. 4 paper. The residue was re-extracted with two additional portions of 300 mL of water and the combined extract freeze-dried to give the aqueous extract.

Chemicals

1,1- diphenyl-2-picryl hydrazyl (DPPH), disodium salt of 3-(2-pyridil)-5,6-bis(4- phenylsulfonic acid)-1,2,4-triazine (ferrozine), Folin Ciocalteu reagent, gallic acid, quercetin, 2,4 dinitrophenyl hydrazine (DNPH), butylated hydroxyanisole (BHA), trichloroacetic acid (TCA) were purchased from Sigma – Aldrich. All other reagents were of analytical grade.

Total phenolic content

Folin Ciocalteu reagent was used for the determination of total phenolic content of the extracts. Briefly, 10 µL of each extract was taken and the volume made up to 2 mL with distilled water. To this mixture, 0.5mL of Folin-Ciocalteu reagent was added and incubated for 3 min. Thereafter, 2 mL of Na_2CO_3 (20 % w/v) was added and the resulting mixture placed in boiling water for 1 min, allowed to cool to room temperature. The absorbance of this mixture was measured at 765nm and normalized by subtracting the absorbance of the control. Total phenolic content was calculated using a calibration curve prepared from gallic acid and expressed in mgGAE/g[8].

Flavonoid content

The modified method of Kumar *et al*[2] was used to evaluate the flavonoid content of the extracts. Briefly, each plant extract (10 μL) was diluted with distilled water to a total volume of 2 mL and equilibrated for 3 min at room temperature. Following this, 3 mL of 5 % $NaNO_2$ and 0.3 mL of 10 % $AlCl_3$ was added and kept for a further 6 min. Thereafter, 2 mL of 1M NaOH was added to this mixture and the final volume adjusted to 10 mL with distilled water. The absorbance of this mixture was read at 510 nm. Flavonoid content was calculated using a standard calibration curve prepared from quercetin and expressed in mgQE/g.

Ascorbic acid content

10 µL of each extract was taken and the volume made to 2 mL with distilled water, 2 mL of 2, 4-dinitrophenylhydrazine (DNPH) and 1 drop of 10 % thiourea were added to the mixture, which was heated in a water bath for 15 min and allowed to cool to room temperature. Following this, the mixture was placed in an ice bath at 0 ^oC and 5 µL of H_2SO_4 (80 %v/v) was added with gentle swirling, and the absorbance taken at 521 nm. Ascorbic acid was used as the standard[2].

DPPH activity

To evaluate the DPPH activity, 1 mL of each extract at varying concentrations was mixed with 1 mL of DPPH solution (0.004 %) in methanol. This mixture was vigorously shaken and allowed to stand in the dark for 30 min. The reduction of the DPPH radical was determined by measuring its absorption at 517 nm. This procedure was repeated for the blank and control and the radical scavenging activity of the extract calculated using the equation:

DPPH scavenging effect (%) = $[(A_{blank} - A_{sample})/A_{blank}]$ x 100.

Sample concentration providing fifty percent inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. BHA was used as standard[9].

Reducing power

The reducing power of the extracts was determined according to the method of Oyiazu[10]. Each extract at varying concentration in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and the mixture incubated at 50 ^oC for 20 minutes. Thereafter, 2.5 mL of 10% trichloroacetic acid (w/v) was added and the mixture centrifuged at 200 g for 19 minutes. The upper layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance measured at 700 nm against a blank. A higher absorbance indicated a higher reducing power. IC_{50} value (mg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation. BHA was used as positive control.

Metal chelating activity

Metal chelating activity was determined according to the modified method of Decker and Welch[11]. Briefly, 0.5 mL of each extract at varying concentrations was mixed with 0.05 mL of 2 mM $FeCl_2$ and 0.1 mL of 5 mM ferrozine and the total volume made to 2 mL with methanol. This mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage

Table 1: Content of phenolics, flavonoids, ascorbic acid and antioxidant activity of mushroom extracts

	Agaricus placomyces			*Cantharellus cibarius*			*Pleurotus ostreatus*			Control	
	AcE	EtE	AqE	AcE	EtE	AqE	AcE	EtE	AqE	BHA	EDTA
Total Phenolics (mgGAE/g)	18.32	31.46	55.63	9.22	16.51	21.78	7.55	36.38	49.21	-	-
Flavonoids (mgQE/g)	3.44	5.26	8.44	2.13	10.32	9.77	1.87	5.71	15.55	-	-
Ascorbic acid (mgAAE/g)	0.27	1.18	1.13	0.33	1.22	1.08	0.56	0.88	1.01	-	-
DPPH activity IC_{50} (mg/mL)	3.83	3.36	2.51	3.88	2.76	2.82	4.37	3.21	2.11	1.45	-
MC activity IC_{50} (mg/mL)	5.95	3.75	3.17	4.54	3.08	2.97	3.57	2.86	2.78	-	1.06
Reducing power IC_{50} (mg/mL)	6.11	5.28	4.04	7.08	5.08	4.31	5.54	3.88	4.39	-	< 1.00

MC = metal chelating.

