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Antioxidant properties of seven wild edible mushrooms from Tanzania

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Consumption of mushroom has increased remarkably because of their desirable aroma, taste and high nutritional content. Wild edible mushrooms (WEM) are well known for their nutritious and antioxidant properties. This study was conducted to measure and compare the antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of six WEM from selected indigenous Tanzanian forests and one domesticated grown form in methanolic extracts. Results show that among the seven mushroom species extracts, the methanolic extract from *Auricularia judae* showed the most potent radical scavenging activity of 93.33% while *Panus conchatus* revealed lowest antioxidant activities of 46.53%. The strongest EC_{50} was observed in *Auricularia judae* 0.08 mg/ml while weakest was in *P. conchatus* >0.3 mg/ml. Nevertheless total phenolics in the methanolic extracts were the highest in *P. tenuiculus* (431.03±3.63 mg GAE/100 g) and lowest in *Macrolepiota procera* (136.21±0.98 mgGAE/100 g). *Lentinus squarrosulus* possessed highest content of β-carotene and flavonoids were 48.15±1.04 mg/100 g and 25.62±1.78 mg/100 g, respectively meanwhile the lowest concentration was found in wild *L. sajor-caju* 5.35±0.68 mg/100 g and 2.49±1.28 mg/100 g, respectively. Comparatively, domesticated mushroom showed higher scavenging ability of 76.61% than its wild counterparts 62.94%. In conclusion, high antioxidant activity in methanolic extract of WEM due to presence of phenolic content can potentially be used as a source of natural antioxidants.

Key words: Wild edible mushrooms, antioxidant activity, polyphenols flavonoids.

INTRODUCTION

Mushrooms have been considered as ingredient of gourmet cuisine across the globe; especially for their unique flavor and have been valued by humankind as a culinary wonder. More than 2,000 species of mushrooms exist in nature, but around 25 are widely accepted as food and few are commercially cultivated. Mushrooms are considered as a delicacy with high nutritional and functional value, and they are also accepted as nutraceutical foods; they are of considerable interest because of their organoleptic merit, medicinal properties, and economic significance (Chang and Miles, 2008; Ergönül et al., 2013). However, there is not an easy

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distinction between edible and medical mushrooms because many of the common edible species have therapeutic properties and several used for medical purposes are also edible [Guillamón et al., 2010). However, wild mushrooms are becoming more important for their nutritional, sensory, and especially pharmacological characteristics. Wild growing mushrooms have a worldwide distribution and have been a popular delicacy in many countries (Valentáo et al., 2005). They can be grouped as "functional food" since their dietary components provides health benefit in cardiovascular and antioxidant properties, which are beyond basic nutrition (Ferreira et al., 2010).

Mushrooms could be an alternative source of new antimicrobial compounds, mainly secondary metabolites, such as terpenes, steroids, anthraquinones, benzoic acid derivatives, and quinolones, but also of some primary metabolites like oxalic acid, peptides, and proteins. They have a great nutritional value since they are quite rich in protein, with an important content of essential amino acids and fiber, poor fat but with excellent important fatty acids content. Moreover, edible mushrooms provide a nutritionally significant content of vitamins (B_1, B_2, B_{12}, C, D, and E) (Heleno et al., 2010; Mattila et al., 2001). Thus, they could be an excellent source of many different nutraceuticals and might be used directly in human diet and to promote health for the synergistic effects of all the bioactive compounds present (Barros et al., 2007; 2008; Ferreira et al., 2009; Pereira et al., 2012).

There are many epidemiological studies that suggest that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer in which polyphenol is linked to the antioxidant properties (Barros et al., 2007; Jagadish et al., 2009). The consumption of dietary antioxidants will help to prevent free radical damage. According to Olajire and Azeez (2011), antioxidants have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions. Among the antioxidant compounds, polyphenols have gained importance due to their large array of biological actions that include free radical scavenging and metal chelation enzyme modulation among others (Rodrigo and Bosco, 2006). The term polyphenol refers to a complex group of compounds that includes in their structure an aromatic ring bearing one or more hydroxyl groups (Elmastas et al*.,* 2007). They include complex structures such as flavonoids and anthocyanins as well as simple phenolic acids and derivatives (Batool et al., 2010). Mushrooms are widely consumed and have been valued as an edible and medical resource. Many studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects (Barros et al., 2007; Oyetayo, 2009). They a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Turkoglu et al., 2007).

