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Antioxidant profile of four selected wild edible mushrooms in Nigeria

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ABSTRACT

Antioxidant activity of four mushrooms was assayed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power, iron chelation, ABTS, DPPH and hydroxyl radical. Furthermore, total phenol and flavonoid contents of these mushrooms extract were also determined. Rutin and quercertin were used as standards. All the four mushrooms exhibited potent and concentration dependent free radical scavenging activity in all the assays tested. However, Pleurotus ostreatus had the highest radical scavenging ability. Reductive ability of these mushrooms were also found to increase with increase in concentration. Total phenol and flavonoid content determination showed that all the mushrooms are rich in phenols and flavonoids. Pleurotus ostreatus had the highest phenolic and flavonoid content among the four mushrooms indicating that it has the highest antioxidant capacity among the mushrooms studied. All the results of the in vitro antioxidant assays reveal potent antioxidant and free radical scavenging activity of P. eryngii, T. robustus, P. cinnabarinus, P. ostreatus. This potent antioxidant activity may be attributed to its high phenolic and flavonoid contents and may be responsible for their nutritional and therapeutic uses.

Keywords: Mushrooms, Phenolics, Flavonoids, *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus, Pleurotus ostreatus.*

INTRODUCTION

Reactive oxygen species (ROS) are generated through normal metabolic processes or as by product of exogenous insult [1,2,3]. ROS include free radicals such as superoxide anion (O_2^{*-}), hydroxyl radical (*OH), as well as non radical molecules like hydrogen peroxides (H_2O_2), singlet oxygen ($^1O_2$) e.t.c. [4,5]. The production of ROS is balanced by the antioxidative defense system in healthy individuals. Oxidative stress is generated when the equilibrium shifts in favor of free radical generation as a result of a depletion of antioxidant levels. Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, protiens, DNA), eventually leading to chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post ischemic perfusion injury, myocardiac infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in human [6,7]. Antioxidants are substances that when present at lower concentration compared to that of an oxidizable substrate, significantly delay or prevent oxidation of the substrate, or compounds that delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidation chain reactions [8,9].

Nowadays, much attention has been given to natural antioxidant because of their associated health benefits. [10]. Mushrooms have been used as food supplement in various cultures because they are rich source of proteins, vitamins, fats, carbohydrates, amino acids and minerals [11]. Some common species like *Pleurotus tuber-regium* has been reported to cure headache, stomach ailments, colds and fever [12]; asthma, smallpox and high blood pressure [12,13]; while other species like *Lentinus tuber-regium* and *Lentinus tigrinus* are used for treating dysentery and for blood cleansing respectively. *Auricularia* species have been used traditionally in the treatment of hemorrhoids and various stomach ailments [14]. Some species are also recommended to anemic patients, owing to their high folic acid content while some are reputed to possess antidiabetic, anti-allergic, anticholesterol, anti-tumor and anti-cancer

properties [11]. Due to the reported nutritional and health benefits of mushrooms and derth of information on *in vitro* antioxidant properties of these mushrooms, this study sought to determine the antioxidant properties of four selected wild Nigerian edible mushrooms (*Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus*

EXPERIMENTAL SECTION

Chemical

All chemicals used, including solvents, were of analytical grade. Thiobabituric acid (TBA), 1, 1-diphenyl-2-picrylhydrazine (DPPH), sodium nitroprusside, ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2$), trichloroacetic acid (TCA) and buthylated hydroxyltoluene (BHT) were obtained from Sigma_Aldrich (St Louis, MO, USA).

Plant collection

The mushrooms samples were collected from logs of woods, palm logs, and humus soil from different locations in Akure, Ondo State, Nigeria and; identified in the Department of Crop Soil and Pest Management, The Federal University of Technology, Akure. The mushroom samples were later de-stalked, washed and sun dried and grounded into a powdery fine texture and stored at room temperature in air tight polythene bag prior to use.

Preparation of extract

Hundred grammes (100.0g) each of the powdered mushrooms were soaked in 1liter of distilled water for 24hours after which they were filtered separately using a Whatman filter paper. The extracts were stored in airtight containers in a refrigerator at -4 ^{O}C until required for use. This serves as the stock solution for all determinations.

Determination of Total Phenolic Content

For the determination of total phenolic content of the extracts , 0.25 mL of the extract was placed in a 25.0 mL volumetric flask and 5.0 mL distilled water was added. Folin-Ciocalteu's phenol reagent (1.25 mL) was added and the resulting solution mixed together. After 2 min, 3.75 mL 20% (w/v) sodium carbonate solution was added, the contents mixed together again and distilled water was added to volume and mixed. The mixture was allowed to stand for 2 h after addition of sodium carbonate solution; and the absorbance of the mixture measured at 760 nm using a Lambda EZ150 spectrophotometer (Perkin Elmer, USA). Tannic acid was used as standard and the results expressed as mg tannic acid equivalents per gram of the sample.

Determination of Total Flavonoid Content

The total flavonoid content of the different mushroom extracts was determined using a slightly modified method reported by[15]. 0.5mL of appropriately diluted sample was mixed with 0.5mL methanol; 50µL of 10% $AlCl_3$; 50µL of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. Quercetin was used as standard and the results were expressed as mg quercetin equivalent per gram of the sample.