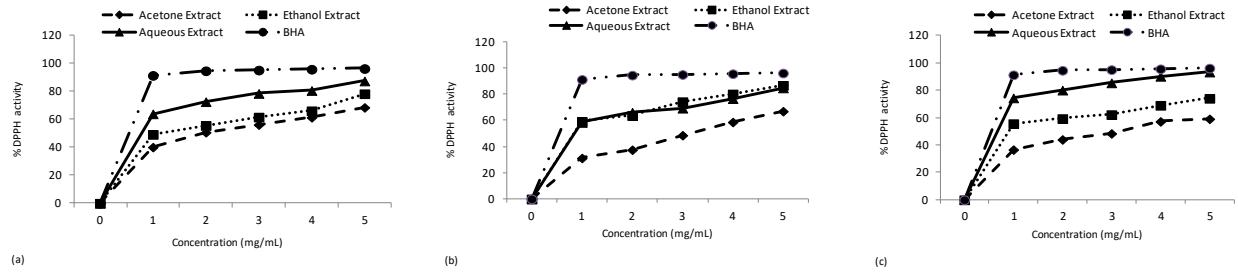

Fig.1: DPPH activity of extracts from (a) *Agaricus placomyces* (b) *Cantharellus cibarius and* (c) *Pleurotus ostreatus.*

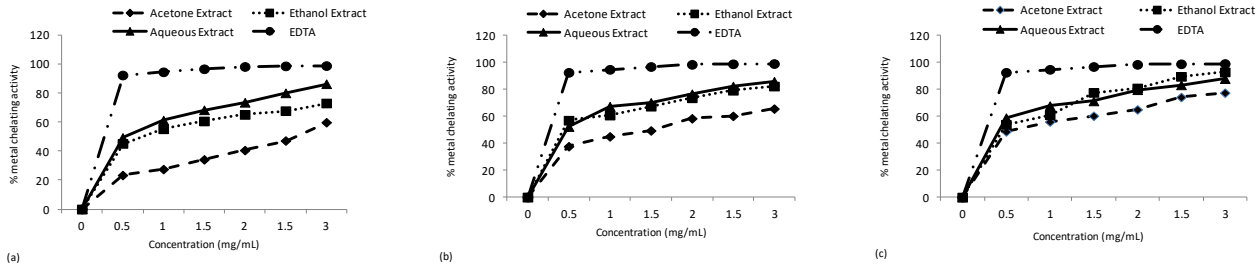

Fig.2: Metal chelating activity of extracts from (a) *Agaricus placomyces* (b) *Cantharellus cibarius and* (c) *Pleurotus ostreatus*

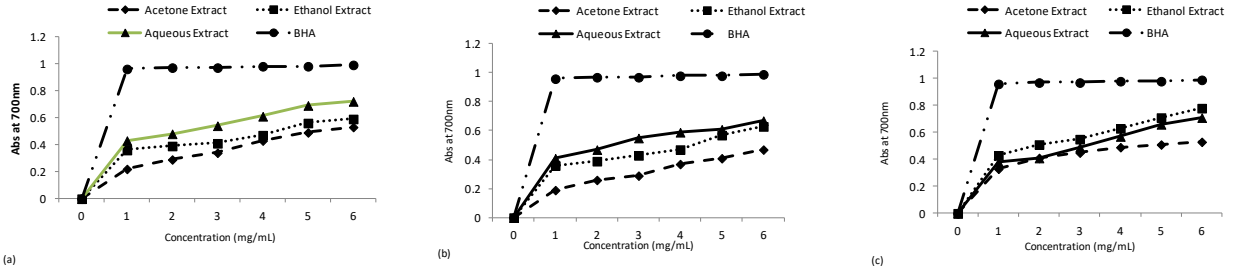

Fig.3: Reducing power of extracts from (a) *Agaricus placomyces* (b) *Cantharellus cibarius and* (c) *Pleurotus ostreatus*

inhibition rate of ferrozine – Fe^{2+} complex formation was calculated using the formula:

Scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where, $A_{control}$ = absorbance of ferrozine – Fe^{2+} complex, and A_{sample} = absorbance of sample. EDTA was used as positive control.

