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Antioxidant activity and nutritional composition of different mushroom extracts grown on different bush encroachers as substrates in Namibia

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Abstract

Mushrooms are a good source of antioxidants with a range of health benefits owing to their bioactive compounds, such as polyphenols, vitamins, and minerals. The objective of this study was to evaluate the antioxidant activity and nutritional composition of mushrooms cultivated on four different encroaching bushes as substrates. Antioxidant activity was measured using reducing power and DPPH scavenging assays. The IC50 values ranged from 0.380±0.098 mg/ml to 0.7780±0.007 mg/ml. The inhibition of the DPPH radical by PS extracts cultivated on *Terminalia sericea* (0.380±0.098 mg/ml) showed the highest activity compared to other mushroom extracts. All the mushroom species grown on different bushes showed an appreciable ability to reduce ferricyanide complex to ferrous form at different levels of concentration (0.063–1.00 mg/ml). TPC varied from 3.93 mg GAE/g to 8.016 mg GAE/g. TFC ranged from 0.515 mg QE/g to 12.1 mg QE/g, which showed a significant difference in the bush species at a p-value of less than 0.01. There was a significant difference in the interaction of bush substrates and mushroom species on crude protein and ash content at p-values of 0.01 and 0.015, respectively. In the current study, crude protein content ranged from 28.0% to 39.0%, with PF grown on *Terminalia sericea* having a high protein content. A significant difference in % NDF on mushroom species was found, where a variation was detected in HK35, while PF, PO, and PS showed a similar performance in % NDF. The study showed that ash content ranged from 5.77% to 17.9%. Percentage moisture ranged from 86.0% to 87.5%. A significant difference in mineral composition, with PO showing a high content of potassium (2.292%), PF showing a high content of sodium (0.059%), and PS showing a high content of phosphorus (0.746%) as compared to other mushroom species. Among the four different bushes, *Combretum collinum* (potassium 2.256%, sodium 0.05%, and phosphorus 0.74%) had the greatest effect on the mineral composition of the different mushrooms cultivated. In conclusion, mushrooms are accessible natural food rich in antioxidants, which may boost the immune system against oxidative damage and can be used as potential sources of therapeutic agents.

Keywords – antioxidants – minerals – therapeutic agents – total flavonoids – total phenolics – oxidative stress

Introduction

Pleurotus species belong to the order Agaricales and the family Tricholomataceae. They are

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called Basidiomycota, which means they produce spores on specialised structures called basidia (Mairami et al. 2022). One of the distinguishing features of *Pleurotus* species is the morphology of their fruit bodies. The fruit bodies typically have an eccentric stalk, which is attached to the cap at an off-center position. The cap is wide and shaped like an oyster shell, hence the common name "oyster mushroom." The widest portion of the cap is usually away from the stalk, giving it a characteristic appearance. Oyster mushrooms are widely cultivated and consumed as a food source. They are known for their delicate flavor and firm texture (Stamets 2000). They grow on various substrates, such as decaying wood, tree stumps, or agricultural by-products. *Pleurotus* species are also valued for their nutritional and medicinal properties**.** They are a good source of protein, dietary fibre, vitamins (such as vitamin B complex), and minerals. Additionally, some studies have suggested that oyster mushrooms possess various bioactive compounds with potential antioxidant, anti-inflammatory, and antimicrobial properties. According to Raman et al. (2021), the production of *Pleurotus* mushrooms has increased significantly in recent years, reaching approximately 27% of global production. Some of the most widely cultivated *Pleurotus* species include *Pleurotus ostreatus*, *Pleurotus eryngii*, *Pleurotus florida*, *Pleurotus citrinopileatus*, *Pleurotus soja-caju* and *Pleurotus pulmonarius*. In addition, different *Pleurotus* hybrids have been developed, including HK35, the most widely grown exotic variety in Europe. With its high yield, adaptable nature, and year-round cultivation potential, HK35 has revolutionised the market, offering top-quality mushrooms in a compact package. *Pleurotus* species are commonly grown in Asia, Europe, and North America, with China, India, and Italy being some of the leading producers. Furthermore, *Pleurotu*s species grow widely in tropical and subtropical areas (Maszlavér & Győrfi 2003).

