Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 2762972, 16 pages
https://doi.org/10.1155/2023/2762972

Research Article

Bioprospecting the Biological Effects of Cultivating *Pleurotus ostreatus* Mushrooms from Selected Agro-Wastes and Maize Flour Supplements

Senzosenkosi Surprise Mkhize,[1] **Mthokozisi Blessing Cedric Simelane**,[2] **Nkoana Ishmael Mongalo**,[3] **and Ofentse Jacob Pooe**[1]

[1]*Discipline of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa*
[2]*Department of Biochemistry, University of Johannesburg, P.O. Box 524, Auckland Park 2006, South Africa*
[3]*College of Agriculture and Environmental Science (CAES) Laboratories, University of South Africa, Private Bag X06, Johannesburg 0710, South Africa*

Correspondence should be addressed to Ofentse Jacob Pooe; pooeo@ukzn.ac.za

Received 11 November 2022; Revised 5 March 2023; Accepted 6 March 2023; Published 29 March 2023

Academic Editor: N. A. DICA Maltar Strmečki

Pleurotus mushrooms are valuable food supplements with health and environmental restorative potential. In this paper, we sought to evaluate the biological activities and profile the bioactive compounds found in *Pleurotus ostreatus* cultivated from agro-waste supplemented with maize flour. We investigated carbon to nitrogen (C/N), antimicrobial, antioxidant, and antimalarial potential for the varying supplementation during mushroom cultivation. GCMS was utilized for screening bioactive compounds found in *P. ostreatus*. Changes in supplementation directly correlate with changes in compound profiling. Nonetheless, some compounds were found to be common amongst the tested mushrooms, including pentadecanoic acid; 9,12-octadecadienoic acid, methyl ester; pentadecanoic acid, methyl ester; octadecanoic acid; and diisooctyl phthalate. The highest antimicrobial potential against Gram-positive *Staphylococcus aureus* was observed when maize flour supplements were increased to 12% and 18%. Our data demonstrated that the observed antioxidant (DPPH, ABTS, and reducing power) and antimicrobial activity could emanate from various supplementation conditions. Furthermore, supplementation has an impact on the mushroom yield and phytochemical profiles of the produced mushroom.

1. Introduction

Mushrooms have established significant value in humans as they are increasingly incorporated into humans since they have profound functional and nutraceutical importance [1]. Amongst the most cultivated mushrooms, the oyster (*Pleurotus*) mushroom ranks as the third most cultivated species following the button and shiitake mushrooms [2]. The *Pleurotus* mushrooms have an added advantage of worldwide distribution since they can grow in temperate to tropical regions with temperatures ranging from 12 to 32°C [3]. Furthermore, the process of cultivation of oyster mushrooms is useful for reducing environmental pollution, and hence it is deemed as one of the environmentally friendly procedures since the mushroom produces mycelia which degrade lignocellulosic waste via a complex enzyme system [4]. For the mushroom mycelia to degrade and utilize lignocellulosic waste for its growth, specific nutrients are needed, and hence the mushroom-growing substrates should be supplemented to increase mushroom yield and improve rapid growth [5, 6] and nutritional property, and hence the nutritional contents of mushroom are influenced by the composition of growing substrates [7].

It has been documented that *Pleurotus* spp. need to be grown on substrates containing carbon, nitrogen, and inorganic compounds for growth [8]. Hence, the addition of supplements is a necessity to enhance the production of oyster mushrooms; however, the supplementation ratio should be of a certain limit to avoid the possibilities of contamination [9] and yield reduction [10]. Therefore, the substrates utilized for mushroom growth should have a well-balanced carbon-to-nitrogen (C/N) ratio that is supplied by nutrients within the substrates [11]. For example, the *P. ostreatus* mushroom requires an optimal C/N ratio for better growth on the substrate [12]. Hence, it was one of the objectives of the study to find an optimal C/N ratio of *Pleurotus ostreatus* mushroom using maize flour as a supplement. However, numerous mushroom species are well known to produce a variety of metabolites that have antioxidant, antimicrobial, antitumor, antihypertensive, antiplatelet aggregation, antihyperglycaemic, antigenotoxic, and antiviral activities [13]. It is worth noting that recently mushrooms have been reported to have compounds such as anthraquinones, flavonoids, and steroids which possess antimalarial activity [14]. Some of these metabolites from mushrooms such as phenolic compounds can be affected by different factors such as substrates and supplements used during cultivation [15]. Some authors have stipulated that supplements such as wheat bran are rich in compounds that have antioxidant activities [16], and hence Magdziak et al. [15] have recently found that the addition of 20% WB caused little effect on the synthesis of low-molecular-weight organic acids within *P. citrinopileatus*.

Hence, our study only focused on growing *Pleurotus ostreatus* mushrooms on sugarcane waste (sugarcane leaves and sugarcane bagasse) substrates supplemented with maize flour, which has been rarely used when compared to other supplements such as wheat bran. To our knowledge, no information confirms the effect of utilizing maize flour as a supplement on factors such as mushroom bioactive compounds, antioxidant properties, antimicrobial properties, and antimalarial properties of the mushroom. Hence, the medicinal benefits of adding maize flour to the mushroom-growing substrates could probably be achieved.

2. Materials and Methods

2.1. The Field and Laboratory Cultivation of P. ostreatus Mushroom. Two different agro-waste substrates (sugarcane bagasse and sugarcane top) were used for the cultivation of *P. ostreatus* mushrooms. A slightly modified method by Mkhize et al. [17] was adopted during the cultivation of the *P. ostreatus* mushroom. The *P. ostreatus* mushroom was obtained from the KwaZulu-Natal (KZN) Department of Agriculture and Rural Development (DARD). (i) Mushrooms were precultured on the media named Potato Dextrose Agar (PDA), incubated under dark conditions for a week at ±25°C, and then stored at −4°C until further used. (ii) Approximately 4-5 pieces of previously grown *P. ostreatus* mushroom on PDA were then inoculated into the fully prepared bird seed grain, and hence mushroom spawn was obtained through the adoption of slightly modified method from Mkhize et al. [9]. (iii) Mushroom-growing base substrates (sugarcane tops and sugarcane bagasse) were thereafter prepared by soaking in water till moisture of 65% was achieved, and the pH of the substrates was balanced by adding standardized formulas of 1% gypsum ($CaSO_4{\cdot}2H_2O$) and 15% $CaCO_3$, and thereafter the substrates were supplemented with maize flour, that is, 0% (no maize flour), 8%, 12%, and 18%, respectively. After supplementation, the substrates were pasteurized at temperatures around 60–65°C for six hours and thereafter given time to cool up to room temperature and inoculated with previously prepared *P. ostreatus* mushroom spawn. After inoculation, the substrates were incubated within the dark environment under ambient temperature (±25°C) until the mycelia colonized the entire substrate. (iv) The fully mycelial colonized substrates were taken out of the dark environment and were stored in the fruiting room which was constructed of 30% gray shade cloth and was timely fogged to maintain 60% moisture, which promoted rapid fruiting of *P. ostreatus* mushroom. The mushrooms were harvested and sun-dried under 30% gray shade cloth.

2.2. The Carbon-to-Nitrogen (C/N) Ratio of Maize Flour-Supplemented Substrates and P. ostreatus Mushroom Yield. The substrates (sugarcane tops and sugarcane bagasse) were initially screened for total carbon and nitrogen, adopting a modified method from Amoah-Antwi et al. [18]. The content of C and N within the abovementioned substrates was analyzed with the combustion method using a machine called CHN analyzer (Leco, Moenchengladbach, Germany). About 3 mg of oven-dried substrates was analyzed in triplicate, with the C/N ratio calculated from the mean of the obtained results.

