

ISSN 2249-8524

Original Article

NUTRITIONAL AND ANTIOXIDANT ANALYSIS OF PLEUROTUS HK 37 GROWN ON AGAVE SISALANA SALINE SOLID WASTE

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Received 03 March 2014; accepted 28 March 2014

Abstract

Mushrooms consumed in recent times have increased both in the amounts as well as the number of species consumed. The main bioactive components in mushroom are phenolic compounds, ascorbic acid, β -carotene and lycopene. Mushrooms are also rich in crude protein, vitamins, amino acids, crude fibre and minerals. The objective of this study was to determine nutritive and antioxidant potential of sun dried Pleurotus HK 37 grown on Agave sisalana waste, grass (*Panicum coloratum*) and in a combined substrate of the two at 50:50 (w/w). Standard procedures were used to determine the proximate chemical composition and antioxidant properties of the samples. Moisture content, crude protein and crude fibre ranged between 12.31-13.61, 17.08-31.14% and 6.12-6.82%, respectively. Macro elements Ca, Mg, Na, K, and P were also found in substantial amounts with K being present in exceedingly higher amount (537.31-631.91 mg/100g) than macro minerals. The samples from the three substrates contained antioxidant β -carotene (4.24-5.07 mg/100g), lycopene (4.44-5.05 mg/100g), Vitamin C (5.07-5.29 \pm 0.02 mg/100g), phenols (350.82-830.97 mg of GAEs/g) and flavanoids (32.21-61.11 mg RE/g). A combined substrate of sisal and grass was found to produce mushroom with high nutritional value although the phenolic content in mushrooms cultivate on sisal substrate was higher. The results further showed that, all the extracts exhibited scavenging ability and metal chelating activity. The findings show that Pleurotus HK 37 can be explored further for pharmaceutical application due to the high antioxidant potential alongside its consumption as a nutritious food.

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Key words: Radical scavenging, antioxidant, free radicals, Pleurotus, phenols.

1.0 INTRODUCTION

Cultivation of the oyster mushroom (*Pleurotus*) has gained popularity in Kenya in recent times. *Pleurotus* popularity in the rest of the world has been attributed to their abilities to grow at a wide range of temperatures and to utilize various lignocelluloses [1]. Members of the genus *Pleurotus* are edible ligninolytic mushrooms [2] and play an important role in managing and recycling of organic wastes as an alternative to other methods of disposal [3]. According to Reis et al. [4], mushrooms are valuable health foods since they are poor in calories, fat, and essential fatty acids, and rich in fibre, proteins, vitamin and minerals [5].

Mushrooms contain important minerals required for normal functioning of the body [6] and at the same time they are devoid of undesirable side effects [7]. *Pleurotus* sp. is promising as medicinal mushrooms, exhibiting antiviral, antitumor, antibiotic, antibacterial, and immunomodulation activities [8].

Oxidation is essential to many living organisms for production of energy for biological processes [9]. Free radical, which are reactive oxygen species (ROS) in cells include superoxide anion (O_2^-), hydroxyl radical (OH \cdot) [10], as well as nonradical molecules like hydrogen peroxide (H_2O_2) and singlet oxygen (1O_2), which are

endogenously constantly produced in the human body. All ROS are extremely harmful to organisms at high concentrations [11]. Free radicals are produced in the normal natural metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS). Once produced, most of the free radicals are neutralized by cellular antioxidant defences (enzymes and non-enzymatic molecules). The un-controlled production of free radicals has been linked to many diseases including several kinds of cancer, diabetes, [12] rheumatoid arthritis, post ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in human [13]. Antioxidants play an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibiting other oxidation reactions by being oxidized themselves [14]. The antioxidants comprising of both synthetic and natural antioxidants in the human diet are of great interest as possible protective agents to help the human body to reduce oxidative damage [15]. The synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been widely used in the food industry to prolong the shelf life, but have been found to be harmful due to their potential toxicity and carcinogenicity [16], currently; much attention has been given to natural antioxidants because of their associated health benefits [17].