Oxidation is a normal body process that enables the transformation of nutrients into energy (Kozarski et al. 2015). The biological processes of living organisms require oxidation. As a result, oxygen radicals or reactive oxygen species are released into the body. However, overcrowding of reactive oxygen species in the body may result in tissue damage, which eventually leads to the development of deadly diseases such as diabetes, cancer, and atherosclerosis, as stated by Sánchez (2017). Naturally, living organisms produce oxidative enzymes such as catalase and superoxide dismutase in defence against free radical damage (Sánchez 2017). Functional foods rich in natural antioxidants contain phytochemicals that combat oxidative stress in the body by helping the maintenance of a proper balance between oxidants and antioxidants (Flieger et al. 2021). Frequent consumption of foods containing natural antioxidants has been associated with a reduced risk of chronic diseases and may help the defence system (Azieana et al. 2017). The antioxidants found in mushrooms are mainly phenolic compounds, which possess specific health benefits and are highly beneficial in the antioxidative defence mechanisms in biological systems (Mwangi et al. 2022).

According to El Sebaaly et al. (2019), mushrooms have valuable nutritional value and are often regarded as meat substitutes with a comparable nutritional value to eggs, milk, meat, and vegetables. Traditionally, mushrooms have been consumed only because of their delicacy; however, their nutritional value has not been explored until recently. Moreover, mushrooms could be part of the solution to malnutrition faced in underdeveloped and developing countries (Ishara et al. 2018). Protein is an essential constituent of mushrooms; they are low in calories and fats. Murcia et al. (2002) reported that mushrooms are the only non–animal food containing vitamin D, hence considered natural sources of vitamin D for vegetarians. Studies reported that mushrooms contain non-starch polysaccharides and beta-glucans that are a good source of soluble and insoluble dietary fibre for humans and thus can be a good remedy against constipation (Cheung 2013). Consumption of mushrooms promotes a healthy, balanced diet as they are rich in minerals such as phosphorus, sodium, calcium, potassium, etc. Minerals play a significant role in human biological processes and metabolism (Mallikarjuna 2013).

Pleurotus mushrooms are highly regarded for their nutritional composition and antioxidant activity. They are low in calories and fat, yet rich in protein, dietary fibre, vitamins (including B vitamins and vitamin C), and minerals (such as potassium, phosphorus, magnesium, zinc, copper, and iron) (Ahmed et al. 2013). These mushrooms contain bioactive compounds like phenolic compounds, flavonoids, tocopherols, and selenium, which contribute to their significant antioxidant

properties (Reis et al. 2012). Additionally, they contain beta-glucans that support immune function and exhibit potential anti-tumor effects. Oyster mushrooms also contain bioactive peptides that have various physiological effects, including antioxidant, antimicrobial, antihypertensive, and immunomodulatory properties (Elkhateeb & Daba 2022). Overall, *Pleurotus* mushrooms offer a nutritious addition to the diet and potential health benefits due to their antioxidant and bioactive properties. Therefore, the study investigated the antioxidant activity and nutritional composition of various edible mushroom species produced from different encroacher bushes as substrates.

Materials & Methods

Mushroom samples and substrates used

The substrate harvesting was carried out in the Okondjatu district of the Otjozondjupa region, Namibia, which was adversely affected by bush encroachment. Different types of encroaching bushes, namely *Senegalia mellifera*, *Grewia flava*, *Combretum collinum*, and *Terminalia serecea*, were harvested, milled, air-dried, packed in bags, and transported to the University of Namibia for mushroom cultivation. Different mushroom strains, including *Pleurotus ostreatus, Pleurotus florida, Pleurotus soja-caju,* and HK35, were collected from the ZERI department at the University of Namibia and propagated on wheat grain under sterile conditions.

Mushroom cultivation

The milled bush substrate was soaked overnight, drained, and mixed with wheat bran. The substrate mixture was packed into plastic bags and pasteurised for 3 hours. After cooling, the bags were inoculated with different mushroom spawns and placed in a dark room at room temperature for colonisation. Once fully colonised, the bags were transferred to a harvesting room, with small holes made for primordial initiation. The bags were watered three times a day to maintain humidity and temperature for fruiting.

Extraction

Dried mushrooms were ground into a fine powder. The weighed powder was then mixed with 99% methanol in a flask and left in a shaker for 24 hours. After this, the sample was filtered with the Whatman Grade 1 paper. Methanol was evaporated under laminar flow. The dried extracts were stored at 4 degrees Celsius in bottles until analysis (Saeed et al. 2012).