The mushroom yield was calculated following a method by Mkhize et al. [9]; hence, the following formula was utilized to calculate the mushroom yield: MY = weight of fresh mushroom harvested/weight of fresh substrate, where MY = mushroom yield in grams.

2.3. P. ostreatus Mushroom Extraction. The *P. ostreatus* extract was prepared through the adoption of a slightly modified method by Chowdhury et al. [19]. The mushrooms which were sun-dried under 30% shade cloth were milled using a milling machine of 30 mm sieves. The mushroom powder of 100 g was then weighed and mixed with 250 ml of methanol solvent, which was thereafter allowed to be shaken at 200 rpm for 24 hours under room temperature (±25°C). After 24 hours, the mixture was allowed to be filtered using Whatman No. 1 filter paper and the filtrate was allowed to evaporate to dryness under the fume hood. The dried extract was thereafter stored in a dark environment under ambient temperature for future analysis.

2.4. Screening of the Bioactive Compounds within P. ostreatus Extract. Screening of bioactive compounds within the mushroom extract was done using gas column chromatography (GCMS) following a modified method by Daffodil

et al. [20]. Firstly, 3 mg of the extract was dissolved in 10% methanol and thereafter mixed with 90% dichloromethane (DCM) and shaken until homogeneity was achieved. The GCMS analysis of mushroom extract was conducted using the Shimadzu GCMS QP2010 SE. The gas chromatograph was interfaced with a mass spectrometer which was equipped with a Zebron ZB-5MSplus column (30 m × 0.25 mm, 0.25 μm). The GCMS detection was through an electron ionization system that had an ionizing energy of 70 eV. The method introduced helium gas as one of the carrier gases, which was set to be at a constant flow rate (1 ml/min), with an injection volume of 8.00 μl and a split ratio of 10 : 1. The injection temperature was at 250°C and the temperature of the ion source was at 28°C. Oven temperature and time were programmed as follows: 110°C (isothermal for 2 min) and was increased at about 10°C/min to 200°C, thereafter 5°C/min to 280°C, and finally ended with 9 min isothermal which was at 280°C. The mass spectra were acquired with 70 eV ionization energy employed, with a scanning interval of 0.5 seconds, in the mass range of 45 to 450 da. The running time for GC was 36 min in an overall state, whereby the relative amount (%) of each component was calculated by comparing its average peak area to the total area. Firstly, all peak areas were added together to obtain the total area of the peaks; thereafter, to calculate the % of any compound in the mixture, its individual area was divided by the total area of the peaks and multiplied by 100. A database from the National Institute of Standard Technology (NIST) mass spectral database 2010 (v11) [21] was used to interpret the GCMS mass spectrum; hence, the database had more than 62000 patterns within the library.

2.5. The P. ostreatus DPPH Scavenging Activity (Antioxidant Activity). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is said to be a stable nitrogen radical that has an absorption around 517 nm. Antioxidant compounds or extracts must be able to give an electron or a hydrogen atom, which therefore converts DPPH into a more stable and diamagnetic molecule [22]. The antioxidant activity of *P. ostreatus* was evaluated using a modified method from Ayeni et al. [23], whereby mushroom extracts were prepared in numerous concentrations which ranged from 10 to 800 μg/ml. An amount of 500 μl of DPPH (0.1 Mm) was mixed with 1 ml of a mushroom extract, which was then allowed to undergo incubation at room temperature for 30–60 minutes under dark conditions. Afterward, the absorbance of the mixture was read using a UV spectrophotometer set at 517 nm. The lower absorbance observed denoted that the extracts had higher radical scavenging activity and vice versa.

The following formula was used to calculate the antioxidant activity of mushroom extracts: % scavenging activity = (Ac − As)/Ac ∗ 100, where Ac = absorbance of the control and As = absorbance of the sample.

2.6. ABTS Radical Scavenging Assay. The method by Re et al. [24] was followed for evaluating the ABTS radical scavenging activity of *P. ostreatus* mushrooms, whereby 7 mM of ABTS stock solution was mixed with 2.45 mM potassium persulfate. The mixture of ABTS and potassium persulfate was incubated in the dark, at room temperature for a period of 12–16 h before further use. The ABTS solution was thereafter diluted with 5 mM phosphate-buffered saline (pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 730 nm. Afterward, 10 μL of mushroom extract was added into 4 mL of the diluted ABTS solution, then left for 30 min, and thereafter the absorbance was measured.

The ABTS radical-scavenging activity of the *P. ostreatus* mushroom extracts was calculated using the following formula: $S\% = (\text{Acontrol} - \text{Asample})/\text{Acontrol}) \times 100$, where Acontrol represents the absorbance of the control (ABTS solution in the absence of mushroom extracts) and Asample represents the absorbance of the test mushroom extracts. The IC50 values were thereafter calculated for every sample.

2.7. P. ostreatus Reducing Power Assay. The ability of *P. ostreatus* mushrooms to reduce free radicals was evaluated following a method from Ayeni et al. [23]. 2.5 mL of *P. ostreatus* mushroom extract (10–800 μg/ml) and 2.5 mL of 0.2 M phosphate buffer (pH 6.6) were mixed with another 2.5 ml of 1% potassium ferricyanide. The whole mixture was allowed to be incubated at temperatures of 50°C for 20 minutes and thereafter was mixed with 10% trichloroacetic acid. The solution was thereafter centrifuged at 1000 rpm for 10 minutes. Approximately 2.5 ml of the supernatant was immediately pipetted out of the solution and mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1%). The absorbance of the solution was observed at 700 nm, and hence higher absorbance denoted better reducing power of the *P. ostreatus* extracts.

2.8. Antimicrobial Screening Activity. Microorganisms, namely, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Cryptococcus neoformans*, were used to screen for the antimicrobial properties of *P. ostreatus* extract, whereby the microplate dilution assay was utilized for minimum inhibitory concentrations (MICs) [25]. The nutrient broth of about 50 μl was added into 96 micro-titer plates, and then 50 μl of *P. ostreatus* extract (5 mg/ml) that was previously dissolved with 1% DMSO solvent was serially diluted downwards in the rows in 96 micro-titer plates. The microbial cultures were grown at 37°C for 24 hours, and then 50 μl of the microbial cultures was set at 0.5 McFarland standard and inoculated into 96 micro-titer plates which was thereafter incubated overnight at temperatures of 37°C. About 40 μl of p-iodonitrotetrazolium violet (INT) reagent (0.2 mg/ml) was thereafter added into all 96 well micro-titer plates and incubated back for 30 minutes at 37°C. Then, the minimal inhibitory concentration (MIC) was recorded as the lowest concentration of *P. ostreatus* extract that managed to inhibit the microbial growth of the above-mentioned microorganisms.

2.9. In Vitro Plasmodium falciparum Asexual Compound Activity Screening. The Institute for Advanced Medical Research and Training provided the *Plasmodium falciparum*

parasites, which were thereafter stored at 37°C within the human erythrocytes (O+/A+). The human erythrocytes were mixed with the complete culture medium which consisted of RPMI 1640 medium (Sigma-Aldrich) that was supplemented as follows:

(i) 20 mM D-glucose (Sigma-Aldrich)
(ii) 25 mM HEPES (Sigma-Aldrich)
(iii) 200 μM hypoxanthine (Sigma-Aldrich)
(iv) 0.2% sodium bicarbonate
(v) 24 μg/ml gentamicin (Sigma-Aldrich)
(vi) 0.5% AlbuMAX II.