Antioxidants are chemical compounds that protect cells from damage by free radicals through inhibiting oxidation or reactions promoted by oxygen or peroxides. These free radicals are capable of damaging all components of body including proteins, lipids, DNA and sugars. Antioxidants are thus important in living organisms because they may delay or stop formation of free radical by giving hydrogen atoms or scavenging them.

Phenolic compounds in mushrooms are known to be excellent antioxidants due to their ability to neutralize excess radicals which are reactive oxygen species in cells. These radicals are constantly produced in the human body (Palacios et al., 2011; Muruke, 2014). Iron chelators are among the important free radical scavengers found in some mushrooms (Pal et al. 2010). Recently, an increasing interest in the consumption of mushrooms has arisen, due to their elevated polyphenol concentration, which correlates with an elevated antioxidant activity (Keles et al*.,* 2011).

Polyphenols generally refer to complex groups of compounds with aromatic ring bearing hydroxyl group. They include complex structures such as flavonoids and anthocyanins as well as simple phenolic acids and derivatives. Polyphenols show chemopreventive, cytostatic, immunomodulatory, bacteriostatic/bactericidal, antifungal, anti-inflammatory, antioxidant and many other pharmacological activities. Phenolic compounds have recently attracted much interest because *in vitro* and *in vivo* studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health (Ramesh and Pattar, 2010). The polyphenols are also well-known for their large array of biological actions that include metal chelation and enzyme modulation activities and free radical scavenging, among others (Rodrigo and Bosco, 2006; Burgosa et al., 2013).

In Tanzania wild edible mushrooms collected during the rainy season have broad cultural acceptance and constitute a traditionally very important nutritious food. Mshandete and Cuff (2007) concluded that the three domesticated edible wild species analyzed are highly nutritious and compare favourably with other foreign edible species. From these analyses it can be concluded that these three edible mushrooms hold tremendous promise in addressing the protein and minerals deficits prevalent in the diets in Tanzania and in other developing countries particularly among the low income families. For the full potential of mushrooms as a nutritional supplement to be realized, intensive efforts should be geared toward their cultivation using mushroom biotechnology adapted locally. A detailed analysis of the amino acid content of the three domesticated mushrooms is suggested to permit more direct comparisons with more popular food sources. Further research is needed