DPPH radical scavenging activity

About 100 µl of distilled water was added to all the wells in the plate. About 100 µl of the 1 mg/ml extract was then added from the stock solution and diluted from 1 mg/ml to 0.5 mg/ml,0.25 mg/ml, and 0.125 mg/ml. A total of 100 µl of vitamin C was added and serially diluted downwards. Then, 100µl of ethanol was added to the wells of the blank extract, and 100 µl of DPPH from stock solution was added to all the wells except the blank extract wells and plate blank wells. The plate was then covered with foil and incubated for 30 minutes. Plate absorbance was read at 520 nm (Kapewangolo et al. 2013).

Reducing power

In 96-well plates, 10 µl of mushroom extract of different concentrations was added to the wells with 25 µl of buffer, and 25 µl of 1% potassium ferricyanide was further added to wells containing extracts. The plate was then incubated for 20 minutes at room temperature. After incubation, 25 µl of 10% trichloroacetic acid (TCA) solution was added to stop the reaction. Then, 85 µl of distilled water and 8.5 µl of 0.1% ferric chloride were added to each well of the extracts. The solution was thoroughly mixed and incubated for another 15 minutes at room temperature. The absorbance of the solutions was measured at 700 nm. Ascorbic acid was used as the positive control (Acharya 2017).

Determination of total phenolic content (TPC)

The TPC of the extracts were analysed according to Saeed et al. (2012), with slight modifications. About 0.5 ml of the sample (1 mg/ml) was mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent and incubated for 5 minutes at room temperature. 5 mL of a 7% Na_2CO_3 solution and 6.5 mL of deionised distilled water were added, respectively. The mixture was left in the dark for 60 minutes at room temperature. Gallic acid was prepared as a positive control at different concentrations (0.063 mg/ml, 0.125 mg/ml, 0.25 mg/ml, 0.5 mg/ml, and 1 mg/mL). The absorbance was measured at 750 nm, and a standard curve from different concentrations of gallic acid was then calibrated to determine the total phenolic content. The total phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per gram of dry matter.

Determination of total flavonoid content (TFC)

This procedure was carried out as stipulated by Josipović et al. (2016). Firstly, 0.4 ml of distilled water and 0.03 ml of a 5% sodium nitrite solution were added to 0.1 ml of an extract solution (1 mg/ml) and incubated for 5 minutes. Afterwards, 0.03 ml of 10% aluminium chloride was added, and the mixture was further incubated for 6 minutes. 0.2 ml of 1 M sodium hydroxide was added, and the volume was adjusted to 1 ml by adding 0.24 ml of distilled water. The absorbance was measured immediately against the reagent blank at 510 nm. Quercetin was prepared as a positive control at different concentrations (0.063, 0.125, 0.25, 0.5, and 1 mg/ml), a standard curve was prepared from different concentrations of quercetin, and the total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of dry matter.

Nutritional and mineral content analyses

Moisture content analysis

Fresh mushroom samples were collected, weighed, and documented. The mushrooms were then air-dried, and the dry mass was recorded. The loss in weight after drying is known as the moisture content, which was calculated using the following equation (Masamba & Kazombo-Mwale 2010).

Moisture % = (Initial Weight (g) - Final Weight (g)) × 100/Sample's Initial Weight (g)

Ash content analysis

The dry crucible and lid were placed in a hot air oven for 20 minutes at 105 degrees Celsius. The crucible and lid were cooled in the desiccator for 20 minutes. The masses of the crucible and lid were taken and recorded. Dried mushroom samples (3 g) were placed in the crucible and the furnace at 700 degrees Celsius for 2 hours. The crucibles were then removed and cooled, and the weight of the curable with ash was recorded (Masamba & Kazombo-Mwale 2010). The total ash was calculated as follows:

Ash % = (weight of crucible with ash (g) – weight of crucible (g)) ×100/ Weight of sample (g)

Neutral detergent fibre (NDF) and Acid detergent fiber (ADF)

The ANKOM 220 Fiber Analyzer unit (ANKOM Technology Corporation, Macedon, NY, USA) was used to analyse neutral detergent fibre (NDF) and acid detergent fibre (ADF). The Ankom filter bags were labelled and weighed. The bags were then filled with 0.50 g of mushroom powder, heat-sealed and weighed. Furthermore, the bags were placed in a bag suspender and transferred to an Ankom machine. The machine was then filled with approximately 2 L of NDF/ADF solution, which covered the top level of the bag suspender. The lid was tightly closed and agitated for 75 minutes for NDF and ADF. The solution was removed from the machine, and boiling distilled water was added to the reservoir to rinse the bags by agitating for 5 minutes. The bags were then removed from the suspender, and excess water was manually removed. The bags

were placed in a 100-degree Celsius oven for 6 hours, and the dry bags were weighed using a desiccator.