Reducing Antioxidant Power

The reducing power of the individual mushroom extracts was determined by assessing the ability of each extract to reduce $FeCl_3$ solution as described by[16]. Appropriate dilution of each extract (2.5 mL) was mixed with 2.5 mL of 200.0mM sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. Each mixture was incubated at 50^{o}C for 20 min and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 353 x g for 10 min. Five millilitres of the supernatant was mixed with an equal volume of water and 1.0 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant power was determined in triplicates and expressed as mg ascorbic acid equivalent/g of the sample.

OH^- Radical Scavenging Ability

The ability of the extract to prevent Fe^{2+}/H_2O_2 induced decomposition of deoxyribose was carried out using the method of [17]. Freshly prepared extract (0-100µl) was added to a reaction mixture containing 120.0 µl, 20mM deoxyribose; 400.0 µl, 0.1M phosphate buffer pH 7.4; 40.0 µl, 20.0 mM hydrogen peroxide and 40.0 µl, 500.0 mM FeSO4; and the volume was made to 800.0 µl with distilled water. The reaction mixture was incubated at 37^{O}C for 30min and then stopped by the addition of 0.5mL of 2.8% TCA. This was followed by the addition of 0.4mL of 0.6% TBA solution. The reaction tubes were subsequently incubated in boiling water for 20min. The absorbance was measured at 532nm in spectrophotometer and the percentage radical inhibition which was determined in triplicates was subsequently calculated.

ABTS Antiradical Assay

Antioxidant activity of the extracts was determined using the 2, 2'-azinobis-(3- ethylbenzothiazoline-6-sulfonic acid) ABTS antiradical assay [18]. The $ABTS^{\bullet+}$ (mother solution) was prepared by mixing equal volumes of 8 mM ABTS and 3 mM potassium persulphate ($K_2S_2O_8$) (both prepared using distilled water) in a volumetric flask and allowed to react for a minimum of 12 h in a dark place. The working solution was prepared by mixing 5.0 mL of the mother solution with 145.0 mL phosphate buffer (pH 7.4). A range of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-carboxylic acid) standard solutions (100-1000 μM) were prepared in acidified methanol. The working solution (2.9 mL) was added to the methanolic extracts (0.1 mL) or Trolox standard (0.1 mL) in a test tube and mixed with a vortex. The test tubes were allowed to stand for exactly 30 min. The absorbance of the standards and samples were measured at 734 nm with a Lambda EZ150 spectrophotometer. The results which were determined in triplicates were expressed as μmol Trolox equivalents/g sample on dry weight basis.

Iron Chelation Assay

2.5 ml of each mushroom extract (1.0 mg/g) was mixed with 2.5 ml of 200.0 mM sodium phosphate at pH of 6.6 and 2.5 ml of 1.0 % potassium ferric cyanide. The reaction mixture was then incubated at 50 ^{O}C for 20 mins; after which 2.5 ml of trichloroacetic acid was added and centrifuged at 659 rpm for 10 mins. 5. 0 ml of the supernatant was mixed with 5. 0 ml of water and 0.1 % FeCl3 was added. The absorbance of the resultant solution was then read at 700 nm [19].

DPPH Antiradical Assay

The stock solution was prepared by dissolving 24.0 mg DPPH with 100.0 mL ethanol and then stored at -20^{o}C until needed. The working solution was obtained by mixing 10.0 mL stock solution with 45.0 mL ethanol to obtain an absorbance of 1.1 units at 515 nm using the spectrophotometer. Phenol extracts (3.0 mL) was allowed to react with 27.0 mL of the DPPH solution for 6 h in the dark and absorbance read at 515nm.

Statistical Analysis

All the experiments were done in triplicates; and the data collected were analysed and the results presented as mean ± SD (n=3). Differences between mean, were analysed by one way analysis of variance (ANOVA) followed by testing for multiple range comparisons between means (Duncan's). The differences between means were reported as statistically significant when $p< 0.05$.

RESULTS

Total phenol content and total flavonoid content

The total phenol content and total flavonoid content *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus* are presented in Table 1. *Pleurotus ostreatus* had the highest total phenol content (66.24±0.00 mg) of tannic acid equivalent/g of the extract as well as the highest total flavonoid (188.65±5.58 mg) of quercertin equivalent/g of the extract when compared to the other three mushrooms.

Reducing Antioxidant Power

Figure 1 showed the reductive ability of *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus.* Absorbance of the solution was increased with increased concentration. A higher absorbance indicates a higher reducing power. *Termitomyces robustus* had the highest reducing ability at all the concentration tested while *Pleurotus eryngii* had the lowest reductive ability at lower concentration but this ability at higher concentration.