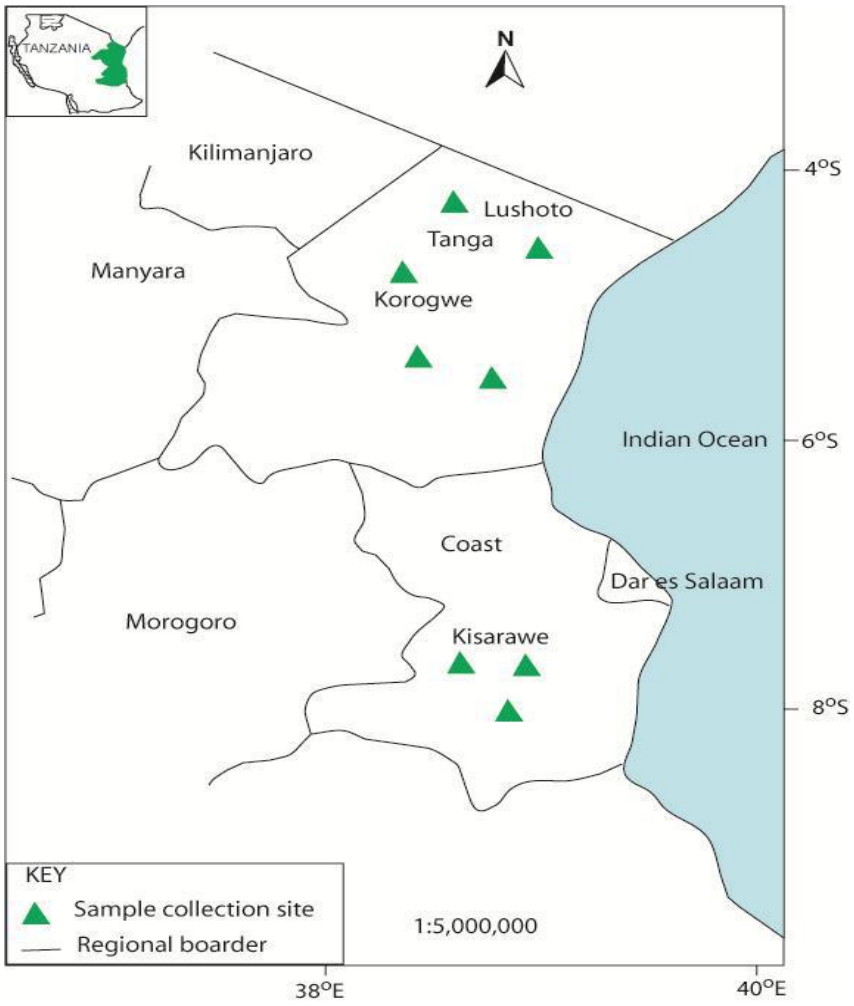

Figure 1. The map showing the sampling sites in Tanzania.

on species like *Pleurotus* and *Volvariella* that have the greatest market potential and efforts should be made to highlight their nutritional properties and advantages. Mushroom research in biochemical composition is at its infancy stage in Tanzania, few studies on antioxidant activities of wild edible mushroom have been conducted in Tanzania.

Among the studies on antioxidant activities of wild edible mushrooms are those of Tibuhwa (2014) who compared antioxidant activities between fresh and dry mushrooms in the genera *Cantharellus* and *Afrocantharellus*; Tibuhwa et al. (2012) established the effect of different post-harvest treatments on nutritive and antioxidant activities of wild edible *Coprinus cinereus,* and Muruke (2014) who determined antioxidant and iron chelating activities of a wild edible *Pleurotus cystidiosus.* This paper presents findings of a study on, antioxidant and ferrous chelating activities of six wild edible mushrooms from selected indigenous Tanzanian forests compared them to the domesticated *L.sajor-caju*.

MATERIALS AND METHODS

Standards and reagents

Methanol was purchased from Fisher Scientific (Leicestershire, UK), Deionized distilled water (ddH_2O), distilled water was prepared with Favorit W4L water distillation system from Genristo (Nottingham, UK). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium acetate ($C_2H_3NaO_2$•$3H_2O$), acetic acid, 2, 4, 6-tripyridyl-s-triazine (TPTZ), hydrochloric acid (HCl), ferric chloride ($FeCl_3$•$6H_2O$), ferrous sulfate ($FeSO_4$•$7H_2O$), Aluminium trichloride ($AlCl_3$), sodium nitrite ($NaNO_2$) were purchased from Sigma Chemical Co. (St. Loius, MO, USA). Folin-Ciocalteu reagent, sodium carbonate, gallic acid, ascorbic acid, sodium hydroxide (NaOH) and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany).

Sample collection

Mushroom samples were collected from different field sites in Tanzania (Figure 1). The study was conducted within natural forests of Lutindi-vue, Shume Magamba, Shaghayu, Kieti and Kunga forests in Tanga region, and Kazimzumbwi forest near Kisarawe town in the Coast region (Figure 1). Samples were collected during

long rainy season from April to May and November to December 2012. Mushroom samples were collected as described Hussein et al. (2014). The collected mushrooms were *Polyporus tenuiculus*, *Lentinus sajor-caju* W (Wild), *Lentinus squarrosulus, Macrolepiota procera, Panus conchatus,* and *Auricularia auricular-judae*. All collected samples were subjected to domestication trials and only one species of *Lentinus sajor-caju* D (Domesticated) was successfully domesticated**.** For the successfully domesticated, *Lentinus sajor-caju* D it was also harvested and dried as well.

Sample preservation and preparation

Mushrooms preservation involved drying by placing them on iron wire mesh and beneath that, the kerosene lamp was used to provide heat (about 40°C for 12 h). The dried mushroom samples were chopped into small pieces then crushed to powder using motor and paste immersed in liquid nitrogen to quicken crushing process.

Quantitative and qualitative determination of antioxidant activity

The antioxidant ability of each species was analyzed using DPPH radical and antioxidant properties were analyzed by determining total phenolic compounds, β-carotene, lycopene and total flavonoid of each extracts.