The abovementioned medium was supplemented in a gaseous environment which consisted of 5% O_2, 5% CO_2, and 90% N_2. The *P. ostreatus* mushroom extract of various concentrations was used to treat the in vitro ring stage erythrocytic *P. falciparum* parasite. The treated parasite cultures included the genotypic drug-sensitive strain, namely, NF54 (200 μl at 1% haematocrit, 1% parasitaemia). The assay included the positive control, namely, chloroquine diphosphate (CQ, 1 μM), and the negative control, namely, RPMI media. The parasite was grown in 96 plates and incubated for 96 hours at 37°C under a gaseous environment with 5% O_2, 5% CO_2, and 90% N_2. After an incubation period of 96 hours, 100 μl of *P. falciparum* parasite was mixed with 100 μl of SYBR Green lysis buffer and thereafter incubated at room temperature for a period. The fluorescence was then measured using a GloMax®-Explorer Detection System with Instinct® Software.

The activity of the extracts was classified below to prioritize them for determination of full dose response: good activity (IC50 that was below 10 μg/ml), moderate activity (IC50 that was between 10 and 20 μg/ml), and no/minimal activity (IC50 that was above 20 μg/ml).

2.10. Data Analysis. The experiments were conducted in triplicate to ensure the accuracy of the obtained data. Statistical packages such as SPSS, original 6.0, and GraphPad Prism were used for capturing and analyzing the data. The one-way analysis of variance (ANOVA), which was followed by Tukey–Kramer multiple comparison tests, was a method of analysis used to determine the statistical difference, with values considered statistically significant when ≤ 0.05.

3. Results and Discussion

Amendment of mushroom-growing substrates through the addition of nitrogen and carbohydrate-rich supplements has significant effects on mushroom quality and yield [26]. Thus, controlling the C/N ratio within the compost (substrate) becomes important to obtain profitable mushroom yields [27]. Hence, within the current study, various levels of maize flour supplements were added into mushroom-growing substrates to improve the yield and medicinal properties of *P. ostreatus* mushrooms. The results depicted in Figure 1 indicate that the yield of *Pleurotus* mushroom was influenced by the addition of maize flour supplement, and hence it was noted that 0% MF (no maize flour) had a significantly lower yield when compared to the rest of the treatments for both substrates.

The two substrates (sugarcane top and bagasse) revealed varying yields of mushrooms, with sugarcane substrates producing higher yields for higher supplementation (12% and 18% MF) when compared to lower supplementation (8% and 0% MF). For the bagasse substrates, the yield was significantly higher for all supplement levels when compared to 0% MF (control). Such differences in yield could be attributed to different factors such as growth factors, the presence of carbohydrates that are not complex [28], and the optimal C/N ratio within the growing substrates. Hence, previous researchers such as Alborés et al. [29] have reported that a lower C/N ratio produces mushrooms of higher yield when compared to substrates with a higher C/N ratio. The results in Figure 1 corroborate with the previous findings, and hence it was noteworthy that bagasse substrates had quite higher C/N ratio when compared to sugarcane top that resulted in mushrooms grown from bagasse to have lower yields compared to sugarcane which gave higher yields. The observed results prove that substrates with low C/N ratios turn to support fruit body formation (yield) better than substrates with high C/N ratios as stipulated by Okere et al. [30]. Furthermore, Chang and Miles [31] recommend a C/N ratio of 32-150 as being the most appropriate in the cultivation of *Pleurotus* spp. Other authors such as Cueva et al. [32] observed the best results when the C/N ratio ranged from 37 to 53 for the mushroom strain 768/12. Thus, the results proved that the composition of the substrates probably influences the mushroom C/N ratio and mushroom yield, and hence it was noted in Figure 1(a) that unsupplemented (0%) substrates (both bagasse and sugarcane) had higher C/N ratio compared to the supplemented substrates. Furthermore, the yield in Figure 1(b) was higher for supplemented substrates compared to the unsupplemented substrates (0%), which means adding supplements to substrates promotes the mushroom yield.

Besides influencing yield, the composition of mushroom-growing substrates and other factors such as pH, growing conditions, and genetic factors may influence the metabolic pathways of *P. ostreatus* mushrooms, thus influencing the phenol content of the mushroom [33, 34]. Hence, during cultivation, the majority of the mushrooms turn to produce some of these valuable secondary metabolites such as phenolic compounds which have health beneficial roles that include antimicrobial, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and cardio protective benefits [35, 36]. Based on the findings in Figure 2, it was observed that the cultivated *P. ostreatus* mushrooms had the antioxidant property, as noted that they can scavenge the free radicals (2,2-diphenyl-1- picrylhydrazyl (DPPH)) in a concentration-dependent manner. Amongst the methanolic extracts tested, it was revealed that all mushroom extracts including the control (0% MF) showed to have antioxidant activity, with maximum activity noted at the highest concentrations (250 μg/ml and 500 µg/ml). For the mushrooms grown in bagasse, it was observed that the *P. ostreatus* mushrooms grown on unsupplemented (0%

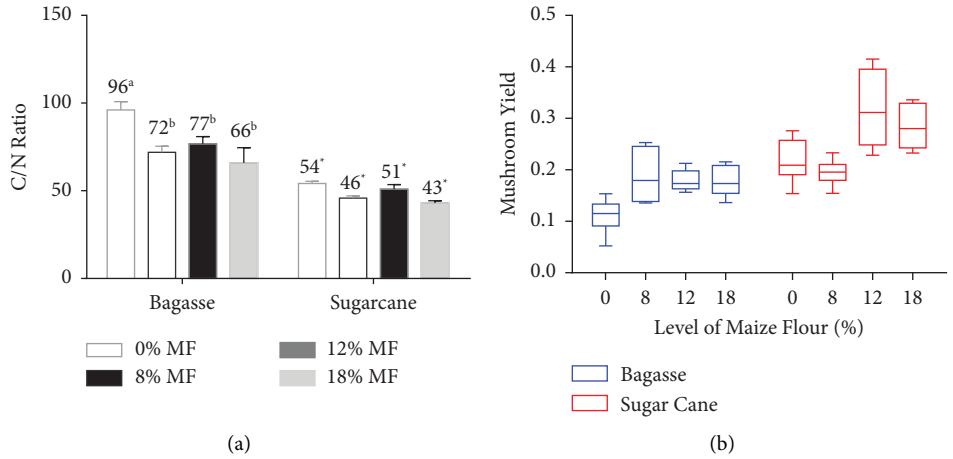

Figure 1: (a) C/N ratio of mushroom-growing substrates, which were supplemented with increasing levels of MF, and (b) the yield of *P. ostreatus* mushroom, which was grown on various supplemented substrates. C/N ratio: carbon-to-nitrogen ratio; MF: maize flour.

MF) bagasse had slightly greater antioxidant activity, especially at lower concentrations when compared to other levels of supplementation. In regard to sugarcane-grown mushrooms, it was also noted that *P. ostreatus* mushrooms grown on 12% MF had slightly greater antioxidant activity, especially at higher concentrations (100–500 μg/ml). The variation in the scavenging of free radicals (DPPH) was probably due to the content of phenolic compounds since the antioxidant activity of the mushroom correlates with the content of phenolics. In general, such findings prove that the locally available substrates (sugarcane and bagasse), which are of low cost, can potentially be used to grow nutraceutical mushrooms with improved antioxidant activity.

