Original Research Article

PROXIMATE NUTRIENT COMPOSITION AND ANTIOXIDANT PROPERTIES OF *PLEUROTUS SAPIDUS* 969 CULTIVATED ON *AGAVE SISALANA* SALINE SOLID WASTE

Abstract

Effects of pure and mixed substrates of sisal waste, grass (*Panicum coloratum*) and a combination of the two substrates at 50:50 (w/w) on nutritional composition, minerals and antioxidant potential of sun dried *Pleurotus sapidus* 969 were investigated in the present study. To determine the proximate chemical composition and antioxidant properties of the samples, standard analytical procedures were employed. Moisture content, crude protein and crude fibre ranged between 11.09-12.80%, 6.4-6.6% and 18.3-30.5%, respectively. Macro elements Ca, Mg, Na, K, and P were also found in substantial amounts with K being present in exceedingly higher amount (541.3-657.1 mg/100g) than the other macro minerals. The samples from the three substrates contained antioxidant *β*-carotene (4.6-6.0 mg/100g), lycopene (4.9-5.1mg/100g), Vitamin C (5.2-5.6 mg/100g), phenols (361.0-859.0 mg of GAEs/g) and flavanoids (33.5-64.0 mg RE/g).Mushroom harvested from mixed substrates contained better nutritional qualities than the pure substrate, although the phenolic content in mushrooms cultivated on sisal substrate was higher. The results further showed that, all the extracts exhibited scavenging ability and metal chelating activity. The findings showed that *Pleurotus sapidus* 969 is rich in nutrients, macro minerals as well as natural antioxidant which could be explored for pharmaceutical applications.

Key words: Sisal, antioxidant, free radicals, *Pleurotus*, flavanoids, phenols

1.0 Introduction

Cultivation of the oyster mushroom, *Pleurotus* spp has increased greatly throughout the world during the last few decades and constitute the second largest variety of mushrooms produced in the world (Mshandete, 2011), with China being the primary source. *Pleurotus* cultivation has the advantage of being cultivable in tropical climates, simple to produce, and compatible with organic substrates rich in lignin and cellulose. Their ability to utilize different substrates has made them the subject of broad research that generally mentions their nutritional quality and the effect of substrate variation on the primary metabolites that are directly related to the nutritional quality. Mushrooms have greatly varied and important uses throughout the world (Wan Rosli, 2011).Mushrooms are valuable health foods since they are poor in calories, fat, and essential fatty acids, and rich in proteins, vitamin and minerals (Reis *et al*., 2012). Moreover, their medicinal properties have been reported such as anti-tumor and immunomodulating effects (Ferreira *et al*., 2010), reduction of blood cholesterol concentrations, prevention or alleviation of heart disease and reduction of blood glucose levels (Jeong*et al*., 2010). These properties of mushrooms have been reported by Ferreira *et al*., (2009), do be as a results of the bioactive products with antioxidant potential (sterols, tocopherols, flavonoids, Carotenoids and phenolic compounds) (Ferreira *et al*., 2009).

Sequences of chemical reactions results in an imbalance between oxidant and antioxidant reactions and is typically referred to as oxidative stress (Poli, *et al*., 2008). Both classes of substances (oxidants and antioxidants) are generated in an oxidation- reduction (redox) set-up, (Ralser, *et al*., 2007) and has been implicated as causes of degenerative diseases such as atherosclerosis, cancer, and tissue damage in rheumatoid arthritis (Jang, *et al*., 2007). Reactive species are commonly identified as substances leading to the oxidation of lipids (lipoxidation), glucose (glycation) and proteins (carbonylation). Maintenance of equilibrium between free radicals production and antioxidant defences is an essential condition for normal organism functioning (Valko*et al*., 2007). Non-controlled production of free radicals has been attributed to various kinds of cancer and diabetes according to Ferreira *et al*. (2009). Natural products with antioxidant activity, in particular mushrooms, are used to aid the endogenous protective system, increasing interest in the antioxidative role of functional foods or nutraceutical products (Reis *et al*., 2011).Antioxidants pay an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Sies, 1997). Many studies have found that some species of mushrooms are having therapeutic properties (Oyetayo, 2009) due to a wide variety of free radicals or reactive oxygen species scavengers which have made them attractive as nutritionally beneficial foods and as a source for drugs development

(Guerra-Dore, 2007). According to Barros *et al.* (2008), mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system. Apart from being delicacy and tasty foods, mushrooms have been reported to have special biochemical compositions, with significant contents of antioxidant compounds, proteins, minerals, vitamins and water, which attract more attention as functional health promoters (Wong and Chye, 2009).