Sample extraction

Extraction was performed according to the method of Tibuhwa (2014). The powdered mushrooms were extracted with 250 ml of methanol. The mixture was placed in a conical flask (wrapped with an aluminum foil) and agitated at 200 rpm with orbital shaker for 48 h. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of ethanol extracts was removed under reduced pressure at 40°C using a rotary evaporator to dryness. The obtained concentrated extracts were stored in dark at 4°C until further analysis.

β-Carotene and lycopene antioxidant activity assays

The assay was carried out according to the method of Nagata and Yamashita (1992). The mushroom extract (100 mg) was shaken with 10 ml of Acetone-hexane mixture (92:3) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. The *β*-carotene and Lycopene content were calculated as:

Lycopene mg/100 mg = $-0.0458A_{663} + 0.204A_{645} + 0.372\ A_{505} - 0.0806A_{453}$

β-carotene mg/100 mg= $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$

Determination of total flavonoid

The $AlCl_3$ method was used for determination of the total flavonoid content of the sample extracts according to the method described by Jaita et al. (2010) and Pitchaon et al. (2007). 1 ml of each extract was diluted with 4.3 ml of 80% aqueous ethanol containing 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate. After 40 min incubation at room temperature, the absorbance was determined calorimetrically at 515 nm against a blank using spectrophotometer (UV-VIS model 6305 Jenway, UK). Total flavonoid concentration was calculated using quercetin standard calibration curve. Quercetin with different concentrations was used as a standard. Data were expressed as quercetin equivalent/100 g of mushroom extracted.

Determination of total phenolic content

Phenolic compounds in the each mushroom extracts was estimated by using Folin-Ciocalteu assay, based on procedures described by Tibuhwa (2014). Each 0.1 g of extract was diluted with 5 ml of methanol. 200 µl of the mushroom extract was transferred into a test tube then mixed thoroughly with 1 ml of Folin-Ciocalteau reagent. After 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added to the mixture. The mixture was agitated for further 30 minutes in the dark and centrifuged at 3300 g for 5 min. The absorbance of mushroom extract and prepared blank were measured at 515 nm using spectrophotometer (UV-VIS model 6305 Jenway, UK). A calibration curve was constructed with different concentrations of gallic acid as standard. The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of extract.

DPPH Free radical scavenging activity assays

The qualitative assays were performed according to the procedure described by Jaita et al. (2010) and Tibuhwa (2014). A series of extracts of methanol (0.006 - 0.3 mg/ml) were prepared. Ascorbic acid was used as a standard control. A measure of 1 ml of the extract was mixed with 1 ml of 0.4 $mmol^{-1}$ methanolic solutions containing 1:1-diphenyl-2picryhydrazyl (DPPH) radical that are very stable. Each free radical scavenging activity assay was done three times from the same extract in order to determine their reproducibility and standard deviation for the three readings were statistically determined. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. The reaction mixture was determined at 515 nm with UV-VIS spectrophotometer (UV-VIS model 6305 Jenway, UK). The percentage of DPPH radical scavenging activity of each extract was determined at these seven concentrations, within the range of dose response and was calculated as:

$$\text{DPPH radical scavenging activity } = \frac{Ao - (A1 - As)}{Ao} X100$$

Where;
A_0 = absorbance of the control solution containing only DPPH;
A_1 = absorbance in the presence of mushroom extract in DPPH solution and;
A_s= the absorbance of the sample extract solution without DPPH.

EC_{50} value (mg/ml) was defined as the total antioxidant needed to decrease the initial DPPH free radicals by 50%. It was determined from the plotted graph of scavenging activity against various concentrations of the sample extracts.

Ferrous ion chelating assay

The chelation of ferrous ion by the extracts was ascertained by the methods of Dinis et al. (1994). One milliliter of extracts with concentration ranging between 0.1 to 0.7 mg/mL was mixed with 3.7 mL of deionized water and then the mixture was reacted with ferrous chloride (2 mmol/L, 0.1 mL). The reaction was initiated by

Table 1. The content of total phenolic, β-carotene, Lycopene, flavonoids and EC_{50} values of DPPH scavenge and Fe^{2+} chelation in the mushroom extract.