Table 1: *In vitro* anti-oxidant indices of the Selected Mushroom Extracts

Property	Mushroom Samples	Levels (mg/g)
Total Flavonoids	*Pleurotus eryngii*	45.94±2.2 7
	Termitomyces robustus	45.94±2.07
	Pycnoporus cinnabarinus	61.97±2.27
	Pleurotus ostreatus	66.24±0.00
Total Phenol	*Pleurotus eryngii*	124.01±0.80
	Termitomyces robustus	103.34±2.39
	Pycnoporus cinnabarinus	162.72±8.77
	Pleurotus ostreatus	188.65±5.58

Total phenol content is expressed in mg Tannic acid Equivalent per gram of the Extract; and flavonoids content in mg Quercetin Equivalent per gram of the Extract

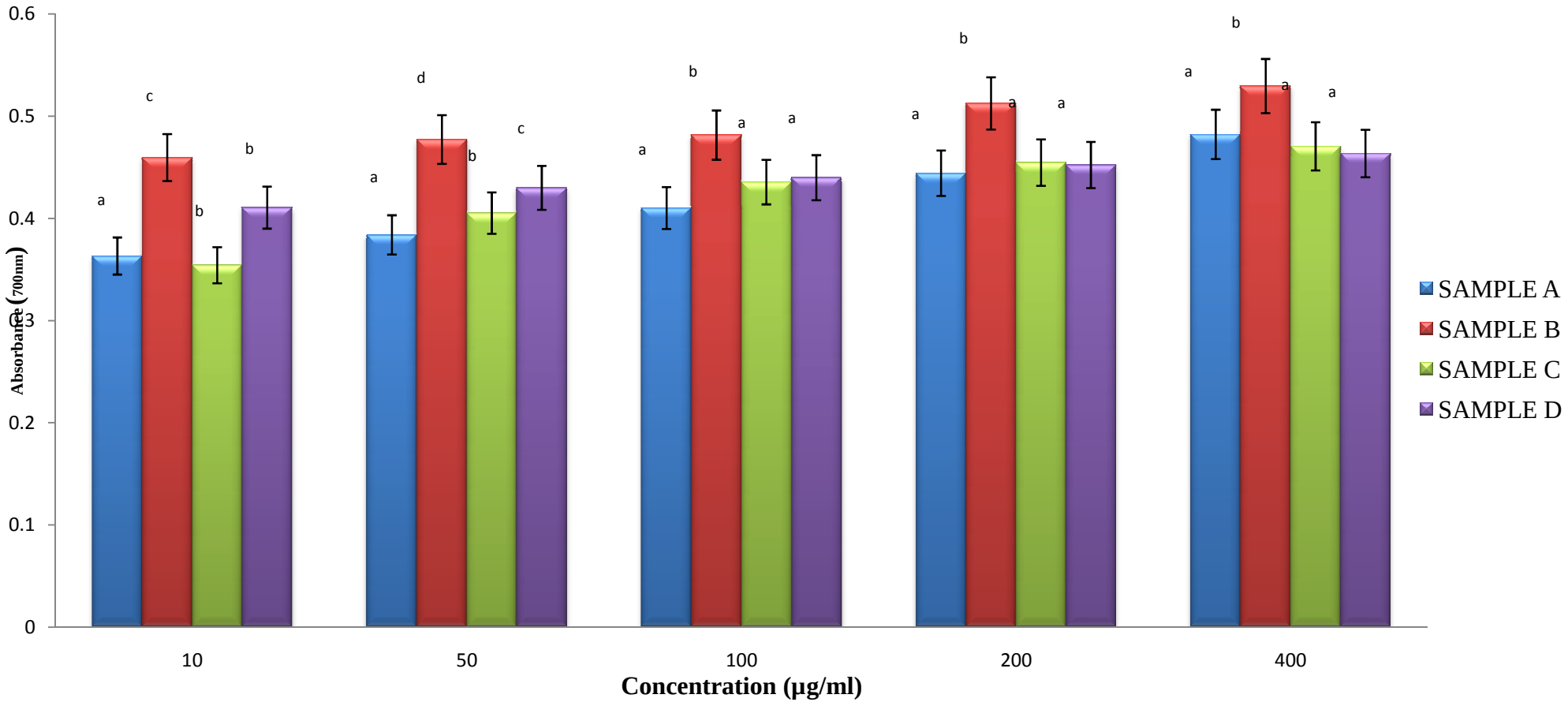

Figure 1: The Reductive Potential of the Mushroom Extracts

Values are the average of triplicate experiments and represented as mean ± standard deviation

Values having the same letter are not significantly different (P≥0.05)

Sample A: *Pleurotus eryngii***Sample B:** *Termitomyces robustus*, **Sample C:** *Picnoporus cinnabarinus*, **Sample D:** *Pleurotus ostreatus*