The chemical composition and nutritional quantity of edible mushrooms has been reported previously (Agahar and Subbulakshmi, 2005). Studies have consistently shown an inverse association between consumption of vegetables and fruits and the risk of certain forms of cancer (Liu, 2003). However the protective effects have been primarily attributed to well-known antioxidants, such as ascorbic acid and other related compounds (Soobrattee, *et al*., 2005).Different mushrooms species have been studied for new therapeutic alternatives and the results proved their bioactive properties (Lindequist*et al*., 2005). Mushrooms are rich sources of nutraceuticals (Elmastas*et al*., 2007), which are responsible for their antioxidant content (Lo and Cheung, 2005). Recent investigations revealed that polysaccharides and extracts of mushrooms had strong antioxidant and no synthase activation properties (Acharya and Rai 2013; Patra *at el*., 2013; Samanta*et al*., 2013).According to Muhammad Nasir*et al*., (2006), there are about 5000 different species of mushrooms, of which at least 1220 are reported to be edible. There are about 40 species under *Pleurotus* mushroom, in that 25 species are commercially cultivated (Singh, 2011). Most of these cultivated mushrooms are consumed as food or food ingredients in various food preparation and processed food products. This has led to the growing interest in the use of edible mushrooms extracts as dietary supplements based on the facts that they have a lot of bioactive compounds.

Pleurotus mushrooms can be grown on various agro-residues (as substrate) as reported by Muthangya *et al.*, (2014).The mushroom cultivation substrate has been reported to influence its growth, yield as well as the functional, organoleptic and chemical composition (Micheal, *et al*., 2011). This study was therefore designed to investigate the nutritive and antioxidant property of *P. sapidus* 969 cultivated on *Agave Sisalana* saline solid waste and on grass (*Panicumcoloratum*) as well as on a mixture of the two substrates at 50:50 (w/w) as reported in Muthangya *et al*.,(2013).

2.1 Samples of *Pleurotus sapidus* 969 Mushrooms

P. sapidus 969 mushroom used in this study were cultivated on pre-treated saline sisal leaf decortications waste as reported in Muthangya *et al.* (2013).Mushrooms were sundried on a fabricated solar drier for 7 hours on a full sunny day before analysis.

2.2 Determination of moisture, crude fibre and macro element content

The sun dried *P. sapidus* 969 mushrooms were analysed for moisture and total fibre content using a Near Infrared Reflectance Spectroscopy (NIRS). The NIRS technique uses near infrared light, instead of chemicals as in conventional "wet chemistry" methods. The samples were prepared and analysed as described by Windhan*et al.*, (1989). The prepared mushrooms samples were analysed for Ca, Mg, Na, K, and P, according to AOAC (2000).

2.3 Crude protein determination

Crude protein in *P. sapidus* 969was determined according to the method previously reported by Tibuhwa *et al*., (2012a). A known weight of each mushroom sample was taken and digested using micro Kjeldahl method. After completion of digestion organic nitrogen was determined calorimetrically using Indophenol-blue method and NH_4^+-N as standard. The absorbance was measured at 660 nm. The total crude protein was obtained and calculated as described in Allen (1989).

2.4 Mushroom crude extracts preparation

Mushroom crude extract was prepared in ethanol according to Tibuhwa, (2012b),with modification, where 1gm of dried whole mushrooms fruiting body was weighed at room temperature (29±3ºC). The samples were finely crushed using motor and pestle, and extracted with 250 ml of ethanol as a solvent. The crushed powder was constantly stirred for 48 hrs and thereafter filtered using Whatman number 4, filter paper. The filtrates were evaporated to dryness in a rotary evaporatorat 90 rpm under reduced pressure and at $40^{o}C$. The concentrated extracts obtained were stored in the dark at $4^{o}C$ until further analysis. The yields of evaporated dried extracts were obtained by gravimetric method. The percentage yield of the extracts was calculated based on dry weight as:

$$\text{Yield (\%)} = \frac{(W_1 \text{ X } 100)}{W_2}$$

Where: W_1 = weight of extract after ethanol evaporation

W_2 = Weight of the ground mushroom powder

2.5 Quantitative Antioxidant assay

2.5.1 Determination of total phenolics content (GAE/g)

The concentration of phenolic compounds in extract of *P. sapidus* 969 mushroom was measured by Folin-Ciocalteu colorimetric method according to the method previously reported by Tibuhwa (2012b), with modification. A blue colour was developed by reaction of phenolic compounds and Folin-Ciocalteu's reagent. The extract solution (1 ml) was mixed with 1 ml of Folin-Ciocalteau reagent and after 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added to the mixture. The reaction was kept in the dark for 30 min with agitation and thereafter centrifuged at 3300 g for 5 min. The absorbance was measured at 765 nm and total phenolic content was expressed as gallic acid equivalent (GAE) to 1 g per extract using gallic acid as a standard.