The consumption of dietary antioxidants can prevent free radical damage [9]. Mushrooms contain various polyphenolic compounds recognized as excellent antioxidants due to their ability to scavenge free radicals by single-electron transfer. Barros et al. [18] reported that 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. The medicinal properties of mushrooms have been reported [19]. Some of the mushroom properties are attributed to bioactive products with antioxidant activity such as sterols, tocopherols, flavonoids, carotenoids and phenolic compounds, vitamin A, C, and E [12]. According to Fu and Shieh [20], several cultivated edible mushrooms such as *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus eryngii* and *Pleurotus ostreatus* have significant antioxidant and free radical scavenging activities. Recent investigations revealed that mushroom polysaccharides and extracts have strong antioxidant and anti-tumor activities [21].

Pleurotus mushrooms, which are members of Basidiomycetes, can be grown on different substrates [22]. To utilize other agro wastes as substrates for cultivation of *Pleurotus* sp., paddy straw, maize stover, coir pith, sugarcane bagasse, wild grasses and mixtures of these wastes have been tested [23]. *Pleurotus* cultivation on different *Agave Sisalana* waste has been reported previously [24, 25, 26]. According to Khare et al. [27], the mushroom cultivation substrate influences its growth, yield and composition while Micheal et al. [28] reported that, different substrates used in cultivating mushrooms do have effect on the functional, organoleptic and chemical

properties of mushrooms. Therefore, the aim of this study was to determine the nutritive and antioxidant property of *Pleurotus* HK 37 cultivated on *Agave Sisalana* saline solid waste and on grass (*Panicum coloratum*) as well as on a mixture of the two substrates at 50:50 (w/w) as reported in Muthangya et al. [25].

2.0 Materials and Methods

2.1 Samples of *Pleurotus* HK 37 Mushrooms

Pleurotus HK 37 mushroom used in this study were cultivated on pre treated saline sisal leaf decortifications waste as reported in Muthangya et al. [25]. Mushrooms were sun dried 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 dried *Pleurotus* HK 37 mushrooms were analyzed 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. [29]. The prepared mushrooms samples were analysed for Ca, Mg, Na, K, and P, according to AOAC [30].

2.3 Crude protein determination

Crude protein in *Pleurotus* HK 37 was determined according to the method previously reported by Tibuhwa et al. [31]. 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 $\text{NH}_4^+\text{-N}$ as standard. The absorbance was measured at 660 nm. The total crude protein was calculated as described in Allen [32].

2.4 Mushroom crude extracts preparation

Mushroom crude extract was prepared in ethanol according to Tibuhwa, [10] with modification, where 1gm of dried whole mushrooms fruiting body was weighed at room temperature ($29\pm 3^\circ\text{C}$). 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 evaporator at 90 rpm under reduced pressure and at 40°C . The concentrated extracts obtained were stored in the dark at 4°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:

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

Where: W_1 = weight of extract after methanol 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 *Pleurotus* HK 37 mushroom was measured by Folin-Ciocalteu colorimetric method according to the method previously reported by Tibuhwa, [10] 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-Ciocalteu 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. [33] as reported in Tibuhwa, [10]. 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 [34]. 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 titrimetrically using 2, 6 DichlorophenoIndophenol methods according to Tibuhwa, [10]. 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 suction 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. [35], and Jaita et al. [33] as previously reported by Tibuhwa et al. [31]. 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 Pleurotus HK 37 extracts to chelate ferrous ions was estimated by the method of Dinis et al. [36]. 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 mM ferrozine (0.2 ml) and the mixture was then shaken vigorously and left to stand at room temperature 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 \pm 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 $p < 0.05$.

3. Results and Discussion

3.1 Composition of sun dried Pleurotus HK 37

The results of moisture content, crude protein, crude total fibre contents and crude extract yield of the samples are shown in Table 1.