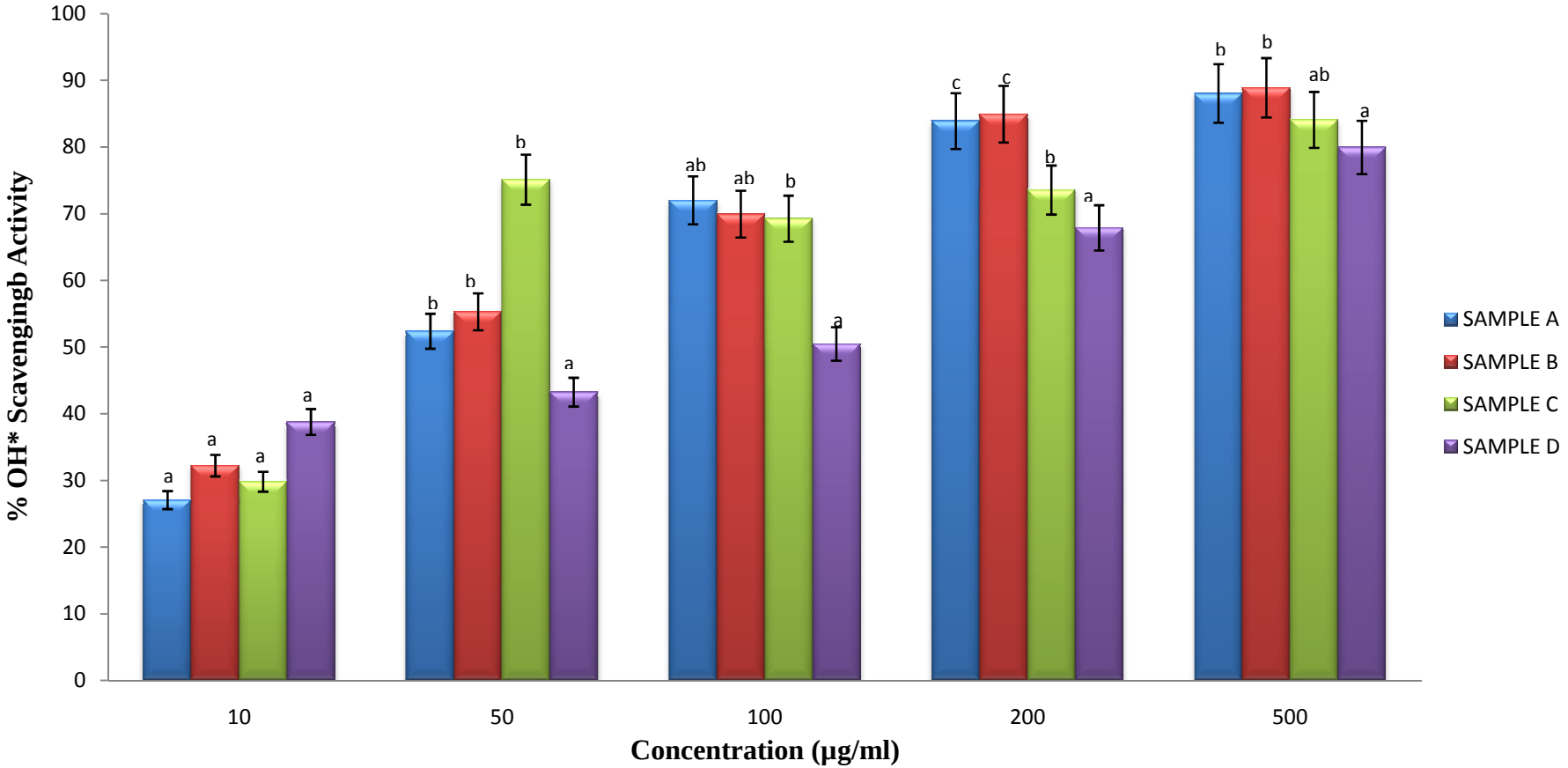

Figure 2: The Hydroxyl Radical Scavenging Activity of the Mushroom Extracts. of 4 wild Mushroom Extracts

Data represents mean ± standard deviation values of 3 to 4 independent experiments performed in triplicates

Values having different letter are significantly different ($P \geq 0.05$)

Sample A: *Pleurotus eryngii*, **Sample B:** *Termitomyces robustus*, **Sample C:** *Picnoporus cinnabarinus*, **Sample D:** *Pleurotus ostreatus.*