Crude Protein

The determination of total nitrogen by the Dumas combustion method is described by Oeno (2002). Mushroom powder samples were taken and analysed at the Ministry of Agriculture, Water, and Land Reform (MAWLR). The determination of total nitrogen by the Dumas combustion method and crude protein was obtained by calculating N x factor 6.25.

Mineral composition

Flame emission spectroscopy was used for the determination of potassium and sodium contents. Phosphorus was determined by colorimetry, and phosphorus concentration in the solution of the digested sample was determined spectrophotometrically as the yellow phospho-vanado-molybdate complex (Poluektov 1973).

Statistical analysis

A general linear model analysis of variance was used for the analysis, using the Statistical Package for Social Sciences version 25 (SPSS). The means that differ significantly were detected using the Duncan multiple range comparison, and the equation for the general linear model used was $y_{ijk}=u + M_i + MB_{ij} + E_{ijk}$.

Where, y_{ijk}= the K^{th} replicate of i^{th} mushroom species in the j^{th} bush substrates

u = Overall mean

Mi = Effect of the i^{th} mushroom species

Bj = Effect of the j^{th} encroaching bush

MiBj = Effect of the interaction between i^{th} mushrooms and j^{th} bush substrate.

The significant difference was tested at p-value ≤ 0.05. Normality assumption was checked using the Shapiro-Wilks test and the histogram residuals; homogeneous variance was tested using Levine's test and the plot of residuals versus fitted values. The minimum inhibitory concentration at 50% (IC50) was calculated using a graph pad prism.

Results and Discussion

Table 1 shows the inhibitory concentration at 50% of methanolic extracts of mushrooms grown on various encroaching bushes. The IC50 refer to the concentration of a mushroom extract that exhibits an antioxidant effect at 50%. The inhibitory concentration at 50% (IC50) values ranged from 0.380± 0.098 mg/ml to 0.780±0.007 mg/ml on various mushroom extracts (Table 1). Moreover, the highest activity was demonstrated by Ps extracts cultivated on *T. sericea* (0.380±0.098 mg/ml) compared to other mushroom extracts cultivated on the four bush encroachers. The PO grown on *T. sericea* showed the least activity (0.780±0.007 mg/ml). However, the activity of standard ascorbic acid (0.023±0.026mg/ml) was higher than that of all extracts tested. These results were similar to those reported by Panthong et al. (2016), with an IC50 ranging from 0.28 mg/ml to 11.45 mg/ml. Figure 1 demonstrates the scavenging of free radicals by mushroom extracts grown on various encroaching bushes at different concentrations. From 0.125 to 1 mg/ml, mushroom extract's capacity to scavenge free radicals keeps increasing as the concentration increases. The PS cultivated on *T. sericea* (79.1%) demonstrated a high percentage of radical scavenging activity at 1mg/ml among the evaluated mushroom extracts. The concentration of inhibition at 1mg/ml was least noticeable in HK35 (66.8%) grown on *G. flava.*

Reducing power ability increased with concentration, as illustrated in Fig. 2. Therefore, the greatest ability for reducing the ferricyanide complex to ferrous form was observed in PO *G. flava* extracts at a concentration of 1.00 mg/ml. HK35 cultivated on *T. sericea* showed the least ability to reduce the ferricyanide complex. However, the results of reducing ability by Vitamin C as a positive control were significantly higher than the activity of mushroom extracts. Moreover, the

findings revealed no detectable variation in the reducing power among several mushroom species grown in various encroaching bushes. This significant finding shows that the combination of substrate species and mushrooms in this set does not significantly affect the reducing power. It is, therefore, advisable to choose the combination based on other practical parameters such as availability, species hardiness, and growth rate.