Sample ID	Total Phenolic content mgGAE100g^{-1}	β-carotene mg 100g $^{-1}$	Lycopene mg 100g $^{-1}$	Total Flavonoids mgQE 100g^{-1}	Percentage of DPPH activity (%)	Percentage of Fe^{2+} chelation activity (%)	EC_{50} DPPH scavenge (mg ml^{-1})	EC_{50} Fe^{2+} chelation (mg ml^{-1})
P. tenuiculus	431.03±3.63	37.10±2.03	15.02±2.05	20.86±2.83	90.71	91.34	0.098	0.59
L. sajor-caju D	240.36±1.08	6.39±1.18	3.10±0.18	3.81±1.58	76.61	91.76	0.19	0.34
L. sajor-caju W	162.74±2.02	5.35±0.68	2.16±0.32	2.49±1.28	62.94	90.78	0.23	0.37
L. squarrosulus	192.43±2.78	48.15±1.04	18.32±1.03	25.62±1.78	76.14	97.21	0.18	0.25
P. cochatus	273.51±2.26	12.55±1.06	5.42±1.50	7.49±2.21	46.53	80.12	> 0.3	0.59
A. judae	214.08±1.70	32.04±0.86	12.68±0.62	16.84±0.99	93.33	96.05	0.08	0.12
M. procera	136.21±0.98	11.57±2.39	5.37±0.55	8.66±1.08	65.41	91.45	0.24	0.13

addition of ferrozine (5 mmol/ L, 0.2 mL) for 20 min. The absorbance at 562 nm was determined spectrophotometrically. EDTA was used as positive control. Chelating activity on ferrous ion was calculated using the equation below:

$$\text{Chelating effect } (\%) = \frac{Ab - As}{Ab} X100$$

Where, Ab is the absorbance of the blank without extract or EDTA; As is the absorbance in the presence of extract or EDTA 4.1.5 determination of mushroom.

Statistical analysis

All analyses were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 20.1). The mean values of data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by the Duncan's multiple range tests at 95% least significance difference ($p< 0.05$). P-Values less than 0.05 were considered statistically significant. The scavenging activities of crude extracts on DPPH radicals were carried out in triplicate and the results were expressed as means ± standard errors. The EC_{50} values (effective concentration at which DPPH/ferrous ion radical were scavenged by 50%) were obtained by interpolation from the linear regression plots. Pearson correlation coefficient was used to determine the relationship between the antioxidant activity, total phenolic and flavonoid content in sample extracts. Differences between means at 5% ($P < 0.05$) level were considered significant.

RESULTS AND DISCUSSION

The analyzed extracts, of all species possessed compounds with antioxidant ability in different concentrations, which varied markedly within the species as shown in Table 1.

Lycopene and β-carotene content (carotenoids)

β-Carotene is precursor for the synthesis of vitamin A, which acts as powerful antioxidants as well (Ross et al., 2011). Lycopene is known to be the most efficient singlet oxygen quencher compared with a variety of carotenoids and α-tocopherol *in vitro* (Tibuhwa, 2014). In this study, the content of β-carotene differed considerably with P= 0.000 between the mushroom species, ranging from 5.35±0.68 mg 100 g^{-1} to 48.15±1.04 mg 100 g^{-1}, highest β-carotene content was found in *L. squarrosulus* and the lowest concentration was in wild *L. sajor-caju* (Table 1). Similarly highest lycopene was observed in *L. squarrosulus* (18.32±1.03 mg 100 g^{-1}) whereas lowest lycopene concentration was recorded in wild *L. sajor-caju* (2.16±0.32 mg 100 g^{-1}). It is very interesting to note that the studied mushrooms had such high amount of lycopene, which is a very good indicator of them being a good source of nutraceuticals. These findings are similar to that reported by Johnsy and Kaviyarasan (2014 a) who reported *β*-carotene and lycopene were 20.03 mg 100 g^{-1} and 37.3 mg 100g^{-1}, respectively. Previous study by Stahl et al. (1998) showed that lycopene was three times more efficient than *β*-carotene in preventing lipid peroxidation in multi lamellar liposomes. Besides, in previous study by Robaszkiewicz et al. (2010) it was established that the content of lycopene was far lower than the concentration of *β*-carotene in the mushrooms where 0.0265 mg 100 g^{-1} for *β*-carotene and 0.0023 mg 100 g^{-1} for lycopene of *M. procera*. Furthemore, Yaltirak et al. (2009) reported presence of antioxidant compounds such as *β*-carotene and lycopene in edible mushroom *Russula delica.* For comparison, the content of carotenoids reported by Ben-Amotz and Fishler (1998) in vegetables ranges from undetectable levels to ~5.28 mg 100 g^{-1} for persimmon, ~53.21 mg 100 g^{-1} for pitango, ~103 mg 100 g^{-1} carrot and ~24.31 mg 100 g^{-1} in tomato. In the present study, phenol was the major antioxidant component followed by *β*-carotene, lycopene and flavonoids (Table 1). This clearly showed that the amounts of *β*-carotene and lycopene in the studied mushrooms are in abundance compared to the concentration reported in some vegetables and other mushrooms, which implies that the studied mushrooms could be a good alternative source of carotenoids.