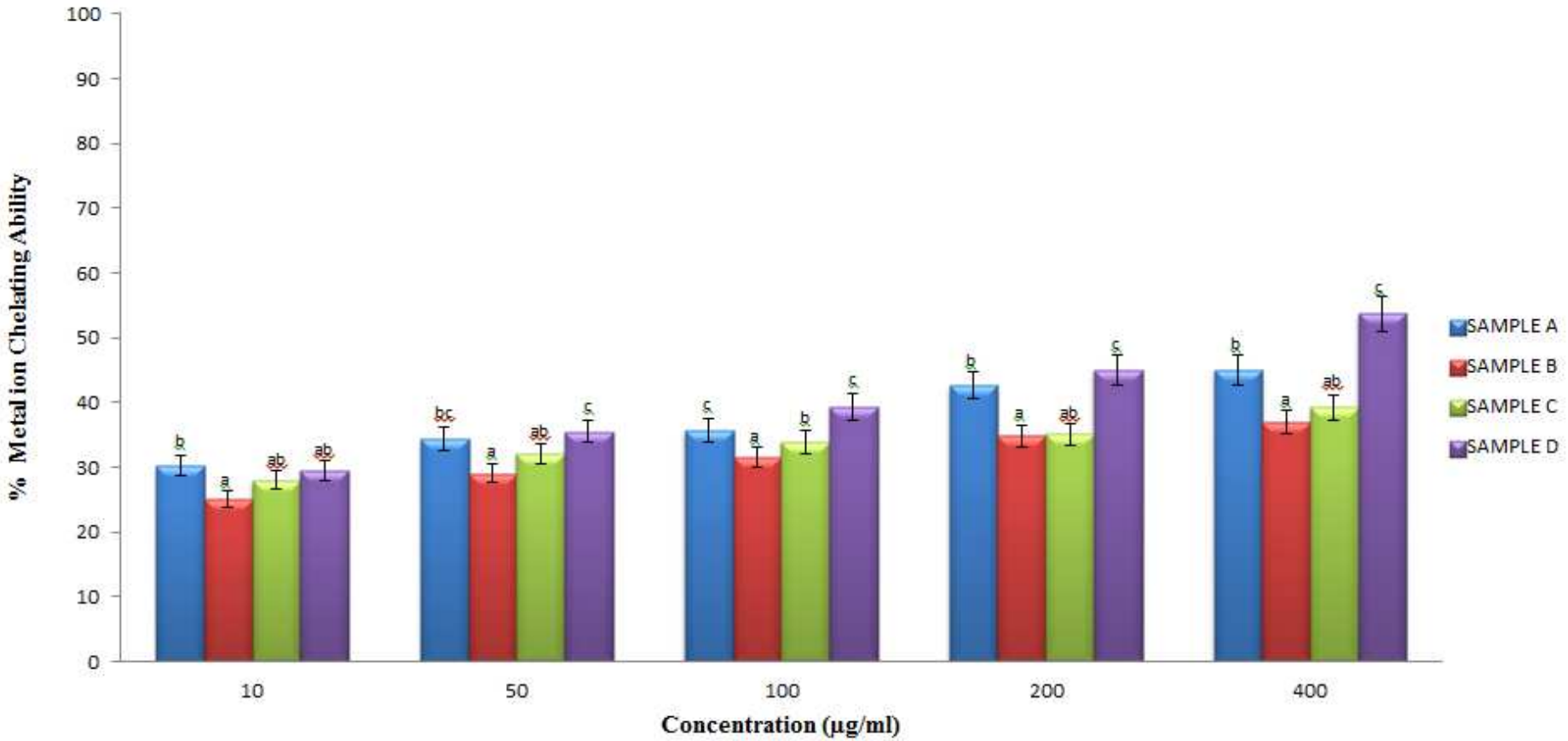

Figure 3: Metal Chelating ability of the Mushroom Extracts. of 4 wild Mushroom Extracts

Data represents mean ± standard deviation values of 3 to 4 independent experiments performed in triplicates

Values having different letter are significantly different ($P \geq 0.05$)

A: *Pleurotus eryngii*, **B:** *Termitomyces robustus*,**C:** *Picnoporus cinnabarinus*, **D:** *Pleurotus ostreatus.*