Free radical scavenging assay

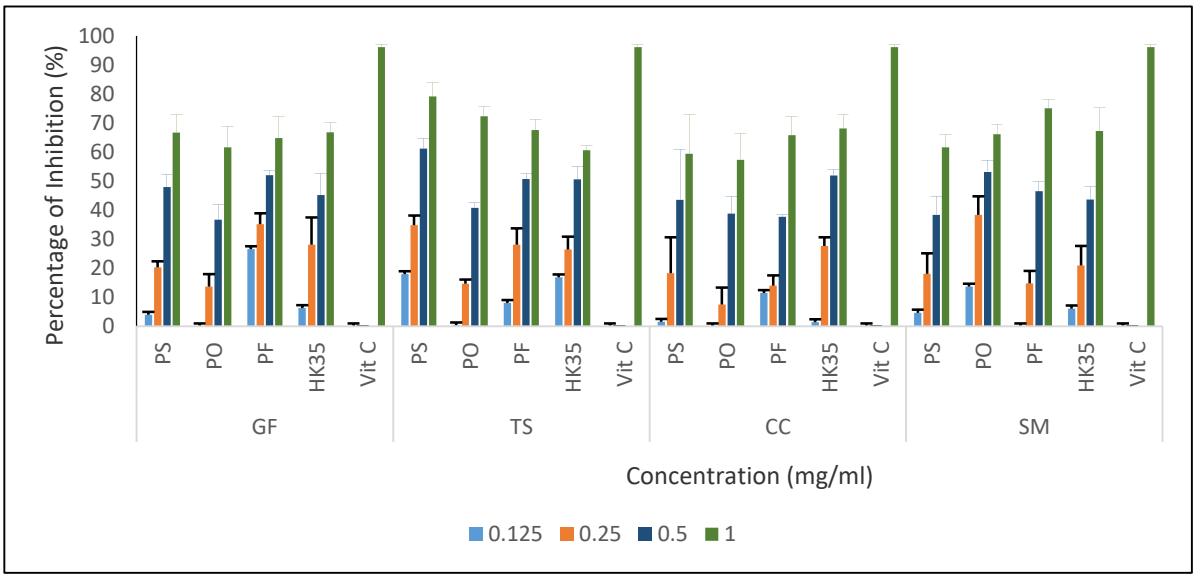

Fig. 1 – DPPH radical scavenging activity of different mushroom species cultivated on encroaching bushes. PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, GF= *Grewia flava*, TS= *Terminalia sericea*, CC= *Combretum collinum*, SM= *Senegalia mellifera*, HK35= *Pleurotus* hybrid.

Reducing power assay

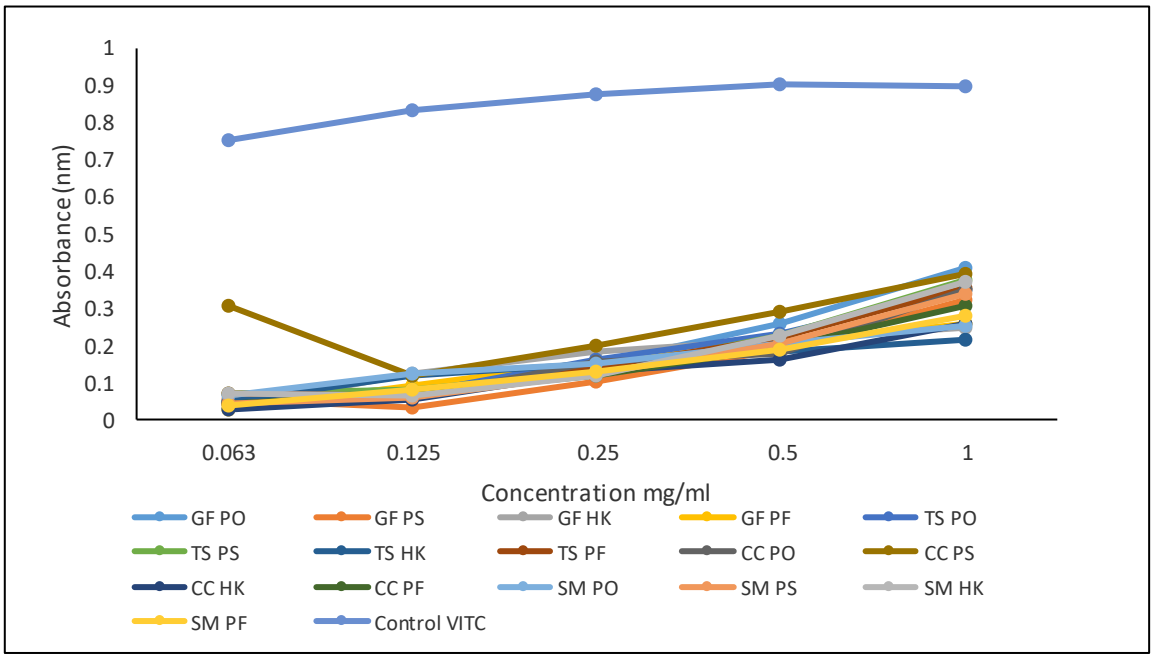

Fig. 2 – Reducing power of different mushroom extracts cultivated on different encroaching bush

substrates. PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida, GF= Grewia flava, TS= Terminalia sericea, CC= Combretum collinum, SM= Senegalia mellifera*

Table 1 Inhibitory concentration at 50% (IC50) of different mushroom species cultivated on different encroaching bushes.