Several publications have indicated that most human diseases are caused by the uncontrolled production of reactive oxygen species (ROS) and other free radicals [37], and hence cells are endlessly exposed to a large number of stressful conditions which lead to the generation of free radicals such as $^{\cdot}OH$ and $^{\cdot}O^{2-}$ that cause oxidative damages in biological systems [38]. Hence, free radicals implicate the progression of several health conditions which include diabetes, cancer, atherosclerosis, cardiovascular diseases, and neurodegenerative disorders [39]. However, *Pleurotus* mushrooms are said to be rich in antioxidants that increase the antioxidative capacity of plasma and therefore minimize the risk of the abovementioned diseases [40]. Thus, numerous biochemical assays have been utilized for screening the antioxidant properties of various mushrooms, one of them being the DPPH assay (the most popular and frequently used) [41] and the other being the ABTS assay. The antioxidant properties of *P. ostreatus* mushrooms were further evaluated using ABTS assay (Table 1), and hence the activity of *P. ostreatus* against ABTS radicals was determined using IC50, which corresponded to the concentration of mushroom extracts that were able to scavenge 50% of ABTS. Therefore, a higher IC50 value denotes the lower antioxidant activity of *P. ostreatus* extracts [42], while a lower IC50 value indicates higher antioxidant activity [43]. The results of the study stipulated that *P. ostreatus* mushrooms grown on sugarcane substrates turn out to have lower potency (>2.5 μg/ml) in scavenging ABTS radicals when compared to the *P. ostreatus* mushrooms cultivated on bagasse substrates. It was further observed that the unsupplemented (0% MF) substrates had lower radical scavenging activity (>2.5 μg/ml) when compared to the maize flour-supplemented bagasse substrates. This was probably due to the presence of bioactive compounds within *P. ostreatus* mushrooms, and thus a previous study by Gupta et al. (2018). also specified that the concentration and efficacy of bioactive compounds vary with the type of mushroom, the type of substrate on which the mushroom was grown, fruiting conditions, and storage conditions of mushrooms. The highest ABTS scavenging activity was observed on the *P. ostreatus* mushroom that was cultivated on sugarcane bagasse supplemented with 12% MF (5.6 μg/ml), followed by 8% MF (7.6 μg/ml), which shows that maize flour supplement affected the antioxidant activity of *P. ostreatus* mushroom. This means that the maize flour boosted the phenolic content of mushrooms since previous research indicated that the maximum ABTS scavenging activity is usually associated with the total phenolic content of mushroom extracts, and hence phenols are considered a major antioxidant component [44]. The ABTS radical scavenging activity observed for *P. ostreatus* mushrooms was probably due to the hydrogen-donating ability and chain-breaking ability of *P. ostreatus* mushroom extracts [45], which means *P. ostreatus* mushrooms grown on maize flour supplemented substrates potentially minimize the risk of human diseases.

Amongst the antioxidant activities of any substance, reducing power is also considered as one of the significant indices [46], which depends on the nature of the compound and its property to prevent the propagation of free radicals, which is achieved through the transference of protons into radical species [23]. All mushroom extracts from differently supplemented substrates were evaluated for their ability to have reducing power, and hence their ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) was monitored. The results in Figure 3 revealed that all extracts have the

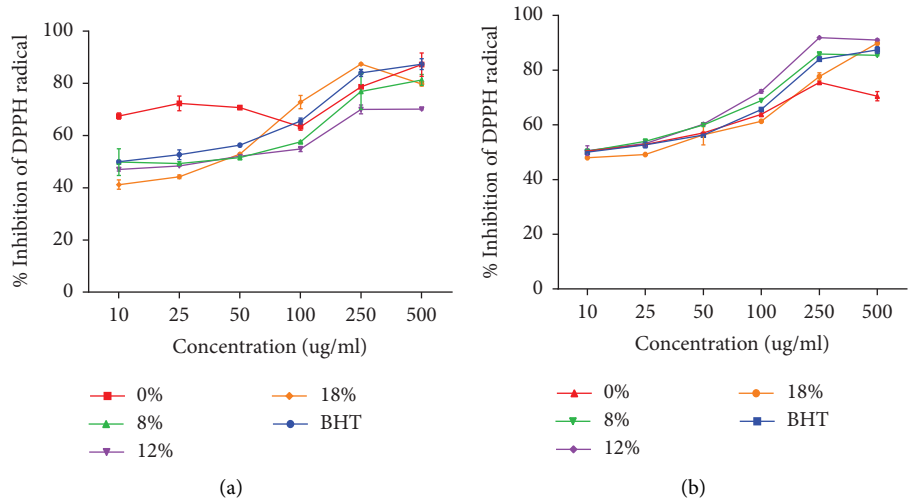

Figure 2: The DPPH radical scavenging activity of *P. ostreatus* mushrooms cultivated on (a) bagasse and (b) sugarcane base substrates supplemented with varying levels of maize flour supplements.

Table 1: Antioxidant activity (ABTS) of methanolic *P. ostreatus* mushroom extract grown on maize flour-supplemented bagasse and sugarcane substrates.

ABTS IC50 (μg/ml): mean IC50 value ± SD		
Maize flour supplement	Bagasse	Sugarcane
0%	>2.5	>2.5
8%	7.6 ± 0.09	>2.5
12%	5.6 ± 0.01	>2.5
18%	44.2 ± 0.01	>2.5
Ascorbic acid	0.32 ± 0.00	

reducing capacity in a concentration-dependent manner, and hence as the concentration increases, the reducing capacity increases. The mushroom extract grown on bagasse substrate supplemented with 18% MF had higher reducing power when compared to other extracts, which might have been due to its content of phenolic and flavonoid compounds which usually play a great role in antioxidant activities [47]. For the mushroom extract grown on sugarcane substrates, it was noteworthy that the unsupplemented substrates (0% MF) had slightly higher reducing power when compared to the supplemented substrates, and this probably meant that the supplements (maize flour) on sugarcane substrate caused some slight decrease in the reducing capacity of the supplemented mushroom extracts. In general, from the results in Figure 3, it could be stipulated that the reducing capacity of the *P. ostreatus* mushroom varies for both substrates and for all supplement levels, and this is an indication that there are some variations in the number of reductones (flavonoids and phenolics) that contribute to the reducing ability (antioxidant) [48], and hence the observed findings are also in line with Mkhize et al. [5] who also observed that the unsupplemented substrates demonstrated higher reducing capacity compared to the supplemented base substrates, which was the case in the current study for the *P. ostreatus* grown on sugarcane base substrates.

Besides the antioxidant activity, it has been documented that several mushroom extracts also possess antimicrobial activity [49], which is due to unique compounds such as alkaloids, peptides, flavonoids, tannins, terpenoids, proteins, and anthraquinones [50]. The study revealed that *P. ostreatus* mushrooms which were grown on sugarcane tops and sugarcane bagasse have antimicrobial activity (0.08-2.5 mg/ml) against various microorganisms; however, the observed activities were noted to vary depending on the type of microorganisms and the level of maize flour supplementation used for substrate supplementation. Hence, it was noted in Table 2 that *P. ostreatus* mushrooms grown in sugarcane top substrates had weaker activity (MIC > 0.625 mg/ml) against *E. coli* microorganisms and *C. neoformans* but had improved or moderate activity (0.1 < MIC < 0.625 mg/ml) and good activity (MIC < 0.625 mg/ml) against *S. aureus* bacteria. Such differences in susceptibility for different microorganisms might be because some microorganisms such as *E. coli*, *C. albicans*, and *C. neoformans* are considered Gram-negative, while other microorganisms such as *S. aureus* are said to be Gram-positive. Thus, the Gram-negative bacteria are known to consist of a cell wall that has an outer membrane made of lipopolysaccharides, which potentially prevents substances from invading the cell wall [51]. The better inhibition of the Gram-positive *S. aureus* bacteria compared to the Gram-negative bacteria corroborates with the findings of Gezer et al. [52] who achieved a similar trend for edible mushrooms found in Turkey. It was further noted from Table 2 that supplementation of sugarcane substrates with MF supplement influenced the susceptibility of certain microorganisms, and higher supplementation such as 12% MF and 18% MF resulted in increased *S. aureus* susceptibility when compared to lower supplements (0% MF and 8% MF). Thus, moderate activity (0.3 mg/ml and 0.16 mg/ml) was observed at lower levels of MF supplementation, whereas good activity (0.08 mg/ml) was observed at higher levels of MF supplementation. Such