2.5.2 Determination of total flavonoid

Determination of total flavanoids was carried out using the aluminium chloride colorimetric method according to Jaita *et al*. (2010), as reported in Tibuhwa (2012b). Each extract (1 ml) was diluted with 4.3 ml of 80 % aqueous ethanol containing 0.1 ml of 10% aluminium nitrate and 0.1 ml of 1M aqueous potassium acetate. The mixture was incubated for 40 minutes at room temperature and the absorbance determined colorimetrically at 415 nm. A standard curve of flavonoids was prepared and concentration of flavonoids in the test samples determined.

2.5.3 *β*-carotene and Lycopene contents

β-carotene and lycopene were determined according to the method of Nagata and Yamashita, (1992). In brief, 100 ml of mushroom extract (10 mg/ml) was vigorously shaken with 10 ml of acetone-hexane mixture (92:3) for 1 min. and filtered through Whatman number 4 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. *β*-carotene and lycopene contents were calculated according to the following equations:

$$\text{Lycopene (mg/100mg)} = 0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$$

$$\beta\text{-carotene (mg/100mg)} = 0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$$

2.5.4 Determination of Vitamin C

The vitamin C content was determined titrametrically using 2, 6 DichlorophenoIndophenol methods according to Plumer (1987).One (1) gram of grounded sample was mixed with 25 ml of 5% metaphosphoric acid solution and shaken for 30 min. The mixture was then filtered through Whatman no. 42 filter paper using sunction pump. Ten (10) ml of the filtrate was titrated against 0.025% of 2.6 Dichlorophenol Indophenol reagents. The amount of vitamin C in each extract was calculated from the equation:

$$\text{Ascorbic acid mg/100g} = \frac{A \times I \times V \times 100}{V_2 \times W}$$

Whereas A = Quantity of ascorbic acid (mg) reacting with 1ml of 2, 6 Indophenol

I = Volume of indophenol (ml) required for the completion of extract titration

V_2 = Total volume of extract

W = Weight of the ground mushroom

2.6 DPPH free radical scavenging activity

The scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined according to the method of Masuda *et al*., (2000), and Jaita *et al*., (2010), as previously reported by Tibuhwa *et al*., (2012a). Each extract (0.01-0.14 mg/ml) was mixed with 1 ml of methanolic solution containing DPPH radicals (0.4 mM). The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was measured at 515 nm. The percentage of DPPH radical scavenging activity of each extract was determined within the range of dose response and was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - (A_1 - A_s) * 100}{A_0}$$

Where A_0 = Absorbance of the control solution containing only DPPH

A_1 = absorbance in the presence of mushroom extract in DPPH solution

A_s = the absorbance of the sample extract solution without DPPH

The EC50 value (total antioxidant necessary to decrease the initial DPPH radical concentration by 50%) was determined from a plot of scavenging activity against the concentration of extracts.

2.7 Chelating effect on ferrous ions

The ability of *P. sapidus* 969 extracts to chelate ferrous ions was estimated by the method of Dinis*et al.*, (1994). The extract (1 mg/ml) was added to a solution of 2 mM ferrous chloride (0.05 ml). The reaction was initiated by the addition of 5 mMferrozine (0.2 ml) and the mixture was then shaken vigorously and left to stand at room temperature (28-30ºC) for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as;

$$\{(A_0 - A_1) / A_0\} \times 100$$

Where A_0 = absorbance of the control

A_1 = absorbance in the presence of the mushroom extract

Statistical analysis

The experimental results were expressed as mean ±SD (Standard deviation) of n=3 measurements. Statistical analysis of the data were carried out using student's t-test and the results were considered significant when *P*= .05.

3. Results and Discussion

3.1 Composition of sun dried *Pleurotus sapidus* 969

The moisture contents of the dried *Pleurotus sapidus* 969were found 11.9-12.8% (Table 1) with nosignificant difference at *P*= .05 level. The highest moisture content was found in *P.sapidus* 969cultivated on a mixture of sisal and grass substrate (1:1), followed by grass and the least was in sisal alone.