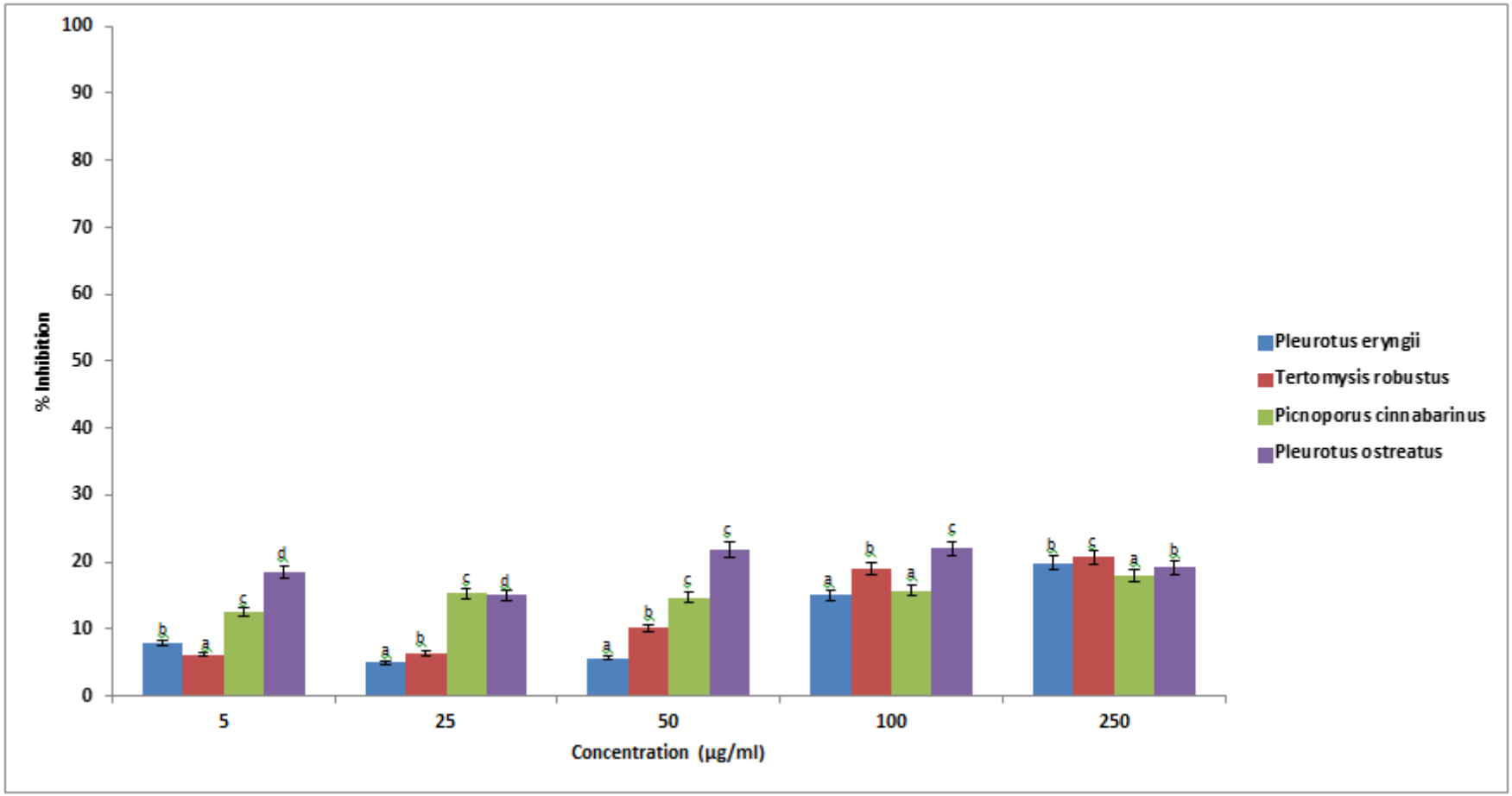

Figure 4 : DPPH Radical Scavenging Activity of 4 wild Mushroom Extracts
Data represents mean ± standard deviation values of 3 to 4 independent experiments performed in triplicates
Values having different letter are significantly different (P≥0.05)

Table 2: The ABTS radical scavenging activity of four selected mushrooms

Extract (100 µg/ml)	TEAC (µmol/g)
Pleurotus eryngii	0.4229^{b}+00.2
Termitomyces robustus	0.4052^{a}+0.010
Picnoporus cinnabarinus	0.4082^{ab}+0.013
Pleurotus ostreatus	0.3935^{a}+0.001

Values are the average of triplicate experiments and represented as mean ± standard deviation. Values having the same letter are not significantly different ($P \geq 0.05$)

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus* were measured deoxyribose method. All showed potent and varying degree of hydroxyl radical scavenging activity at various concentrations Figure 2.

ABTS radical scavenging activity

ABTS radical scavenging activity of *P. eryngii, T. robustus, P. cinnabarinus,* and *P. ostreatus* were shown in Table 2. All the mushrooms extract exhibited potent ABTS radical scavenging activity in concentration dependent manner with *P. ostreatus* having the most radical scavenging activity at low concentration.

Metal chelating activity

Figure 3 depicts the iron chelating ability of *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus*. The ferrous ion-chelating effect of all mushrooms increased well with increasing concentrations. *Pleurotus ostreatus* had highest iron chelating ability compared to other mushrooms extracts in this study.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus* are presented in Figure 4. *Pleurotus ostreatus* had the highest DPPH radical scavenging ability at the concentration studied compared with the other three mushroom samples.

DISCUSSION

Under increasing situation of excess of free radicals generation, which may distrupt normal functions of human organism due to the damage of cellular lipids, proteins, and DNA, the interest in antioxidant abilities of various foods has increased for years. This trend recently includes recently also the wild growing mushrooms [20].. Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and abiotic stress condition such as infection, water stress, and cold stress [21] and can protect the human body from free radicals, whose formation is associated with the normal natural metabolism of aerobic cells. Polyphenols are common constituents of the human diet, present in most foods and beverages of plant origin. They are capable of removing free radicals, chelating metal catalysts, activating antioxidant enzymes, reducing α-tocopherol radicals, and inhibiting oxidases. They are considered to contribute to the prevention of various degenerative human diseases such as Alzheimer's diseases [22,23,24]. The *in vitro* antioxidant properties of the extracts of the selected mushrooms revealed that phenolics are found in large quantities in the mushrooms; the high total phenolic content (124.01±0.80, 103.34±2.39, 162.72±8.77, 188.65±5.58) mg/g tannic acid equivalents for *Pleurotus eryngii, Termitomyces robustus, Piccnoporrus cinnabarinus,* and *Pleurotus ostreatus* respectively suggests that the total phenolic concentrations in the mushroom samples are in the order; *Pleurotus ostreatus >Piccnoporrus cinnabarinus >Termitomyces robust s> Pleurotus eryngii* (Table 1). This finding indicates that the mushroom species are a significant source of phenolics. The antioxidant activity of the mushroom extracts may be due to the hydroxyl groups in the phenolics [25]. A similar finding was reported in the extracts of *Eucop Mia ulmoides* (Du-zhong) and *Acacia confusa* in which enriched phenolics correlated well with their antioxidant activities [26,27].