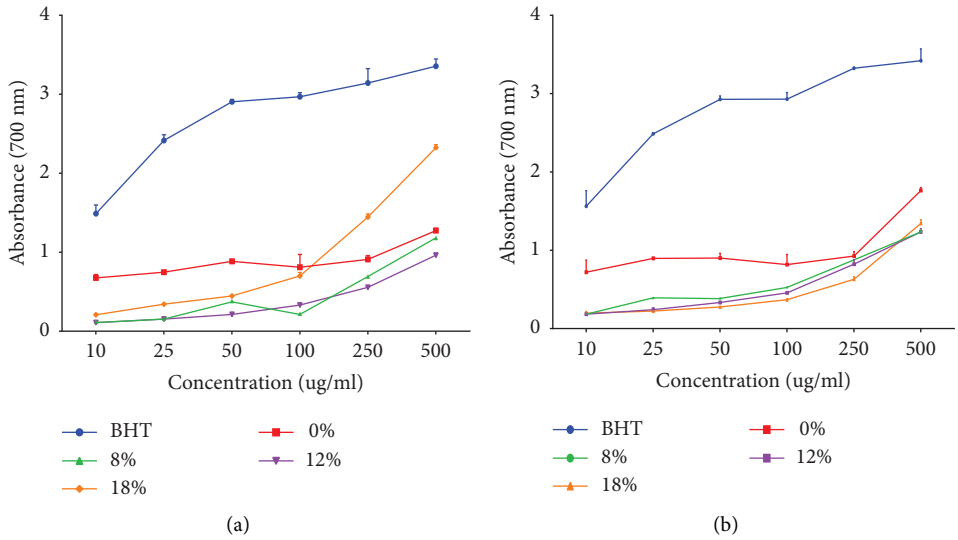

Figure 3: Reducing power of *P. ostreatus* grown on (a) bagasse and (b) sugarcane substrates supplemented with maize flour supplement.

Table 2: Minimum inhibitory concentration (MIC) of methanol extract of *P. ostreatus* mushrooms grown on sugarcane substrates supplemented with varying levels of maize flour.

MF supplement level	Test organisms			
	E. coli	*S. aureus*	*C. albicans*	*C. neoformans*
0%	2.50	0.31	1.25	2.5
8%	2.5	0.16	2.5	2.5
12%	2.5	0.08	0.63	1.25
18%	2.5	0.08	1.25	1.25
Control drugs				
Vancomycin	0.002	0.001	—	—
Streptomycin	0.025	0.013	—	—
Amphotericin	—	—	0.012	0.004

Table 3: Minimum inhibitory concentration (MIC) of methanol extract of *P. ostreatus* mushrooms grown on bagasse substrates supplemented with varying levels of maize flour.

MF supplement level	Test organisms			
	E. coli	*S. aureus*	*C. albicans*	*C. neoformans*
0%	2.5	0.63	2.5	1.25
8%	2.5	0.63	0.31	2.5
12%	0.16	0.63	2.5	2.5
18%	0.31	1.25	1.25	2.5
Control drugs				
Vancomycin	0.002	0.001	—	—
Streptomycin	0.025	0.013	—	—
Amphotericin	—	—	0.012	0.004

variation in antimicrobial activity might be probably due to the type and amount of bioactive compounds which vary with environmental conditions such as soil nutrients, precipitation, and temperature, which influence the content of secondary metabolites and biological activities of the plants [53]. Hence, Kim et al. [54] also testified that the high inhibition observed for RD mushroom extract was due to high phenolic compounds.

Table 4: The *in vitro* antimalarial activity of *P. ostreatus* mushrooms cultivated on sugarcane substrates with varying levels of maize flour supplement (at 20 and 10 μg/ml).

Dual screening of mushroom extracts		
	Asexual parasites, SYBR Green	
MF supplement level	% inhibition ± SD	
	20 μg/ml	10 μg/ml
0%	0.00 ± 7.27	2.74 ± 1.97
8%	3.71 ± 8.93	6.58 ± 1.58
12%	1.45 ± 4.87	5.11 ± 1.17
18%	7.04 ± 4.16	4.55 ± 2.20
CQ (1 μM)	100	100

Table 5: The *in vitro* antimalarial activity of *P. ostreatus* mushrooms cultivated on bagasse substrates with varying levels of maize flour supplement (at 20 and 10 μg/ml).

Dual screening of mushroom extracts		
	Asexual parasites, SYBR Green	
MF supplement level	% inhibition ± SD	
	20 μg/ml	10 μg/ml
0%	0.00 ± 10.59	0.00 ± 4.10
8%	0.00 ± 8.46	0.00 ± 2.18
12%	3.83 ± 6.40	3.02 ± 4.74
18%	0.00 ± 3.37	0.00 ± 4.63
CQ (1 μM)	100	100

Furthermore, the results in Table 3 also proved that supplementation of bagasse substrates with MF caused a major influence on the susceptibility of different microorganisms towards mushrooms grown on varying levels of supplemented bagasse substrates. For example, *E. coli* was less or weakly sensitive to lower levels of MF (0% and 8% MF), whereby at higher levels of MF supplementation (12% and 18% MF), moderate susceptibility of *E. coli* was observed. However, unsupplemented and low supplementation (0%, 8%, and 12%

Table 6: GCMS profiling of methanolic extracts of *P. ostreatus* mushroom grown on bagasse substrates supplemented with varying levels of maize flour supplement.

MF supplement level (%)	Peak	RT (min)	Compound	Molecular formula	Area %	Height %	Mol wt
0	1	26.553	4-Pyrimidinecarboxylic acid, 2,6-bis[(tert-butyldimethylsilyl)oxy]	$C_{23}H_{46}N_2O_4Si_3$	12.38	12.62	498
	2	27.675	1,1,3,3-Tetraallyl-1,3-disilacyclobutane	$C_{14}H_{24}Si_2$	7.30	5.36	248
	3	28.701	Octadecanoic acid, 7-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	8.57	6.90	314
	4	29.336	Dimethylmalonic acid, dodecyl pentafluorophenyl ester	$C_{23}H_{31}F_5O_4$	8.94	7.58	466
	5	30.770	gamma.-Tocopherol	$C_{28}H_{48}O_2$	49.87	44.31	416
	6	30.885	Ginsenol	$C_{15}H_{26}O$	6.94	14.33	222
	7	32.372	alpha.-Tocopherol-.beta.-D-mannoside	$C_{35}H_{60}O_7$	5.99	8.90	592
8	1	4.449	Succinimide	$C_4H_5NO_2$	0.13	0.18	99
	2	4.721	Octanoic acid	$C_8H_{16}O_2$	0.02	0.05	144
	14	10.994	4-Trifluoroacetoxytridecane	$C_{15}H_{27}F_3O_2$	0.02	0.06	296
	16	11.875	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.11	0.25	228
	17	12.155	n-Nonadecanol-1	$C_{19}H_{40}O$	0.01	0.05	284
	18	12.543	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	0.02	0.06	256
	23	13.827	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.12	0.31	270
	25	14.170	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	0.14	0.20	240
	27	14.320	Dibutyl phthalate	$C_{16}H_{22}O_4$	0.26	0.48	278
	28	14.658	Pentadecanoic acid	$C_{15}H_{30}O_2$	13.49	7.94	242
	33	15.345	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	0.01	0.03	266
	35	15.523	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	0.05	0.09	310
	36	15.635	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	0.08	0.14	310
	39	16.256	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	0.15	0.34	296
	41	16.490	Eicosane	$C_{20}H_{42}$	0.01	0.03	282
	42	16.628	Methyl stearate	$C_{19}H_{38}O_2$	0.02	0.06	298
	44	17.157	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	7.61	10.60	280
	47	17.430	Palmitoleic acid	$C_{16}H_{30}O_2$	5.96	14.0	254
	48	17.568	Octadecanoic acid	$C_{18}H_{36}O_2$	2.74	3.32	284
	50	17.810	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	17.810	1.19	294
	54	18.086	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	0.59	0.45	280
	64	18.780	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentyl	$C_{25}H_{42}O_2$	0.04	0.07	374
	69	19.510	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	0.02	0.02	266
	77	20.269	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	0.05	0.09	281
	86	22.651	Diisooctyl phthalate	$C_{24}H_{38}O_4$	0.15	0.30	390
	87	22.895	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	$C_{20}H_{38}O_2$	0.01	0.02	310
	91	23.838	cis-9,10-Epoxyoctadecan-1-ol	$C_{18}H_{36}O_2$	0.01	0.02	284
	126	34.330	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	$C_{28}H_{46}O$	0.10	0.08	398
	129	35.041	7,22-Ergostadienone	$C_{28}H_{44}O$	0.04	0.06	396
	131	35.830	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	$C_{30}H_{50}O$	0.31	0.24	426
12	1	26.330	Ethanol, 2-[2-[2-[2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy] ethoxy]ethoxy	$C_{22}H_{38}O_5$	24.38	14.19	382
	2	26.555	Silane, dimethyl(docosyloxy)butoxy-	$C_{28}H_{60}O_2Si$	24.38	27.30	456
	3	30.801	N-(6,7,9,10,12,13,15,16-Octahydro-5,8,11,14	$C_{16}H_{23}NO_6$	27.54	31.25	592
	4	32.395	alpha.-Tocopheryl acetate	$C_{31}H_{52}O_3$	7.49	14.56	472
	5	34.245	1-Propanol, 3-(octadecyloxy)-	$C_{21}H_{44}O_2$	15.74	12.69	328