Flavonoids

In this study the highest total content of flavonoids methanolic extracts was found in *L. squarrosulus* and *P. tenuiculus* while the lowest in wild *L. sajor-caju* and domesticated *L. sajor-caju* (Table 1). However, Gan et al. (2013) reported *Agaricus brasiliensis* had higher amount of flavonoids contents compared to that reported in this study. The study by Tibuhwa (2014) reported higher flavonoid amount from fresh form of *Afrocantharellus splendens* and *Cantharellus rufopunctatus* (155.16 mg 100 g^{-1} and 134.31 mg 100 g^{-1}, respectively), which are higher than recorded in this study. High flavonoids level may help provide protection against oxidative stress induced diseases by contributing along with other antioxidant vitamins, and enzyme to the total anti oxidative defense system of the human body. Many studies have attributed that antioxidant properties are due to the presence of flavonoids (Tripathy et al*.,* 2014) hence may be a reason for the high lipid peroxidation inhibition found in certain species of the studied mushrooms.

Total phenolic content

Total polyphenols were the major naturally occurring antioxidant components found in the methanolic extracts from wild edible mushrooms studied. All studied mushrooms were contained phenolic compound though there were significant variations (P-value 0.000). The highest amount of phenolic content was found in *P. tenuiculus* (431.03±3.63 mgGAE 100 g^{-1}) > *P. conchatus* (273.51±2.26 mgGAE 100 g^{-1}) > domesticated *L. sajor-caju* (240.36±1.08 mgGAE 100 g^{-1}) >*A. judae* (214.08±1.70 mgGAE 100 g^{-1}) >*L. squarrosulus* (192.43±2.78 mgGAE 100 g^{-1}) > wild *L. sajor-caju* (162.74±2.02 mgGAE 100g^{-1}) and the least was in *M. procera* (136.21±0.98 mgGAE 100 g^{-1}). In the present study phenolic acids were the major compound present in methanolic extracts than other compounds studied.This is in agreement with previous report (Ferreira et al*.,* 2009, Johnsy and Kaviyarasan, 2014a) on the antioxidant compounds found in mushrooms. It was further noted that the amounts of total phenolic obtained in this study were low compared to previous studies on wild and cultivated edible mushrooms. For example, Yim et al. (2009) reported an amount of of phenolic content in *Lentinus ciliatus* was 801.08 mg GAE 100 g^{-1} and in cultivated species of *Pleurotus ostreatus* was 1046.87 mgGAE 100 g^{-1} which are relatively higher than what were observed in this study. Inspite of comparatively low amount of phenolic content observed in the studies species as compared to already published work as noted above, the results in this study show a low positive correlations between phenol content and antioxidant activities with. Pearson correlation analysis revealed total phenolic contents of the extracts has strong correlation with DPPH (r = 0.809). The higher the content of phenol in the mushroom extract, the stronger antioxidant activities. This observation is inline with that of Velioglu et al*.* (1998), Wong and Chye (2009) and Heleno et al., (2012) who reported antioxidant activity of mushroom to be positively correlated with the content of their phenolic compounds.

Currently there are no established standard dietary intakes of phenolic compounds, however, the findings of this study show that the phenolic concetration in the studied mushrooms have potential antioxidant activity. Tibuhwa (2014) reported that the American Cancer Society(ACS) established 100 mg per day of flavonoids as an adequate amount for the prevention of cancer and deteriorating illness. Similarly, Tripathy et al. (2014) reported similar range of phenolic concetration to have good antioxidant activity which are of the same range with our studied mushrooms. Moreover, it is well known that phenolics lead the amplification of proliferation inhibition Liu et al. (1997) and play an important role in stabilizing lipid peroxidation Duh et al. (1999).