Bush species substrate	Mushroom Species	IC50 mg/ml
Combretum collinum	HK 35	0.430±0.900
	PF	0.723±0.017
	PO	0.770±0.029
	PS	0.703±0.001
Grewia flava	HK 35	0.575±0.103
	PF	0.476±0.098
	PO	0.727±0.028
	PS	0.476±0.090
Terminelia sericea	HK 35	0.479±0.014
	PF	0.462±0.017
	PO	0.780±0.007
	PS	0.380±0.098
Senegalia mellifera	HK 35	0.607±0.014
	PF	0.562±0.014
	PO	0.478±0.025
	PS	0.703±0.104
Vitamin C		0.0235±0.053

NB: PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Phytochemicals content

There was no significant difference in TPC and TFC on the effect of the interaction of bush substrates and mushroom species (Table 2). The different oyster mushroom species exhibited a TPC ranging from 3.93 mg GAE/g to 8.016 mg GAE/g. These results were comparable to those reported by Rajoriya et al. (2015) for medicinal wild *Ganoderma lucidum* extracts, which ranged from 8.44 to 11.60 mg GAE/g. However, the TPC values for PO grown on farm waste, sawdust, and peanut waste were lower, ranging from 2.672 ± 0.003 mg GAE/g to 1.073 ± 0.028 mg GAE/g, as reported by Yilmaz et al. (2017). The TFC values ranged from 0.515 mg QE/g to 12.1 mg QE/g. The TFC values were similar for *G. flava* and *S. mellifera*, while *C. collinum* and *T. serecea* had different TFC values. *Terminalia serecea* had the lowest TFC value (0.922 mg QE/g), while *C. collinum* had the highest TFC value (0.922 mg QE/g) (Table 2). Furthermore, the significant difference in TPC was due to the substrate used (encroaching bushes) and mushroom species at p-values <0.01 (Table 3), while the significant difference in TFC was due to mushroom species at a p-value of 0.18 (Table 3).

Nutrient composition

The current study showed that mushrooms could potentially be a good source of protein, typically comprising 28.93% to 39.1% of their dry weight (Table 4). The study found a significant difference in the interaction of bush substrates and mushroom species on protein and ash content. The interaction of bush substrates and mushroom species showed no significant difference in the percentage of NDF, ADF and moisture (Table 4). However, a significant difference in the main effects of NDF was observed, which was due to the mushroom species used (Table 5). Elattar et al. (2019) reported ADF and NDF content of PO within a range of 22.8%–28.5% and 48.7%–59.4%, respectively. Comparing these results to the current study, ADF content was in close proximity, ranging from 29.4% to 34.2%, while NDF content was relatively higher than that of the current study (27.7% to 34.8%). These results are similar to those of Naeem et al. (2020), who reported % ash of edible mushrooms in the range of 6.2%–8.26%, and Bhattacharjya et al. (2015), whose study showed % ash of PO grown on sawdust to be in a range of 9.0%–13.0%. The current results showed that different bush species performed differently on the ash content of different mushroom

species. *Senegalia mellifera* showed the highest ash content in HK35 (17.9%), while the least was detected in PO (5.77%) cultivated on *C. collinum*. Moisture content ranged from 86.0% to 87.5%, and there was no significant difference in the moisture content of the mushroom species cultivated on different bush species. According to Alam et al. (2008), the moisture percentage ranged from 86.0% to 87.5%, similar to the current study.

Table 2 The effects of the interaction of bush substrates and mushroom species on phytochemicals

Bush species Substrate	Mushroom Species	TPC (mg GAE/g)	TFC (mg QE/g)
Combretum collinum	HK	5.21	10.16
	PF	3.93	7.40
	PO	4.49	9.07
	PS	4.37	12.1
Grewia flava	HK	5.34	3.64
	PF	4.42	6.01
	PO	4.93	2.72
	PS	4.87	2.78
Terminelia sericea	HK	7.37	1.46
	PF	6.11	0.970
	PO	6.22	0.760
	PS	5.84	0.510
Senegalia mellifera	HK	7.18	1.19
	PF	5.88	1.02
	PO	6.91	0.980
	PS	8.02	0.890
SEM		0.741	1.44
p -value		0.912	0.48

NB: PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*

Table 3 The main effects of bush substrates and mushroom species on total phenolic and flavonoid contents.