Table 1. Composition of sun dried Pleurotus HK 37 samples and crude extract yields (%)

Cultivation substrate	Moisture (%)	Crude Proteins (%)	Total fibres (%)	Crude extract yield (%)
Sisal	12.73 \pm 0.01	17.80 \pm 0.48	6.12 \pm 0.01	16.60 \pm 0.56
Grass	13.61 \pm 0.04	23.28 \pm 0.23	6.64 \pm 0.01	12.31 \pm 0.77
Sisal: Grass	12.31 \pm 0.77	31.14 \pm 0.20	6.82 \pm 0.01	12.66 \pm 0.49

Determination of percentage crude fibres in the dried mushrooms samples indicated an insignificant difference with the total crude fibres being in the range of 6.12%-6.82%, when the mushroom was grown in the three substrates. The results obtained by this study for the dried Pleurotus HK 37 are within the range of those reported

previously for other *Pleurotus* species. Oyetayo and Ariyo [37], working on *Pleurotus ostreatus* reported the moisture content to be within 9.00-10.72%. While previously, Chang and Miles [38] had reported the moisture content of dried mushrooms to be in the range 9 - 13%. The protein content among edible mushrooms has been documented to vary due to various factors including; the type of mushrooms, the stage of development and level of nitrogen available [39]. Mshandete and Cuff [40] also suggested that besides mushrooms strain specificity, protein content could also vary with substrates used for mushroom cultivation. Moreover, the nature of the protein in the substrate has direct influence on the protein content of the fruiting bodies [41]. *Pleurotus ostreatus* growing in the wild have been reported to contain 16.35% protein [42], while when cultivated on different wood substrate, it has been reported to contain 20.03 to 20.11% [37]. The present results showed that, protein content of *Pleurotus* HK 37 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. The protein content of 31.14% in this study is within the range of 18.07-37.00% reported for wild edible mushrooms of different species [5]. The total fibre obtained in this study showed insignificant variations among the three substrates used in cultivation, an observation in line with Sales-Campos et al. [43], who reported a variation in fibre content while working on several *Pleurotus* sp. grown on crushed sugar cane, elephant grass and banana tree leaves. The fibre content was within the range (5.4–30.0%) reported by other authors for *Pleurotus* sp. [44] cultivated on different substrates.

3.2 Macro-minerals elements

Pleurotus HK 37 mushroom samples analysed in this study contained macro-minerals including; calcium, magnesium, sodium, potassium and phosphorus (Table 2).

Table 2. Macro mineral content in *Pleurotus* HK 37 fruiting bodies cultivated on sisal, grass and sisal:grass substrates

Mushroom cultivation substrate	Macro-minerals (mg/100g)				
	Ca	Mg	Na	K	P
Sisal	6.16 ± 0.18	15.77 ±0.46	15.20 ±0.04	618.48 ±1.54	113.08 ±1.05
Grass	7.15 ±0.08	16.89 ±0.57	14.57 ±0.33	537.31 ±1.83	121.78 ±0.47
Sisal : Grass	7.18 ±0.04	17.62 ±0.42	15.41 ±0.42	631.91 ±0.20	130.76 ±0.47

The highest amount of Ca (7.18 mg/100g) was recorded in the *Pleurotus* HK 37 samples from sisal:grass substrate, followed by grass (7.15 mg/100g) and lastly sisal (6.16 mg/100g). Mg concentration followed the same trend as Ca, being the highest in sisal:grass samples (17.62 mg/100g) and the least in samples obtained from sisal substrate (15.77 mg/100g). The value of Na, K and P in the *Pleurotus* HK 37 were found to be in the range of 14.57-15.41, 537.31-631.91 and 113.08-130.76 mg, respectively.