Flavonoids have been said to decrease capillary fragility and exert a cortisone-like effect on tissues [28]; and protect against cancer and heart diseases [29]. It therefore implies that the high flavonoids content in the mushroom extracts (Table 1); might be responsible for the therapeutic effect (antihypertensive) of some mushroom species earlier reported [25,28,30,31]. The highest concentration of flavonoids was recorded for *P. ostreatus* extract, followed by *P. cinnabarinus extract*; while the least concentration of flavonoids was recorded for both the *P. eryngii* and *T. robustus* extracts (Table 1).

Reducing power of some medicinal plants has been used as one of the indices of their antioxidant ability. A report by [32] revealed that reducing property can be a novel antioxidative defense mechanism; this is possible through the

ability of the antioxidant compound to reduce transition metals. Antioxidant property of phenolics is due to their redox properties which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [33]. The result of the reducing power of the various mushroom extracts showed that *T. robustus* had the highest reducing power as the concentration increases while the lowest reducing power with increasing concentration is recorded for *P. cinnabarinus* extract (Figure 1).

The hydroxyl radical scavenging activity of the mushrooms as shown in Figure 2 revealed that the least ability of the mushroom extracts to reduce hydroxyl radicals to harmless water molecules (27.20 % at a concentration of 10 µg/ml of mushroom extract) was observed in *Pleurotus eryngii* extract while *Termitomyces robustus* had the highest ability to scavenge or fix hydroxyl radicals (86.09 % at a concentration of 500 µg/ml of mushroom extract). The mushroom extracts appeared to exert potent antioxidant power by acting as a scavenger against OH* free radical, therein providing antioxidant defense systems. The finding also showed that the OH* scavenging activity of the mushroom extract increases with increasing concentration of their extracts.

The ABTS radical scavenging activity is a more sensitive radical that is used for the estimation of antioxidant activity. The decoloration of ABTS radical cation is an indication of the potency of antioxidants to donate electrons or hydrogen atoms to deactivate these radical species. The reduced ABTS radical is colourless in a colour-quenching reaction [34,35]. The ability of the individual mushroom extracts to decolorize the ABTS solution show the potency of these mushrooms in scavenging organic radicals, i.e., ABTS* [32]. The result suggests that the extracts of the mushroom samples can prevent or reduce oxidative damages caused by organic radicals.

Iron is essential for proper cell functions, such as oxygen transport, cellular respiration, and is a co-factor for a number of iron metallic enzymes. However, an excess of free iron can cause the generation of reactive oxygen species from the Fenton reactions, which is a transition metal–catalysed decomposition of hydrogen or lipid peroxide into highly reactive and biological damaging hydroxyl and lipid peroxyl radicals [36]. Metal chelating ability results of the mushroom extracts showed that the highest value (53.78 % at 400 µg/ml) was recorded for the *Pleurotus ostreatus* extract while the weakest metal chelating ability (25.18 % at 10 µg/ml) was recorded for *Termitomyces robustus* since the metal chelating ability value was also the lowest among the four mushroom species (Figure 3). It has been observed that Metal ion chelating antioxidants would also remove the oxidative damage from other less prominent but equally damaging pro-oxidant metal ions such as Cu [36]. Thus, the iron chelating capacity of the mushroom species would prevent transition metals to participate in the initiation of oxidative stress.

DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) is known to be a stable radical at room temperature, which accepts electron or hydrogen radical to become a stable diamagnetic molecule [37]. It has been used to determine the antioxidant activity of various neutral products [38]. The effect of antioxidants on 1, 1-diphenyl-2-picryl hydrazyl (DPPH) had been thought to be due to their hydrogen donating ability [39]. The highest scavenging activity (22.0 %) was recorded for *Pleurotus ostreatus* in the DPPH radical scavenging activity results, while least radical scavenging activity value (5.0 %) was recorded for *Pleurotus eryngii* (Figure 4). The extracts of the mushroom species of interest could be potential sources of natural antioxidant. This finding was supported by [40], who suggested that the strong anti-radical potency possessed by *Dorstenia psilurus* and *Dorstenia ciliate* against DPPH test might be the basis of for their strong therapeutic efficacy in traditional medicine.

The antioxidant investigations on the different mushroom species revealed that all the mushrooms possess high reductive potential; metal chelation, and hydroxyl radical scavenging activities etc., with high concentration of total phenol and total flavonoids.

These bioactive compounds together with the high antioxidant activities obtained in these species of mushrooms may be responsible for their nutritional and therapeutic uses.

These results therefore revealed the fact that *Pleurotus eryngii*, *Termitomyces robustus*, *Piccnoporrus cinnabarinus*, and *Pleurotus ostreatus* contain valuable bioactive agents that could find a tremendous application in drug development.

REFERENCES

[1] LF Shyur; T Jieh-Hen; C Je-Hsin; C Chih-Yang; L Chiu-Ping, *International Journal of Applied Science and Engineering*, **2005**, 3, 3: 195-202.
[2]DG Deavall; EA Martin; JM Horner; R Roberts, *Journal of Toxicology*, Volume **2012**, 1-13.
[3] OO Elekofehinti; IG Adanlawo; A Fakoya; JA Saliu; SA Sodehinde, *Curr Resear J Biol Sci***2012**, 4, 530-533.
[4] CH Foyer; J Harbinson, Eds.,pp.1–42, CRC Press, Boca Raton, Fla, USA, **1994**.