Bush Species Substrate	***TPC***	***TFC***
Combretum collinum	4.50^{b}	9.68^{a}
Grewia flava	4.89^{b}	3.79^{c}
Terminelia Sericea	6.38^{a}	0.922^{b}
Senegalia mellifera	7.00^{a}	1.017^{c}
SEM	0.371	0.718
p-value	<0.01	<0.01
Mushroom Species		
HK	6.28^{a}	4.11
PF	5.09^{c}	3.85
PO	5.64^{ab}	3.38
PS	5.77^{ab}	4.07
SEM	0.37	0.72
p -value	0.18	0.88

NB: Mean values that bear different superscript letters are significantly different ($\alpha \leq 0.05$). PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Table 4 The effects of the interaction of bush substrates and mushroom species on nutrient composition

Bush species substrate	Mushroom Species	% Crude Protein	% NDF	% ADF	% Ash	% Moisture
Combretum collinum	HK 35	37.4^{ab}	33.5	32.0	6.30^{d}	86.4
	PF	30.1^{c}	30.5	30.9	16.2^{ab}	87.1
	PO	29.7^{cd}	30.1	30.8	5.77^{d}	87.2

Table 4 Continued.

Bush species substrate	Mushroom Species	% Crude Protein	% NDF	% ADF	% Ash	% Moisture
	PS	38.0^{ab}	28.8	34.3	8.53^{bcd}	86.3
Grewia flava	HK 35	31.1^{c}	34.8	31.6	7.20^{d}	85.9
	PF	36.6^{b}	28.1	29.4	6.30^{d}	87.4
	PO	36.1^{b}	30.8	29.9	8.37^{bcd}	86.7
	PS	37.3^{ab}	30.7	32.0	8.10^{bcd}	87.4
Terminelia sericea	HK 35	38.3^{ab}	33.7	31.4	17.20^{a}	86.5
	PF	39.0^{a}	28.9	30.0	6.43^{d}	87.4
	PO	36.3^{b}	31.7	34.2	10.4^{abcd}	87.0
	PS	36.2^{b}	29.5	30.0	7.87^{cd}	86.7
Senegalia mellifera	HK 35	28.0^{d}	33.5	31.4	17.9^{a}	86.8
	PF	31.2^{c}	27.7	29.4	15.7^{abc}	87.1
	PO	31.2^{c}	30.9	32.0	12.6^{abcd}	87.5
	PS	36.4^{b}	30.6	30.3	8.20^{bcd}	87.0
SEM		0.690	2.02	2.64	2.47	0.755
P -value		<0.01	0.990	0.966	0.015	0.986

NB: Mean values that bear different superscript letters are significantly different ($\alpha \leq 0.05$). PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Table 5 The main effects of bush substrates and mushroom species on nutrient composition

Bush species substrate	%Crude Protein	% NDF	% ADF	% Ash	% Moisture
Combretum collinum	33.8^{c}	30.7	32.0	9.2^{b}	86.7
Grewia flava	35.3^{b}	31.1	30.7	7.5^{b}	86.9
Terminalia Sericea	37.5^{a}	30.9	31.4	10.5^{ab}	86.9
Senegalia mellifera	31.7^{d}	30.7	30.8	13.6^{a}	87.1
SEM	0.346	1.009	1.32	1.24	0.377
p -value	<0.01	0.988	0.890	0.011	0.906
Mushroom Species					
HK 35	33.7^{b}	33.9^{a}	31.6	12.1^{a}	86.4
PF	34.2^{b}	28.8^{b}	29.9	11.2^{ab}	87.5
PO	33.3^{b}	30.9^{b}	31.7	9.30^{ab}	87.1
PS	37.0^{a}	29.9^{b}	31.6	8.18^{b}	86.9
SEM	0.346	1.009	1.32	1.236	0.377
p -value	<0.01	0.008	0.726	0.121	0.425

NB: Mean values that bear different superscript letters are significantly different ($\alpha \leq 0.05$). PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Mineral composition