Table 6: Continued.

MF supplement level (%)	Peak	RT (min)	Compound	Molecular formula	Area %	Height %	Mol wt
18	2	11.887	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.11	0.26	228
	4	13.130	Pentadecanoic acid	$C_{15}H_{30}O_2$	2.65	4.69	242
	5	13.839	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.13	0.32	270
	7	14.328	Dibutyl phthalate	$C_{16}H_{22}O_4$	0.12	0.26	278
	11	16.170	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	2.94	6.71	294
	12	16.260	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.65	1.48	296
	13	16.361	Dihydroqinghaosu	$C_{15}H_{24}O_5$	0.14	0.23	284
	14	16.624	Methyl stearate	$C_{19}H_{38}O_2$	0.16	0.40	298
	17	17.324	Octadecanoic acid	$C_{18}H_{36}O_2$	2.38	2.69	284
	23	18.215	Ethyl 9,12-hexadecadienoate	$C_{18}H_{32}O_2$	0.19	0.29	280
	33	21.724	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	0.23	0.39	224
	37	22.630	Diisooctyl phthalate	$C_{24}H_{38}O_4$	0.23	0.45	390
	38	23.399	4,22-Stigmastadiene-3-one	$C_{29}H_{46}O$	0.11	0.22	410
	55	34.995	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	0.15	0.16	326

Table 7: GCMS profiling of methanolic extracts of *P. ostreatus* mushroom grown on sugarcane substrates supplemented with varying levels of maize flour supplement.

MF supplement level (%)	Peak	RT (min)	Compound	Molecular formula	Area %	Height %	Mol wt
0	1	12.894	Phytol, acetate	$C_{22}H_{42}O_2$	0.75	1.30	338
	2	12.990	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	0.38	0.57	268
	3	13.911	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl	$C_{18}H_{30}O$	0.39	0.68	262
	4	16.405	Cyclopentadecanone	$C_{15}H_{28}O$	0.45	1.03	224
	5	22.868	Pentadecanal-	$C_{15}H_{28}O$	0.43	0.86	226
	6	25.160	cis-11,14-Eicosadienoic acid, tert	$C_{26}H_{50}O_2Si$	0.42	0.32	422
	7	25.380	2-Isoamyl-6-methylpyrazine	$C_{10}H_{16}N_2$	6.59	7.61	164
	8	25.485	p-Cresyl isovalerate	$C_{12}H_{16}O_2$	2.82	2.27	192
	9	26.022	Butyldimethylsilyl	$C_{19}H_{36}O$	1.18	1.77	280
	10	26.783	8-Nonene-2,4-diol, 8-methyl-, (R∗,S∗)-	$C_{10}H_{20}O_2$	1.09	1.05	172
	11	28.844	2-Methyl-Z,Z-3,13-octadecadienol	$C_{27}H_{46}O_2$	0.85	1.13	402
	12	29.261	(Phenylthio)acetic acid, tridec-2-ynyl ester	$C_{21}H_{30}O_2S$	1.58	1.65	346
	13	30.417	.beta.-Tocopherol	$C_{28}H_{48}O_2$	8.93	9.33	416
	14	30.759	.gamma.-Tocopherol	$C_{28}H_{48}O_2$	54.94	53.50	416
	15	31.964	Hexahydropyrimidine-2,4,6-trione, 5-ethyl-1-methyl	$C_{27}H_{34}N_4O_3$	0.93	1.03	462
	16	32.388	Vitamin E	$C_{29}H_{50}O_2$	13.20	11.74	430
	17	33.716	Ethyl-3.alpha.,5.alpha.-cyclocholest-22(E)-en-6-one Octadecanoic acid, 7-hydroxy-, methyl ester	$C_{29}H_{46}O$	1.66	1.47	410
	18	35.328	Cholest-6-one, 3-chloro- 3-chloro-17-(1,5-dimethylhexyl)-10,13-dimethylhexadecahydrocyclopenta [a]phenanthren-6-one $$ 3-Chlorocholes	$C_{27}H_{45}C_lO$	3.42	2.68	420
8	1	6.581	2,4-Decadienal	$C_{10}H_{16}O$	0.02	0.07	152
	2	9.690	Tridecanoic acid, 12-oxo-	$C_{13}H_{24}O_3$	0.01	0.04	228
	3	9.829	Trifluoroacetic acid,n-tridecyl ester	$C_{15}H_{27}F_3O_2$	0.03	0.12	296
	6	12.162	n-Nonadecanol-1	$C_{19}H_{40}O$	0.03	0.13	284
	7	13.175	Pentadecanoic acid	$C_{15}H_{30}O_2$	2.81	5.42	242
	8	13.731	2-Dodecen-1-yl(-)succinic anhydride	$C_{16}H_{26}O_3$	0.01	0.05	266
	11	14.656	Pentadecanoic acid	$C_{15}H_{30}O_2$	17.39	15.40	242
	13	15.515	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	0.06	0.15	268
	9	14.177	Palmitoleic acid	$C_{16}H_{30}O_2$	0.19	0.31	254
	15	15.814	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	0.05	0.12	652
	17	16.168	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.10	0.41	294
	18	16.260	8-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.03	0.13	296
	20	16.615	Methyl stearate	$C_{19}H_{38}O_2$	0.01	0.03	298
	21	17.311	Ethyl 9,12-hexadecadienoate	$C_{18}H_{32}O_2$	58.53	31.17	280
	23	17.511	Octadecanoic acid	$C_{18}H_{36}O_2$	2.48	4.80	284
	24	17.620	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl	$C_{21}H_{36}O_4$	0.54	1.23	352
	25	17.754	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	1.32	1.66	294
	32	18.297	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	1.01	1.36	264
	37	19.189	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl	$C_{35}H_{68}O_5$	0.03	0.09	568
	42	20.251	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	0.06	0.14	281
	44	21.744	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{35}NO$	0.15	0.46	281
	47	22.643	Diisooctyl phthalate	$C_{24}H_{38}O_4$	0.08	0.25	390
	49	22.965	Decanedioic acid	$C_{10}H_{18}O_4$	0.01	0.04	202
	50	23.396	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.	$C_{28}H_{48}O_4$	0.04	0.12	448
	57	28.148	15,17,19,21-Hexatriacontatetrayne	$C_{36}H_{58}$	0.04	0.11	490
12	1	26.330	Ethanol, 2-[2-[2-[2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy] ethoxy]ethoxy	$C_{22}H_{38}O_5$	24.38	14.19	382
	2	26.555	Silane, dimethyl(docosyloxy)butoxy-	$C_{28}H_{60}O_2Si$	24.38	27.30	456
	3	30.801	N-(6,7,9,10,12,13,15,16-Octahydro-5,8,11,14	$C_{16}H_{23}NO_6$	27.54	31.25	592
	4	32.395	alpha.-Tocopheryl acetate	$C_{31}H_{52}O_3$	7.49	14.56	472
	5	34.245	1-Propanol, 3-(octadecyloxy)-	$C_{21}H_{44}O_2$	15.74	12.69	328

Table 7: Continued.