Table 1. Composition (%)of sun dried *Pleurotus sapidus* 969 and crude extract yields, Mean±SD, n=3).

Cultivation substrate	Moisture (%)	Total fibres (%)	Crude Proteins (%)	Crude extract yield (%)
Sisal	11.9±0.03	6.4±0.1	18.3±0.4	17.0±0.4
Grass	12.2±0.01	6.6±0.2	23.3±0.2	17.9±0.3
Sisal: Grass	12.8±0.04	6.5±0.2	30.5±1.2	13.7±0.4

The fibre content was found highest in *P. sapidus* 969 (6.6g/100g) cultivate on grass. The variation in fibre contentbetween the mushrooms from the three different substrates was not statistically significant at *P*=.05. Comparison of the results of protein content of the mushroom from the three substrate showed a significant difference at *P*= .05 with the highest crude protein content being recorded from the mushrooms in the combined substrate of sisal and grass (30.5%).

The results of obtained by this study for the dried *Pleurotus sapidus* 969 are within the range of those reported previously for other *Pleurotus* species. Muthangya et al. (2014), working on *Pleurotus* HK 37 from the same substrate reported results, which were within the range of those obtained in this study.Oyetayo and Ariyo,(2013), working on *Pleurotus ostreatus*reported the moisture content of dried samplesto be within 9.00-10.72%. While previously, Chang and Miles(2004), reported the moisture content of dried mushrooms to be in the range 9 - 13%. Sales-Campos *et al*., (2011), reported a variation in fibre content while working on several *Pleurotus* sp. grown on crushed sugar cane, elephant grass and banana tree leaves on the other hand the results obtained on the fibre content was within the range (5.4–30.0%) previously reported by other authors for *Pleurotus* sp. (Kurtzman, 2005) cultivated on different substrates.The protein contents of mushrooms are reported to vary according to genetic structure of species, physical and chemical differencesin growing medium (Akyüz, and Kirbaˇg, 2010), cultivation time and strain (Bernaś, *et al*., 2006; Mshandete and Cuff, 2007), as well as the stage of development and level of nitrogen available (Chang and Miles, 2004). The mushroom protein contents that were found in this study (Table 1) are in agreement with the range of mushroom protein contents reported in the literature (Bernaś*et al*., 2006) varying between 17 and 42.5%, but higher in *P.sapidus* 969cultivated on grass and on a combined substrate of grass and sisal than the value (20.28%)

reported by Bonatti *et al.*(2004) for *Pleurotus ostreatus*cultivated on cotton waste. The present results showed that, protein content of *P.sapidus* 969 was significantly higher when the mushroom was cultivated on a combination of sisal and grass than that obtained for the mushroom grown on separate substrates.

3.2 Macro-minerals elements

Pleurotus sapidus 969mushroom samples analysed in this study contained macro-minerals including; calcium, magnesium, sodium, potassium and phosphorus (Table 2).

Table 2. Macro-minerals composition of *P.sapidus* 969(g/100g of dried sample)Mean±SD, n=3

Cultivation substrate	**Macro-minerals (mg/100g)**				
	Ca	**Mg**	**Na**	**K**	**P**
Sisal	6.1±0.4	16.21±0.6	15.17±0.1	614.5±1.9	117.7±0.9
Grass	7.7±0.3	17.8±0.5	14.2±0.3	541.3±2.2	123.4±0.9
Sisal : Grass	7.6±0.1	18.1±0.4	16.4±0.2	657.1±4.8	131.7±2.0