There was no significant difference in mineral composition (Table 6). Furthermore, the mineral composition of mushrooms grown on *C. collinum* ranged from 2.107% to 2.407% potassium, 0.033% to 0.077% sodium, and 0.760-0.723% phosphorus. According to Mallikarjuna et al. (2013), factors such as mushroom species, morphology, quality of mycelium, and substrate composition can influence mineral accumulation in mushrooms. The potassium and calcium composition of mushroom extracts varied significantly depending on the mushroom species and bush substrates (Table 7). The results showed that *C. collinum* had the highest mean content of potassium, sodium was highest in *C. collinum* and *G. flava*, and phosphorus was highest in *C. collinum* and *T. sericea*. In terms of mushroom species, PO had the highest potassium content, PF had the highest sodium content, and PS had the highest phosphorus content. Overall, the mineral composition of the different mushrooms cultivated was greatly influenced by the type of bush substrate and mushroom species used. Minerals play a crucial role in various physiological processes such as metabolic reactions, bone formation, nerve impulse transmission, and maintaining water and salt balance in the body (Mairami et al. 2022). Therefore, minerals are

essential for the growth, development, and maintenance of the body. The presence of different minerals significantly affects the growth, health, and quality of mushrooms. Potassium maintains proper water balance and prevents dehydration, while calcium strengthens cell walls for disease resistance. Balancing these minerals in the growth medium ensures optimal conditions for robust mushroom growth, development, and high-quality yields (Nagulwar et al. 2020).

Table 6 The effects of the interaction of bush substrates and mushroom species on mineral composition

Bush species substrate	Mushroom Species	%Potassium	% Sodium	%Phosphorus
Combretum collinum	HK	2.41	0.033	0.760
	PF	2.11	0.077	0.723
	PO	2.28	0.050	0.727
	PS	2.23	0.043	0.753
Grewia flava	HK	1.89	0.060	0.710
	PF	2.04	0.053	0.713
	PO	2.19	0.040	0.743
	PS	2.02	0.050	0.733
Terminelia sericea	HK	2.21	0.040	0.730
	PF	1.91	0.050	0.740
	PO	2.29	0.040	0.720
	PS	1.98	0.040	0.757
Senegalia mellifera	HK	2.02	0.040	0.530
	PF	1.86	0.057	0.433
	PO	2.40	0.040	0.737
	PS	1.90	0.043	0.740
SEM		0.019	0.002	0.014
p -value		<0.001	< 0.001	<0.001

NB: Mean values that bear different superscript letters are significantly different ($\alpha \leq 0.05$). PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Table 7 The main effects of bush substrates and mushroom species on mineral composition.

Bush Species substrate	% Potassium	% Sodium	% Phosphorus
Combretum collinum	2.26^{a}	0.051^{a}	0.741
Grewia flava	2.04^{c}	0.051^{a}	0.725
Terminelia Sericea	2.10^{b}	0.042^{b}	0.737
Senegalia mellifera	2.05^{c}	0.045^{b}	0.610
SEM	0.009	0.001	0.007
p -value	<0.001	<0.001	<0.001
Mushroom species			
HK 35	2.13^{b}	0.043^{b}	0.683^{b}
PF	1.98^{d}	0.059^{b}	0.653^{a}
PO	2.29^{a}	0.042^{b}	0.732^{a}
PS	2.03^{c}	0.044^{a}	0.746^{a}
SEM	0.009	0.001	0.007
p -value	<0.001	<0.001	<0.001

NB: Mean values that bear different superscript letters are significantly different ($\alpha \leq 0.05$). PS= *Pleurotus sajor-caju*, PO= *Pleurotus ostreatus*, PF= *Pleurotus florida*, HK35= *Pleurotus* hybrid

Conclusion

In conclusion, the present study provides valuable insights into the effect of different bush substrates and mushroom species on antioxidant activity and nutritional and mineral composition in cultivated mushrooms. The results demonstrated that mushroom extracts had varying levels of inhibitory concentration at 50%, depending on the type of bush encroacher. The highest activity

was observed in PO grown on *T. sericea*, while the lowest was in PO extracts cultivated on *C. collinum*. The reducing power ability increased with concentration, while Vitamin C had significantly higher activity than mushroom extracts. Flavonoids and phenolics were found to be natural antioxidants in mushrooms. The results suggest that the mineral composition of mushrooms is significantly influenced by the bush substrate and mushroom species. *Combretum collinum* had the greatest effect on mineral composition in mushrooms, with a high content of potassium, sodium, and phosphorus. These findings can have important implications for the production of nutrient-rich mushrooms with a specific mineral profile, as well as for the utilisation of encroaching bushes as a potential substrate for mushroom cultivation. Overall, this study contributes to the growing body of research on the potential of mushroom extracts as natural antioxidants, highlighting the importance of considering the substrate species when selecting oyster mushrooms for cultivation. In addition, further research in this area can shed light on the specific mechanisms that govern the nutrient accumulation in mushrooms and the potential benefits for human health.

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