Minerals in human diets have been reported to be essential constituents for metabolic reactions, healthy bone formation, transmission of nerve impulses, regulation of water and salt balance [45]. The mineral contents of *Pleurotus* HK 37 from the two different substrates and their combinations in this study did not vary significantly. The values of calcium detected in these mushrooms (6.16-7.18 mg/100 g) are significantly higher than 11.95-29.79 ppm (equivalent to 1.195-2.979 mg/100g) reported for three edible mushrooms in Turkey by Caglarlrmak et al. [46]. These results are an indication that *Pleurotus* HK 37 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. [47]. Mg, an essential co-factors for certain enzymes in various biochemical pathways was detected in *Pleurotus* HK 37 and the levels of Mg were quite higher than those reported (1.69-3.57 mg/100g) for *Pleurotus ostreatus* cultivated on different woody substrates [37]. Na and K are important in the maintenance of osmotic balance between cells and the interstitial fluid in animal systems [42]. In this study, the levels of K are present in exceedingly higher amount (537.31-631.91 mg/100g) than Na (14.57-15.20 mg/100g). 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 [47]. The results of phosphorus in this study (113.08-130.76 mg/100g) compare well with 122.28 mg/100g reported for a wild *P. ostreatus* [42]. The differences in phosphorus content have again been previously attributed to substrates used for growing the mushrooms [48].

3.3 Antioxidant contents of *Pleurotus* HK 37

3.3.1 Total Phenol and Flavonoid contents

The total phenolic and flavonoid content in *Pleurotus* HK 37 analysed in this study, are shown in Figure 1.

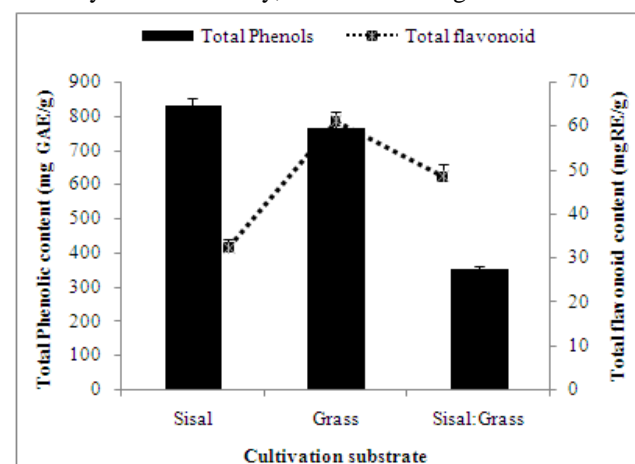


Fig. 1. Total phenol and flavonoid content of *Pleurotus* HK 37, Values are expressed as mean ± SD mg of Gallic acid equivalent per gram of dry weight (mg GAE/gm). Error bars indicate standard error of the mean of the replicates.

The total phenolic and flavanoids contents in the mushroom samples were 830.97, 764.03 and 350.82 mg of GAEs/g and 32.21, 61.11 and 48.47 mg RE/g in the mushrooms

grown on sisal, grass and sisal:grass substrates, respectively. Phenolic compounds have been reported to be of great interest due to their possible use as dietary supplements or food preservatives [52]. Phenolic compounds have been reported in mushrooms previously [10] and linked to various biological functions including antioxidant activity [49]. Flavonoids in mushrooms on the other hand have been reported to possess a number of beneficial effects on human health, including antioxidant, anti-inflammatory, antiallergic, antiviral, and anticarcinogenic activities. Phenol and flavonoids have been reported to scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [50]. Phenolic compounds have also been associated with antioxidant activity and stabilization of lipid peroxidation. Previous studies have shown that food consumption with high phenolic content can reduce the risk of heart disease [51]. From this study, the high levels of phenols and flavonoids make these mushrooms favourable for nutritional and therapeutic application.

3.3.2 β -carotene, Lycopene and Vitamin C content

Antioxidant components such as β -carotene, lycopene and vitamins C have previously been isolated from Pleurotus mushrooms. The quantity of these antioxidant phytochemical components have been reported to vary from strain to strain, the substrate used for cultivation as well as added nutrient supplement. 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 [52]. The quantities of β -carotene, Lycopene and Vitamin C content of Pleurotus HK 37 analysed in this study are presented in Figure 2.