The highest amount of Ca (7.7 mg/100g) was recorded in the *P. sapidus* 969 samples from grass substrate, followed by sisal:grass (7.6 mg/100g) and lastly sisal (6.1 mg/100g). Mg concentration was the highest in sisal:grass samples (18.1 mg/100g) and the least in samples obtained from sisal substrate (16.21 mg/100g). The value of Na, K and P in the *P.sapidus* 969were found to be in the range of 14.2-16.46, 541.2-657.1 and 123.4-131.7 mg per 100g, respectively. Minerals in human diets are essential constituents for metabolic reactions, transmission of nerve impulses, healthy bone formation, regulation of water and salt balance Kalac, and Svoboda, (2000). The mineral contents of *P. sapidus* 969from the two different substrates and their combinations in this study did not vary significantly at *P*=.05. The results of the macro-minerals elements composition of *P.sapidus* 969 are within the range as those reported by Muthangya*et al*., (2014), from dried samples of *Pleurotus* HK 37 cultivated on the same substrates, although slightly higher. The values of calcium in this study are an indication that *P. sapidus* 969 is a valuable food for formation and maintenance of bone and normal function of nerves and muscles in humans and other vertebrates as reported by Wani*et al.*(2010). Mg, an essential co-factors for certain enzymes in various biochemical pathways was detected in *P. sapidus* 969and the levels of Mg were quite higher than those reported (1.69-3.57 mg/100g) for *Pleurotus ostreatus*cultivated on different woody substrates(Oyetayo and Ariyo, 2013). Na and K are important in the maintenance of osmotic balance between cells and the interstitial fluid in animal systems (Afiukwa, 2013). These results indicate that these mushrooms could play a role in human health by lowering blood pressure, reducing the risk of osteoporosis and in maintaining bone health (Wani*et al.*, 2010).The results of phosphorus in this study (123.4-131.7mg/100g) compare well with 122.28 mg/100g reported for a wild *P. ostreatus*(Afiukwa, 2013).The differences in phosphorus contents in mushroom have been attributed previously to substrates what about mushroom species/strain since they differ in substrate utilization/absorption and translocation of biomaterials from substratesused for growing the mushrooms according to Ahmed, (2009). *Pleurotus* species canprovide a useful source of phosphorus, potassium, calcium, and magnesium. Thus, the inclusion of *P.sapidus* 969in the diet could be oneof the strategies for combating macronutrient deficiencies

3.3 Antioxidant contents of *Pleurotus sapidus* 969

3.3.1 Total Phenol and Flavonoid contents

The total phenolic and flavonoid content in *Pleurotus sapidus* 969analysed in this study are shown in Figure 1. The total phenolic and flavanoids contents in the mushroom samples were 859.0, 784.7 and 361.0 mg of GAEs/g and 33.5, 64.0 and 53.8 mg RE/g in the mushrooms grown on sisal, grass and sisal:grass substrates, respectively.

The findings of this study is supported by previous findings of Phenolic compounds in mushrooms as reported by Tibuhwa, (2012b)and linked to various biological functions including antioxidant activity Phenolic compounds are well known secondary metabolites commonly found in plants and mushrooms and reported to have vital biological functions including antioxidant activity (Dimitrios, 2006). Knowing the amount of total phenolic compounds in mushrooms is of great importance in their nutritional and functional characterization since the profile of the phenolics has been reported to be species-specific Banerjee*et al*., (2012). Phenolic compounds have been reported to be of great interest due to their possible use as dietary supplements or food preservatives,Jayakumar *etal.,* (2009). Several species of mushroom have been reported to contain a wide variety of free radicals or reactive oxygen species scavengers, which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development (Guerra-Dore, 2007). Barros *et al.* (2008) reported that mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation oftriglycerides in the food system.Flavonoids have been reported to decrease capillary fragility and exert a cortisone-like effect on tissues (Gonzalez-Nunez*et al*., 2001) and protect against cancer and heart diseases (Filippos*et al*., 2007). It therefore implies that the high flavonoids content in the mushroom extracts might be responsible for the therapeutic effect of some mushroom species earlier reported (Ogbonnia, *et al*., 2008).