Scavenging activity of DPPH radical

Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. In this study, studied mushrooms showed good ability of scavenging DPPH radical though there were significant variations (P-value 0.000). At 0.3 mg/mL the methanol extract of *A. judae* showed the highest scavenging activity 93.33% followed by *P. tenuiculus* with 90.71%. The lowest activity was observed in *P. conchatus* having 46.53% as shown in Figure 2. However the lowest EC_{50} was observed in *A. judae* 0.08 mg/mL which was the strongest. The weakest EC_{50} was in *P. conchatus* >0.3 mg ml^{-1} (Table 1). These findings are similar to those explained by Sarikurkcu et al. (2008) who found that the radical scavenging activities of the wild edible mushrooms were relatively high.

Interestingly, although the scavenging abilities of wild form of *L. sajor-caju* were found lower (62.94%) than other studied mushrooms, its counterpart (the domesticated form) was found to have relatively higher scavenging activity of 76.61%. This could be due to the reason that, mushroom absorbs nutrients from the substrates they grow. Thus possibly the substrate used for cultivation of mushrooms contained more nutrients compared to the substrate where the mushroom was collected. In addition, maturation stages of fruiting bodies could affect antioxidant contents which results to low scavenging activities (Gan et al., 2013). The scavenging ability for the wild form is in agreement with previous work by Johnsy and Kaviyarasan (2014b) who also reported that the methanol extract of *L. sajor-caju* portrayed antioxidant activity being able to scavenge

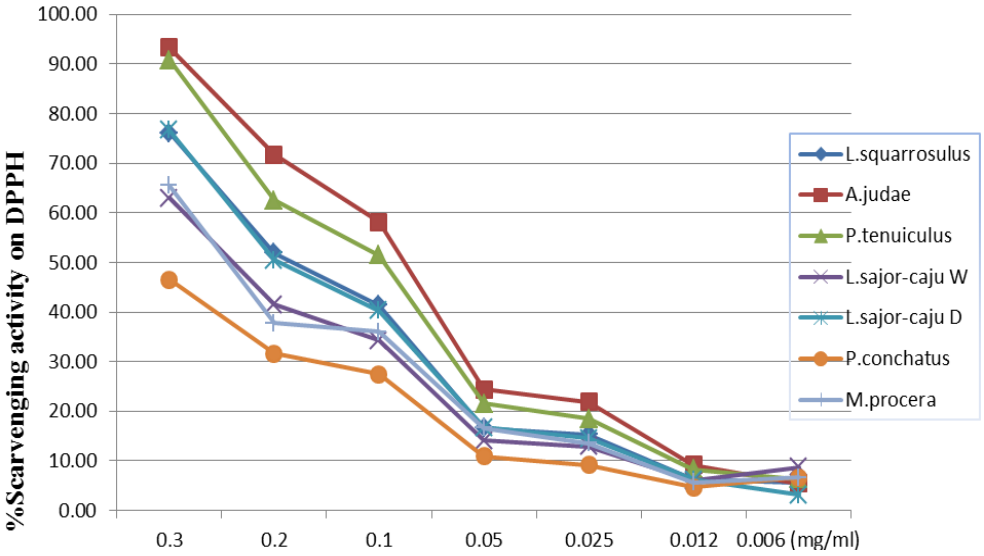

Figure 2. DPPH Scavenging ability of mushroom studied at different concentration.

more than 62.19% of the DPPH radical but at a concentration of 0.2 mg ml^{-1}. Several studies (Abdullah et al., 2011; Gan et al., 2013) also reported edible mushrooms to have high radical scavenging activity of DPPH radical.

Obviously, the extracts contained antioxidant components, which could react rapidly with DPPH radicals, and reduce most DPPH radical molecules. This result reveals that the extracts are free radical inhibitor or scavenger, acting possibly as primary antioxidants. Various extracts might react with free radicals, particularly the peroxy radicals, which are the major propagators of the autoxidation chain of fat, thereby terminating the chain reaction (Brand-Williams et al., 1995).