MF supplement level (%)	Peak	RT (min)	Compound	Molecular formula	Area %	Height %	Mol wt
	1	4.727	Octanoic acid	$C_8H_{16}O_2$	0.10	0.30	144
	2	4.818	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	0.06	0.17	102
	3	4.880	Benzoic acid	$C_7H_6O_2$	0.06	0.11	122
	5	5.888	Nonanoic acid	$C_9H_{18}O_2$	0.12	0.28	158
	6	6.242	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	0.06	0.19	152
	7	6.562	2,4-Decadienal	$C_{10}H_{16}O$	0.09	0.25	152
	8	7.120	Decanoic acid, silver(1+) salt	$C_{10}H_{19}AgO_2$	0.02	0.07	278
	10	9.575	Dodecanoic acid	$C_{12}H_{24}O_2$	0.04	0.09	200
	12	9.782	Fumaric acid, ethyl 2-methylallyl ester	$C_{10}H_{14}O_4$	0.06	0.20	198
	13	9.821	n-Pentadecanol	$C_{15}H_{32}O$	0.07	0.26	228
	14	9.855	Diethyl phthalate	$C_{12}H_{14}O_4$	0.04	0.14	222
	16	11.883	Tetradecanoic acid	$C_{14}H_{28}O_2$	0.30	0.92	228
	17	12.155	Z-5-Nonadecene	$C_{19}H_{38}$	0.04	0.18	266
	18	12.626	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.01	0.04	242
	19	13.211	Pentadecanoic acid	$C_{15}H_{30}O_2$	4.51	6.68	242
	21	13.830	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.04	0.15	270
	25	14.322	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	0.44	0.83	278
	30	15.420	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	0.01	0.03	436
18	31	15.530	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	0.07	0.16	268
	36	16.258	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.06	0.20	296
	37	17.280	Ethyl 9,12-hexadecadienoate	$C_{18}H_{32}O_2$	49.82	25.51	280
	38	17.330	14-Pentadecenoic acid	$C_{15}H_{28}O_2$	5.94	18.75	240
	39	17.476	Octadecanoic acid	$C_{18}H_{36}O_2$	1.54	3.21	284
	44	17.808	9-Octadecyne	$C_{18}H_{34}$	0.41	0.61	250
	57	20.167	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	0.24	0.60	294
	58	20.246	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	0.08	0.20	281
	59	20.886	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.06	0.18	322
	63	21.738	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	0.09	0.22	298
	64	21.830	cisZ-11,12-Epoxytetradecan-1-ol	$C_{14}H_{28}O_2$	0.02	0.04	228
	66	22.641	Diisooctyl phthalate	$C_{24}H_{38}O_4$	0.09	0.26	390
	67	22.883	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	0.03	0.10	294
	70	23.836	Isopropyl linoleate	$C_{21}H_{38}O_2$	0.02	0.08	322
	72	24.626	Rhodopin	$C_{40}H_{58}O$	0.19	0.35	554
	77	25.666	8-Hexadecenal, 14-methyl-, (Z)-	$C_{30}H_{50}$	0.04	0.08	410
	79	26.609	Squalene	$C_{30}H_{50}$	0.02	0.06	410
	85	28.880	9(11)-Dehydroergosteryl benzoate	$C_{35}H_{46}O_2$	0.98	1.58	498
	122	37.993	Globulol	$C_{15}H_{26}O$	0.05	0.09	222

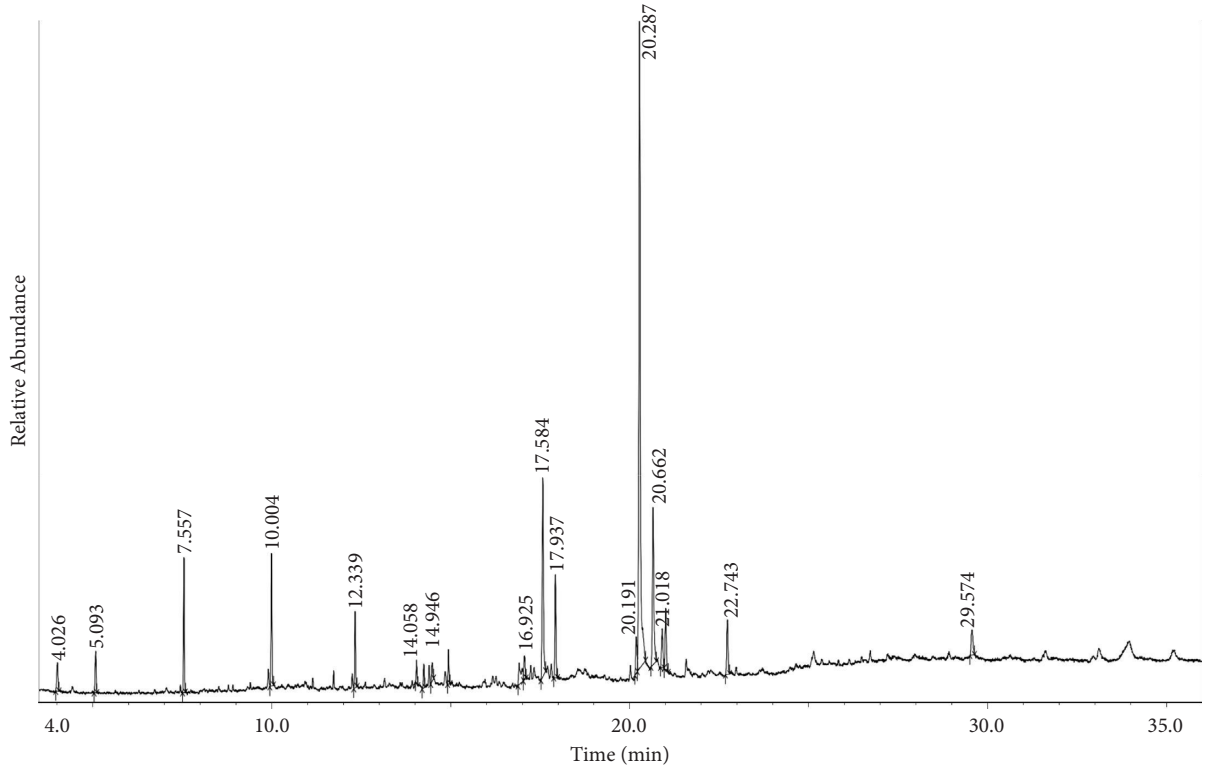

Figure 4: GCMS chromatogram of methanolic extract of *P. ostreatus* mushroom cultivated on sugarcane substrates supplemented with maize flour supplement.