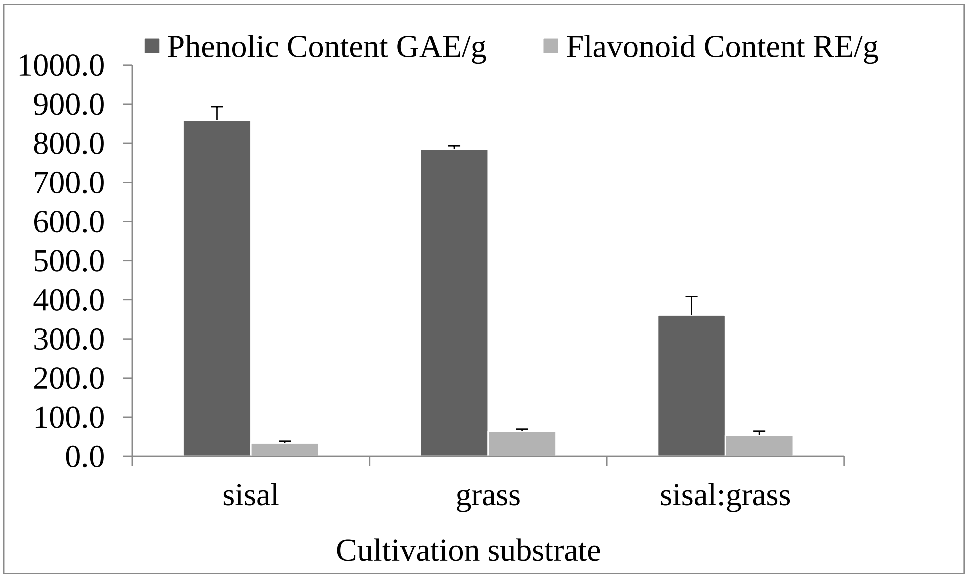

Fig. 1. Total phenol and flavonoid contents of *P. sapidus* 969, Values are expressed as mean ± SD mg of Gallic acid equivalent per gram of dry weight (mg GAE/gm).

Previous studies have shown that food consumption with high phenolic content can reduce the risk of heart disease (Singla*et al*., 2010). From this study, the high levels of phenols and flavanoids make *P. sapidus* 969 favourable for nutritional and therapeutic application as supported by the findings of Ferreira*et al*., (2007).

3.3.2 *β*-carotene, Lycopene and Vitamin C content

Carotenoids are natural colorants, stabilizers and active in the protection process of human body cells, where they balance and offset the destructive effects of free radicals Jayakumar *etal.,* (2009).The quantities of *β*-carotene, Lycopene and Vitamin C content of *P. sapidus* 969analysed in this study are presented in Figure 2. The content of *β*-carotene was in the range of 4.6 mg/100g to 6.0 mg/100g, lycopene was in the range of 4.9 mg/100g to 5.1 mg/100g, while vitamin C was in the range of 5.2 mg/100g to 5.6 mg/100g in the three substrates.Carotenoids are major antioxidants with known health benefits, while diets high in lycopene; a cyclic isomer of *β*-carotene has been linked to reduction of prostate cancer and cardiovascular diseases (Raoand Agarwal, 2000); whereas, Ascorbic acid is reported to directly interact with radicals in plasma, preventing damage to red cell membranes (Jayakumar *etal.*, 2009).

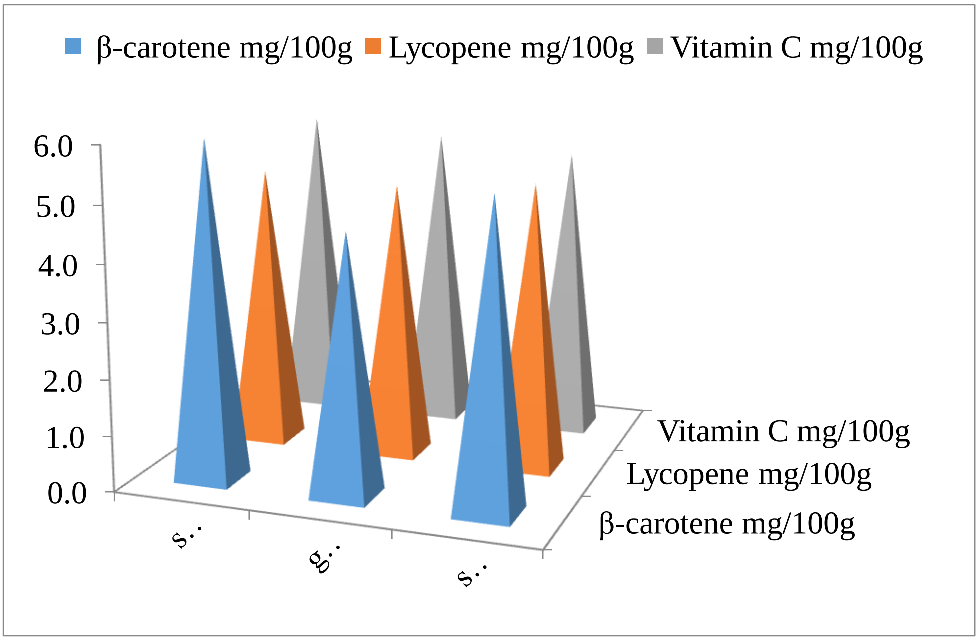

Fig. 2. Total *β*-carotene, Lycopene and Vitamin C, content of *P. sapidus* 969 Values are expressed as mean ± SD mg/100g.