It is well known that natural antioxidants can be used to replace the synthetic antioxidants in the food industry such as butylatedhydroxytoluene (BHT), butylatedhydroxylanisole (BHA) and tertiary butylhydroquinone (TBHQ) which may possess mutagenic activity (Batool et al., 2010). This study thus encourages people to consume edible mushroom as part of their daily meals for better health.

Chelating abilities on ferrous Ions

The range of the mean of Fe^{2+} varied significantly among studied mushrooms with P= 0.000. The Fe^{2+} binding capacity of mushroom extracts was tested at concentrations range between 0.1 to 0.7 mg ml^{-1}. The higher chelating ability was observed in *L. squarrosulus* and *A. judae* at 0.7 mg/mL, 97.21% and 96.05%, respectively, while the lowest was in *P. conchatus* 80.12% as shown in Figure 3. The lowest EC_{50} was in *A. judae* 0.12 mg ml^{-1} with the highest being *P. conchatus* 0.60 mg ml^{-1}.

The metal chelating capacity is important since it reduces the concentration of transition metals that may act as catalysts to generate the first few radicals to initiate the radical-mediated oxidative chain reactions in biological and/or food systems. Ion chelating agents also may inhibit the Fenton reaction and hydroperoxide decomposition (Pal et al., 2010). In this study, it was interesting to note that the chelating abilities increased as concentration increased (Figure 3). Similarly Mau et al. (2001) has reported methanolic extract of the wild edible mushrooms were better chelator of ferrous ion than those of commercial mushrooms. These results shows that wild edible mushroom have potential nutrients and bioactive molecules which can be used to improve health and prevent many diseases.

Conclusions

Findings of this study showed that studied WEM exhibited a substantial amount of antioxidant activity with significant amounts of *β*-carotene, flavonoids, total phenolics and high ability to scavenge DPPH radical. Therefore, these mushrooms can be used as an easily available source of natural antioxidants and as a promising food supplements in an effort to curtail nutrition insecurity.

This study suggests that high antioxidant activity in methanol extract of mushrooms can potentially be used as a source of natural antioxidants due to presence of phenolic compounds since mushrooms are readily available and acceptable to the public.

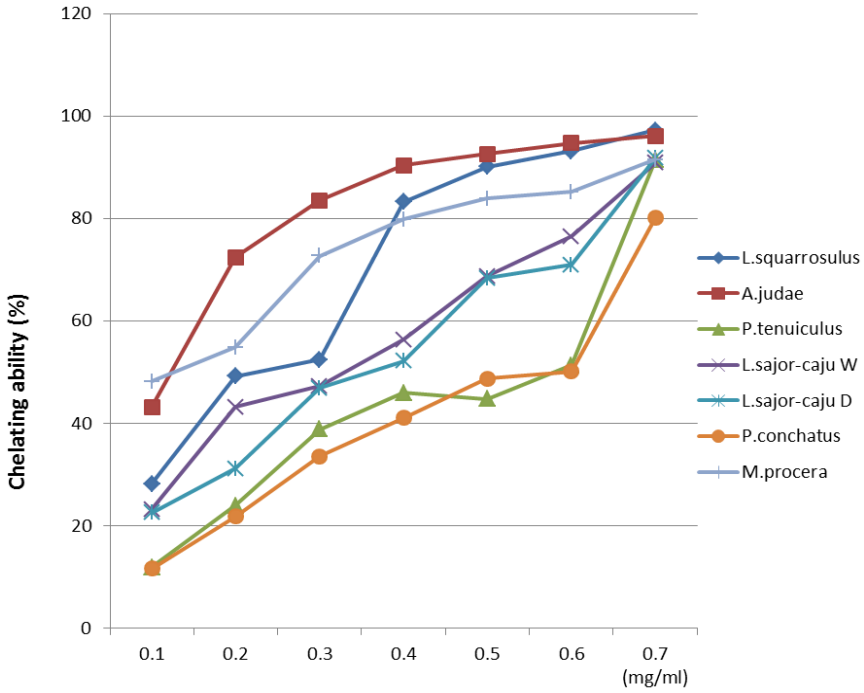

Figure 3. Ferrous iron chelating ability of extracts of studied mushroom.

Conflict of interests

The authors did not declare any conflict of interest.

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