Statistical analysis

Microsoft Excel 2007 was used for all statistical analysis. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Contents of total phenolics in the mushrooms varied with solvent with *A. placomyces* aqueous extract having the highest (55.63 mgGAE/g) and *Pleurotus ostreatus* acetone extract the least (7.55mgGAE/g) (Table 1). Generally, observed trend was aqueous extract (aqE) > ethanol extract (EtE) > acetone extract (AcE), with mushrooms showing order *Agaricus placomyces* > *Pleurotus ostreatus* > *Cantharellus cibarius*. Also, phenolics in the aqueous extracts from *A. placomyces* and *P. ostreatus* were at least two times higher than in *C. cibarius*, whereas 36.38 mgQE/g phenolic content was obtained in the ethanol extract of *C. cibarius* indicating a higher concentration than in the other mushrooms. Contents of flavonoids were lower than values for phenolics, and varied between 1.87 to 15.55 mg QE/g, with the aqueous extract from *P. ostreatus* having the highest value and the acetone extract from *C. cibarius* the least.

In comparison with other works, our values are higher than reports by Keles *et al*[12], Wang and Xu[13] and Hussien *et al*[14], but had lower phenolic content than the methanolic extracts from *C. cibarius*[7]. In addition, higher total phenolic contents have been reported in the aqueous extracts compared to other solvents[15]; this is in consonance with our results. Ascorbic acid content was low (0.27 to 1.18 mgAAE/g) with a slightly higher concentration (1.22 mgAAE/g) in the ethanol extract of *C. cibarius* (Table 1). Generally our result indicate that *A. placomyces*, *P. ostreatus* and *C. cibarius* from this habitat are rich sources of polyphenolic compounds which can scavenge free radicals and thus protect against oxidative stress and cell damage.

In order to access the radical scavenging activity of the extracts, we evaluated their DPPH radical scavenging activity as well as their metal chelating and ferric reducing properties, which are good indicators of antioxidant activity.

In the DPPH assay, the extracts were efficient scavengers of free radicals, however, this varied with concentration. With regards to *A. placomyces*, the acetone, ethanol and aqueous extracts scavenged 40.1, 49.2 and 63.9 % respectively of the DPPH radical at 1 mg/mL; this increased to 68.5, 73.8 and 87.4 % respectively at 5 mg/mL (Fig. 1). Similar results were obtained for *C. cibarius* whose acetone, ethanol and aqueous extracts scavenged 67.1, 86.9 and 84.9 % of the DPPH radical at 5mg/mL respectively. However, lower scavenging activities were

observed for the acetone and ethanol extracts from *P. ostreatus* at 5 mg/mL (59.2 and 74.2% respectively), but its aqueous extract exhibited the highest scavenging activity at 5 mg/mL (93.4%) which was comparable to that of BHA (96.3%). Furthermore, the DPPH activity was also expressed as IC_{50} values (mg/mL), the effective concentration of the extracts that showed 50% inhibition of the radical. Lower IC_{50} values indicate higher antioxidant activity. Based on IC_{50} values (Table 1), our extracts exhibited high DPPH radical scavenging activities (IC_{50} = 2.11 to 4.37 mg/mL), with the aqueous extract from *P. ostreatus* as the most potent. IC_{50} values for methanol extract of *C cibarius* in other studies varied from 2.68 mg/ml[16] to 16.31 mg/ml[3]. Kozarski *et al*[3] reported higher IC_{50} values of 16.31 mg/mL for methanolic extract from *C. cibarius* while Aina *et al*[16] reported comparable value of 2.68 mg/mL for methanol extract from *C. cibarius.* Generally,
our aqueous and ethanol extracts from *P. ostreatus*, had lower IC_{50} values than reports by Chirinang and Intarapichet[15].

These results show that our extracts can effectively scavenge free radicals either by donating hydrogen or by forming nonradical species to reduce oxidative stress and minimize damage to biological systems. This suggests that when consumed, the mushrooms can attenuate oxidative stress associated with free radicals in biological systems. From this we can infer that, fractionated and purified extracts of the mushrooms responsible for the observed activity are potential natural sources of compounds as dietary supplements to produce nutraceuticals and functional foods.

Metal chelating ability is significant in reducing the potential of transition metals (such as iron) to catalyse oxidative chain reactions in biological systems initiated by free radicals[3]. It is evident from our results, that the mushroom extracts are good metal chelators. Ethanol extract from *P. ostreatus* exhibited a pronounced chelating ability of 92.7% at 3 mg/mL, whereas a minimal activity was shown by the acetone extract of *A. placomyces* (23.4% at 0.5 mg/mL) (Fig. 2). Overall, based on IC_{50} values, the aqueous extracts were the most potent metal chelators with the order: *P. ostreatus* > *C. cibarius* > *A. placomyces*. In comparison with other works, our values are similar to reports by Barros *et al* [17].