The results of *β*-carotene, lycopene and vitamin C obtained in this study are within the range of those reported previously by Muthangya*et al*. (2014), from sun dried samples of *Pleurotus* HK 37 cultivated on similar substrates. The presence of these compounds in *P. sapidus* 969 is an indication that these mushrooms are equipped with antioxidant properties.Jayakumar *etal.*, (2009) reported similar findings of carotenoid and ascorbic acid compounds from *P. ostreatus*mycelium extracts. The quantities of these compounds in various extracts has been suggested to be influenced by the culture medium usedfor producing the mycelium (Petre*et al.*, 2010), a similar scenario observed in this study where different substrates were used to cultivate *P. sapidus* 969. These findings support Barros *et al.*, (2007), who reported that the carbon sourceand especially the nitrogen sources has a direct influence on the quantum of biologically activesubstances in the extracts.

3.4 Antioxidant activities

3.4.1 DPPH Free radical scavenging activities

The result from this study showed that, the free radical scavenging activity of *P. sapidus* 969extract from the three cultivation substrates increased with increasing concentration of extract indicating the concentration dose dependency of anti-oxidative activities (Figure 3). This observation concur with that of Banerjee*et al*., (2012) who also noted a similar trend of anti-oxidative activities dose dependency and associated it with the presence of reductones that are reported to be the terminators of free radical chain reactions.

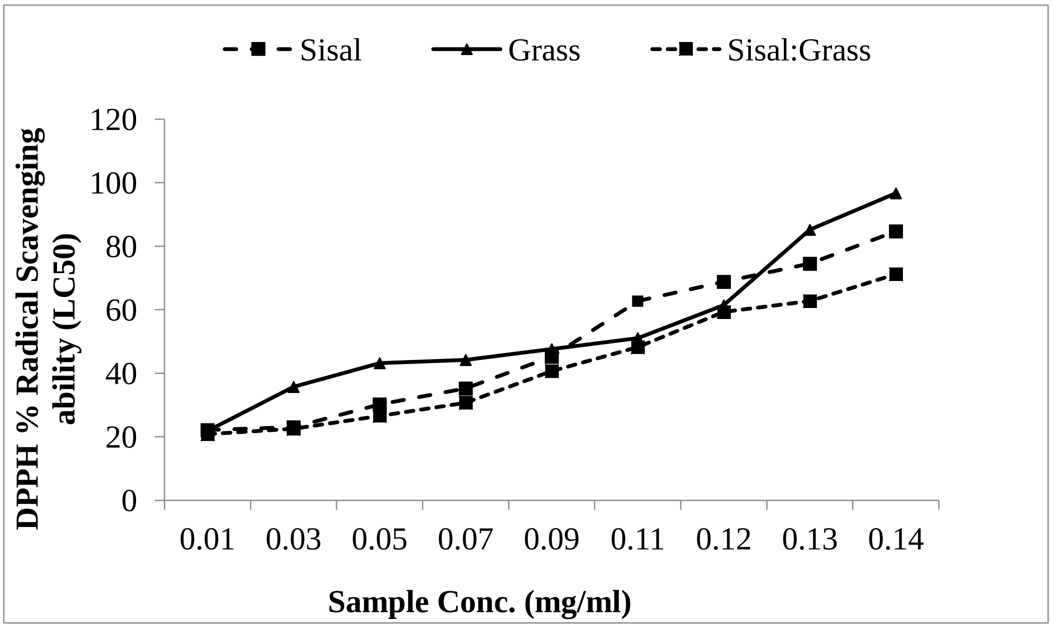

Fig. 3. DPPH radical scavenging activity (%) of *P. sapidus* 969 (ethanolic extract) cultivated on sisal grass, sisal:grassat 1:1 Values recorded are (mean ± SD, n=3).

In this study, the maximum scavenging activity values were at a dilution of 0.14mg/ml. The mushroom extracts from grass substrate showed the highest percentage (96.7%) scavenging power while the extracts from sisal and sisal:grass had 84.7% and 71.2%, respectively.However, the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%, determined from plotted graph of scavenging activityagainst different concentration of the extracts, showed the extract from sisal had the highest ability (EC50 <

0.09 mg/ml) followed by that from grass (EC50 < 0.11 mg/ml) while the extracts from sisal:grass had the least ability of (EC50 < 0.13 mg/ml), a similar observation reported by Muthangya *et al*., (2014), working dried samples of *Pleurotus* HK 37 cultivated on the same substrates.These result shows that the *P. sapidus* 969mushroom studied have high scavenging ability compared to other mushrooms. Although in this study, mushrooms fromsisal:grass had the least ability of (EC50 < 0.13 mg/ml), this value is still better compared to other well appreciated antiradical mushrooms. Filipa*et al*., (2011) established EC50 values in *Paxillusinvolutus*and *Pisolithusarhizus*of (EC50 = 0.61 and EC50 = 0.56 mg/ml), respectively which show them having relatively low free radical scavenging ability compared to mushrooms from sisal:grass with least ability in this study. The higher content of phenolic compounds in mushrooms cultivated on sisal substrate could be the cause of the high total antioxidant necessary to decrease the initial DPPH radical concentration by 50% an observation in line with the findings of Abdullah *et al*.(2011), working on of Brazilian button mushrooms.