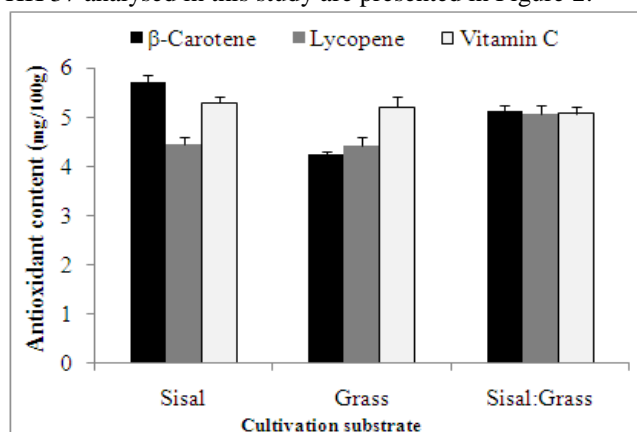


Fig. 2. Total β -carotene, Lycopene and Vitamin C, content of ethanolic extract of Pleurotus HK 37. Values are expressed as mean \pm SE mg/100g. Error bars indicate standard error of the mean of the replicates.

The content of β -carotene was in the range of 4.24 ± 0.06 mg/100g to 5.07 ± 0.06 mg/100g, lycopene was in the range of 4.44 ± 0.01 mg/100g to 5.05 ± 0.20 mg/100g, while vitamin C was in the range of 5.07 ± 0.04 mg/100g to 5.29 ± 0.02 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 [53]; whereas, Ascorbic acid is reported to directly interact with radicals in plasma, preventing damage to red cell membranes [52]. The results of β -carotene and lycopene obtained in this study are within the range of those reported previously for Pleurotus squarrosulus [54] and much higher than the findings of Vamanu, [55] on Pleurotus ostreatus PQMZ91109 mushrooms. However, the vitamin C content reported in the present study is lower than that reported for Pleurotus ostreatus PQMZ91109 cultivated on wheat and paddy straw [56].

3.4 Antioxidant activities

3.4.1 DPPH Free radical scavenging activities

Radical scavenging of Pleurotus HK 37 extracts after cultivation on the three substrates were evaluated through scavenging activity on DPPH radicals. The free radical scavenging activity of the extracts increased with increasing concentration (Figure 3) an observation previously reported by Tibuhwa [10] and Banerjee, [57] both working on different mushroom species. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. DPPH is a stable free radical with a characteristic absorbance at 515 nm, which decreases significantly on exposure to radical scavengers by providing hydrogen atom or electron to become a stable diamagnetic molecule. The use of stable DPPH radical has the advantage of being unaffected by side reactions, such as enzyme inhibition and metal chelation.

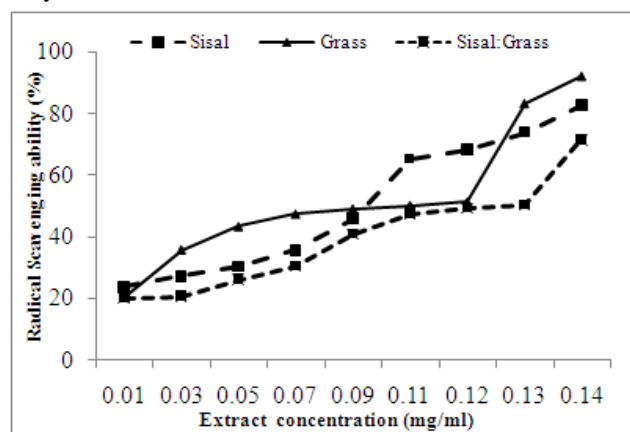


Fig. 3. DPPH radical scavenging activity (%) of Pleurotus HK 37 (ethanolic extract) cultivated on sisal, grass and sisal:grass at 1:1

The maximum scavenging activity values in this study were at a dilution of 0.12 mg/ml. The mushroom extracts from grass substrate showed the highest percentage (92.03%) scavenging power while the extracts from sisal and sisal:grass had 82.5% and 71.28%, respectively. The total antioxidant necessary to decrease the initial DPPH radical concentration by 50% based on the concentration-scavenging activity response curves of Pleurotus HK 37 showed the extract from sisal had the highest ability ($EC_{50} < 0.09$ mg/ml) while that from grass ($EC_{50} < 0.11$ mg/ml) followed, and the extracts from sisal:grass had the least ability ($EC_{50} < 0.13$ mg/ml). Previous studies on P.