The reducing power of an extract is a measure of its ability to donate a single electron or hydrogen atom for reduction, as such serve as an important indicator of antioxidant activity. In this study, our extracts had potent reducing power which increased in a dose-dependent manner (Fig. 3). At 6 mg/mL, aqueous extract from *A. placomyces* had significantly ($p < 0.1$) higher reducing power (abs = 0.72) than ethanol and acetone extracts. With regards to *C. cibarius*, comparable reducing power was observed between the aqueous and ethanol extract, and the difference was not significant ($p < 0.1$). However, the optimum reducing power was exhibited by the ethanol extract from *P. ostreatus* (abs = 0.78), and corroborates with our IC_{50} value (IC_{50} = 3.88 mg/mL) (Table 1). A higher IC_{50} value has been reported for *C. cibarius*[3].

CONCLUSION

In this investigation, *A. placomyces*, *P. ostreatus* and *C. cibarius* harvested within the Niger Delta region of Nigeria were rich sources of total phenolics and flavonoids, and their content were solvent- dependent. In addition, the extracts were potent free radical scavengers, with promising metal chelating abilities and good reducing powers. Overall, the aqueous and ethanol extracts were the most active with potentials to attenuate oxidative stress and cellular damage caused by accumulated free radicals. Purified extracts from these mushrooms are thus, good sources of natural compound to produce nutraceutical and functional foods.

REFERENCES

1. T Islam, X Yu, B Xu. Phenolic profiles, antioxidant capacities and metal chelating ability of edible mushroos commonly consumed in China. LWT – Food Science and Technology, 2016; 72:423-431.
2. V Kumar, M Lemos, M Sharma, V Shiram. Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. Free radicals and Antioxidants, 2013; 3: 55-60.
3. M Kozarski, A Klaus, J Vunduk, Z Zizak, M Niksic, D Jakovljevic *et al*. Nutraceutical properties of the methanolic extract of edible mushroom *Cantharellus cibarius* (Fries): primary mechanism. Food and Function, 2015; 6: 1875-1886.
4. OV Oyetayo. Medicinal uses of Mushrooms in Nigeria: Towards full and sustainable exploitation. African Journal of Complementary and Alternative Medicine, 2011; 8(3):267-274.
5. SK Sharma, N Guatam. Chemical, bioactive and antioxidant potential of twenty wild culinary mushroom species. Biomed Research International, 2015; 346508: 1-12.
6. S Panthong, N Voonsathorn, S Chuchawankul. Antioxidant activity, anti-proliferative activity, and amino acid profiles of extracts of edible mushrooms. Genetics and Molecular Research, 2016; 15(4): 123-127.
7. HR Unekwu, JI Audu, MH Makun, EE Chidi. Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. Asian pacific Journal of Tropical Disease, 2014; 4(Suppl1):S153-S157.
8. H Gul, I Bhakshu, F Ahmed, I Qureshi, I Ghazi. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using in vitro assays. BMC Complementary and Alternative Medicine, 2011; 11:64-69.
9. B Subhasree, R Baskar, RL Keerthana, RL Susan, P Rajasekran. Evaluation of antioxidant potential in selected green leafy vegetables. Food Chemistry, 2009; 115: 1213 -1220.
10. M Oyiazu. Studies on products of browning reactions: Antioxidative activities of products of browning

reaction prepared from glucosamine. Japanese Journal of Nutrition, 1986; 44: 307-315.
11. EA Decker, B Welch. Role of ferritin as lipid oxidation catalyst in muscle food. Journal of Agricultural and Food Chemistry, 1990; 38: 674-678.
12. A Keles, I Koca, H Genccelep. Antioxidant properties of wild edible mushrooms. Journal of Food Processing and Technology, 2011; 2(6):1-6.
13. X Wang, B Xu. Distribution of antioxidant activities and total phenolic contents in acetone, ethanol, water and hot water extracts from 20 edible mushrooms via sequential extraction. Austin Journal of Nutrition and Food Sciences, 2014; 2(1):1-5.
14. JM Hussien, DD Tibuhwa, AM Mshandete, AK Kivaisi. Antioxidant activity of seven wild edible mushrooms from Tanzania. African Journal of Food Chemistry, 2015; 9(9): 471-479.
15. P Chirinang, K Intarapichet. Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus sapidus* and *Pleurotus sajor-caju*. Sciences Asia, 2009; 35:326-331.
16. DA Aina, SG Jonathan, OJ Olawuyi, DO Ojelabi, BM Durowoju. Antioxidant, antimicrobial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius* - a Nigerian mushroom. New York Science Journal, 2012; 5(10):114-120.
17. L Barros, M Ferreira, B Queiros, ICFR Ferreira, P Baptista. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chemistry, 2007; 103:413-419.