3.4.2 Chelating ability of ferrous ions

Figure 3 depicts the iron chelating ability of *Pleurotus sapidus* 969 cultivated onthe different substrate under investigation in this study. The ferrous ion-chelating effect of all samples increased well with increasing concentrations (Figure 4). *P. sapidus* 969 from sisal substrate had highest iron chelating ability (90.8% at 0.12 mg/ml), while the weakest metal chelating ability (82.2%) was recorded for samples from a combined substrate of sisal and grass.

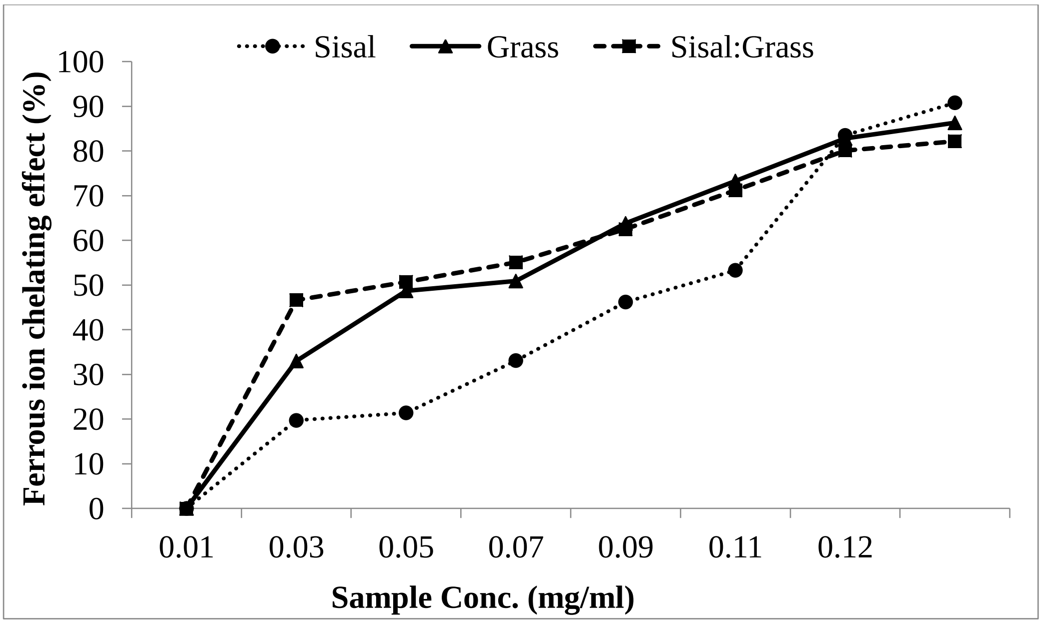

Fig. 4. Ferrous ion chelating effect (%) of *P. sapidus* 969 (ethanolic extract) cultivated on sisal grass, sisal:grass at 1:1.

Extract from samples cultivated on sisal substrates recorded 86.3% metal chelating ability at the same concentration. 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 (Halliwell, 2001). Thus, the iron chelating capacity of the mushroom species would prevent transition metals to participate in the initiation of oxidative stress.

Conclusions

It was observed in our studies that fruiting bodies harvested from different substrates varied in their biochemical analysis. It might be due to the variability of the substrates to provide different nutritional elements to mushroom grown on these substrates. Among the substrates investigated in this study, a combination of Sisal and grass gave the best overall composition of all the nutrients.The nutritional and antioxidant investigations on the mushroom cultivated on the different substrate revealed that all the mushrooms possess high reductive potential and metal chelation activities, with high concentration of macro nutrients, proteins, total phenol and total flavonoids. These bioactive compounds together with the high antioxidant activities of *P. sapidus* 969 could be explored as a natural rich antioxidant food, which may enhance the immune system against oxidative damage, or it may be utilized as a potential source for drug development.

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