ostreatus indicate its EC50 on DPPH radical scavenging activity at 1.8 ± 0.02 mg/ml, while Tibuhwa [10], working on different Termitomyces sp. reported the highest scavenging ability at $EC_{50} < 0.1$ mg/ml. The extracts of the Pleurotus HK 37 mushroom in this study could be potential sources of natural antioxidant a finding supported by Kansci et al. [58], who suggested that the strong anti-radical potency possessed by Dorstenia sp. against DPPH test might be the basis for their strong therapeutic efficacy in traditional medicine.

3.4.2 Chelating ability of ferrous ions

Pleurotus HK 37 extracts chelating capacity increased with the increasing concentration (Figure 4) with chelating ability in the range of 6.21% and 91.2%. Transition metals act as the catalysts for the initial formation of radicals. Chelating agents may reduce free radical damages in human body by stabilizing the transition metals in living systems and inhibit a generation of radicals.

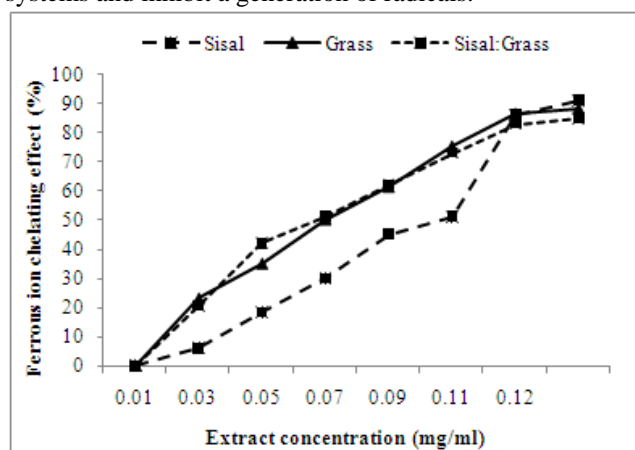


Fig. 4. Ferrous ion chelating effect (%) of Pleurotus HK 37 (ethanolic extract) cultivated on sisal, grass and sisal:grass at 1:1.

Metal chelating ability results of the mushroom extracts showed that the highest value (91.2% at 0.12 mg/ml) was recorded for the Pleurotus extract when cultivated on sisal substrate while extracts from grass and sisal:grass substrates had 88.17% and 85.2%, respectively at the same concentrations. The weakest metal chelating ability (6.21% at 0.01 mg/ml) was recorded for extract from sisal substrate at the least concentrations. Metal chelating ability of other Pleurotus sp. has been previously reported. The extract from *P. ostreatus* [59] was found to be 62.5% (100 µg/mL) and Jayakumar et al. [52] reported the activity as 60.68% (10 mg/ml) for extracts of *P. ostreatus* basidiocarp. The results of this study indicate that Pleurotus HK 37 extract is a good metal chelater, and would prevent transition metals in initiating the oxidative stress in human cells.

Conclusion

The results obtained from this study suggest that the extract from Pleurotus HK 37 has significant nutritional components and antioxidant activity. The mushrooms cultivated on the three different substrates possess high metal chelation and radical scavenging activities with high

concentration of total phenol and total flavonoids. The content of β -carotene, lycopene and vitamin C were very low leaving phenolic compounds to be the possible major antioxidant in the mushroom extracts. The consumption of these mushrooms could therefore enhance the immune system against oxidative damage.

Acknowledgement

The authors wish to acknowledge the Bio-resource Innovation Network for Eastern Africa Development (Bio-Innovate) project for financial support, Mr. Charles Kweyunga of Botany Department University of Dar es Salaam as well as Ms. Evaline James Mfuru of Ministry of Livestock and Fisheries Development, Tanzania for their assistance in the analysis. We also acknowledge the Management of Kilifi Plantation limited, Kenya for the mutual collaboration during this study.

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Source of support: Nil; Conflict of interest: None declared