[5] P Sharma; AB Jha; RS Dube; M Pessarakli, *J Botan*., **2012**; 217037:1-26.
[6] S Bhuvaneswari; S Murugesan; TS Subha; R Dhamotharan; N Shettu,*Journal of Chemical and Pharmaceutical Research*, **2013**, 5(3):82-85.
[7] F Yun-Zhong; Y Sheng; W Guoyao, *Nutrition*, **2002**, 18, 872- 879.
[8] G Sanjoy; S Soneswar; A. A. Mao;S Tapan, *Journal of Chemical and Pharmaceutical Research*,**2013**, 5(4):33-40
[9] MT Olaleye; AC Akinmoladun; AA Ogunboye, *Food. Chem. Toxicol*., **2010**, 48(8-9), 2200-2205.
[10] N Huda-Faujan; A Noriham; AS Norrakiah; AS Babji, *African Journal of Biotechnology* **2009**, 8 (3) 484-489,
[11] MM Jiskani, *The dawn Economic and Business Review*, **2001**, 15-21.
[12] BA Oso, *Mycologia*, **1977,**69, 271-279.
[13] IA Fasidi; KS Olorunmaiye , *Food chemistry*, 1994, 50, 397-401.
[14] ST Chang; JA Buswell; *Microbiology and Biotechnology*, **1996,** 1(12), 473-476.
[15] A Meda; CE Lamien; M Romito; J Millogo; OG Nacoulma, *Food Chem***2005,** 91, 571-577.
[16] M Oyaizu, *Japanese Journal of Nutrition*, 1986, 44, 307-315.
[17] B Halliwell; JMC Gutteridge, Free radicals in biology and medicine(3rd ed.). Oxford University Press, 1999.
[18] JM Awika; LW Rooney, *Phytochemistry*, 2004, 65(9): 1199-1221.
[19] R Pulido; L Bravo; F Saura-Calixto , *Journal of Agriculture and Food Chemistry*, **2000,** 48, 3396-3402.
[20] M Esrefoglu, *World Journal Gastroenterology*, **2012,** 18 (39), 5533-5541.
[21] G Oboh; JBT Rocha, *European Food Research and Technology*, **2007b** 225 (2) 239-247.
[22] D Amic; D Davidovic-Amic; D Beslo; N Trinajstic , *Croatia Chemica Acta*, 2003, 76, 55-61.
[23] AC Akinmoladun; EM Obuotor; EO Farombi, *J.Med. Food*, **2010** 13(2), 441-451
[24] OO Elekofehinti ; IJ Kade, *Der Pharma Lett.*, **2012**, 4, 1352-1359.
[25] T Hatano; R Edamatsu; M Hiramatsu; A Mori; Y Futija; T Yasuhara; T Yoshida; T Okuda, *Chemical and Pharmaceutical Bulletin*, **1989,** 37, 2016-2022.
[26] GC Yen; CL Hsieh, *Journal of Agriculture and Food Chemistry*, **1998**, 46, 3952-3957.
[27] ST Chang; JH Wu; SY Wang; PL Kang; NS yang ; LF Shyur, *Journal of Agriculture and Food Chemistry*,**2001**, 49, 3420-3424.
[28] D Gonzalez-Nunez; J Claria; F Rivera; E Poch, *Hypertension*, **2001**, 37, 334-338.
[29] V Filippos; T Emmanaouil; D Carl; V Guenter; K Georg; P Nikolas, *Biotechnology Journal*, **2007**, 2(10), 1214-1234
[30] IO Fasidi1996 *Food Chemistry*, **1996** 55,161 – 163.
[31]S Ogbonnia; AA Adekunle; MK Bosa; VN Enwuru, *African Journal of Biotechnology*, 2008,7,701-705.
[32] N Nenadis; W Lan-Fen; M Tsimidou; HY Zhang , *J Agric. Food Chem*., **2004,** 52: 4669 - 4674.
[33] CA Rice-Evans; NJ Miller; G Paganga, *Free radical Biology and Medicine*, **1996** 20, 933-956.
[34] B Bhuvaneswari; R Hari; VR Suguna, *Asian J Pharm Clin Res.* **2002**, 5,147-150.
[35] OO Elekofehinti; JP Kamdem; IJ Kade; IG Adanlawo ; JBT Rocha, *Asian Journal of Pharmaceutical and Clinical research*, **2013,** 6, 249-254.
[36] B Halliwell., **2001,** *Drugs and Aging*, **2001** 18(9), 685–716.
[37] JR Soares; TCP Dins; AP Cunha, *Free Radical Research*., **1997,** 26**,** 469-478.
[38] C Hu; DD Kitts, *Journal of Agriculture and Food Chemistry*., **2000,** 48, 1466-1472.
[39] MLiu; XQ Li ; C Weber ; RH Liu, *Journal of Agricultural and Food Chemistry*., **2002,** 50, 2926-2930.
[40] G Kansci ; E Dongo; C Genot , *Nahrung*.,**2003,** 47, 434-437.