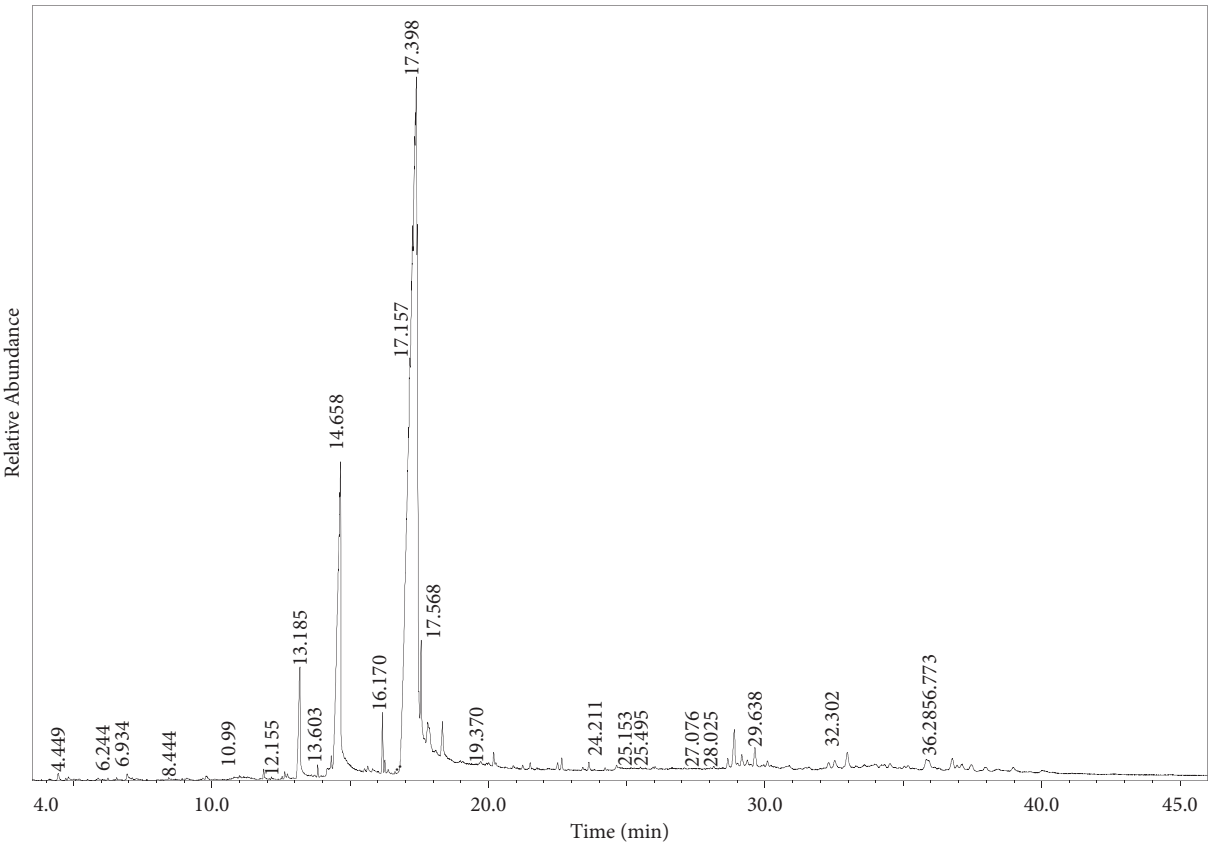

Figure 5: GCMS chromatogram of methanolic extract of *P. ostreatus* mushroom cultivated on bagasse substrates supplemented with maize flour supplement.

MF) had moderate activity towards *S. aureus* when compared to 18% MF. It was also noted that *P. ostreatus* extracts have lower activity towards *C. albicans* and *C. neoformans*. Such variations in antimicrobial activity could potentially be due to variations in the concentrations of bioactive metabolites such as phenolics and flavonoids, which are known to have strong biological activity [55]. The antimalarial potential of mushroom extracts was evaluated in the current study; thus, it was worth noting that all mushroom extracts had weak anti-plasmodium activity (Tables 4 and 5), which contradicts to the literature that stated that mushrooms possess antimalarial activity [14]. Previous researchers have obtained possible antimalarial activity of various mushrooms such as *Pleurotus ostreatus* [56], *G. lucidum,* and *T. pfeilii* [57]. The possible reason for such weak antimalarial activity observed in the current study could be attributed to the fact that cultivated edible mushrooms are known to have lower concentrations of secondary metabolites [58], since the mushroom-growing substrates may not necessarily provide all nutrients required by the mushrooms [59].

The abovementioned biological activities (DPPH, ABTS, reducing power, and antimicrobial) of *P. ostreatus* mushroom could be due to the types of compounds present within the extracts. Hence, Tables 6 and 7 and the chromatogram in Figures 4 and 5 prove that all *P. ostreatus* extracts had varying biological active compounds of known activity. These compounds were selected since they had known activities such as antioxidant and antimicrobial. Hence, GCMS results in Tables 6 and 7 proved that various levels of MF supplementation result in variations in biologically active compounds within the mushroom. However, some compounds such as pentadecanoic acid; 9,12-octadecadienoic acid methyl ester; pentadecanoic acid methyl ester; octadecanoic acid; and diisooctyl phthalate were found to be the most common ones amongst certain supplement levels. The majority of these compounds such as pentadecanoic acid, benzoic acid, Z-5-nonadecene, and dihydroqinghaosu are well known to have antimicrobial activity [60] and some antimalarial activity [61]. Furthermore, the antioxidant activity of some of these compounds such as 9-octadecenoic acid methyl ester [62], 9,12-octadecadienoic acid, methyl ester [63], and squalene [64] have been reported by various authors.

Hence, this proves that the type of supplement used on the growing substrates has a major influence on the type of compounds present within the mushroom, which could potentially influence the biological activity of mushrooms. However, it could be recommended that the bioactive compounds from these mushroom extracts should be purified, isolated, and characterized based on their biological activity, which could be either in vitro or in vivo. It could prove to be useful in filling the gap of using synthetic compounds for medicinal purposes since synthetic antioxidants such as BHT have proven to cause chronic cytotoxicity at high concentrations [65].

4. Conclusion

The study revealed that maize flour supplements promote mushroom yield and better C/N ratio, which are optimal for the growth and yield of *P. ostreatus* mushrooms. The observed biological activities of the mushroom extracts were linked to the level of maize flour supplement used upon growing substrates. Hence, it could be concluded that supplementing the mushroom-growing substrates with maize flour supplements potentially influences the biological activities of the mushrooms, such as antioxidant and antimicrobial activity, probably through promoting variations in bioactive metabolites found in mushrooms. However, a correlation study needs to be further conducted to fully understand the abovementioned phenomenon. The observed activities were potentially contributed by a variety of compounds such as phenolics, flavonoids, vitamin E, and other compounds which were synthesized by various mushroom extracts. Some of these compounds such as pentadecanoic acid, benzoic acid, Z-5-nonadecene, dihydroqinghaosu, 9-octadecenoic acid, methyl ester, 9,12-octadecadienoic acid, methyl ester, and squalene, to name a few, had known biological activities. Hence, it can be recommended for future studies that some of the natural compounds, especially the ones with known antimicrobial, antimalarial, and antioxidant activity, should be isolated and purified, which would greatly minimize the use of synthetic compounds such as butylated hydroxytoluene (BHT) that have great toxicity within the human body. Furthermore, the aspects of gene-encoding enzymes that synthesize a plethora of potential secondary metabolites should be explored to gain more bioactive compounds which could potentially be novel in fighting against pandemic diseases such as cancer, diabetes, malaria, and sexually transmitted diseases to name a few. Furthermore, future studies should focus on the activation of silent genes through various strategies such as the one-strain compound approach, which could also promote novel natural new compounds that have various biological activities.

Data Availability

The study data in this paper come from the image classification datasets of the Kaggle platform and Baidu AI Studio platform: Large Scale Fish dataset (https://www.kaggle.com/crowww/a-large-scale-fish-dataset); Weather dataset (https://aistudio.baidu.com/aistudio/datasetdetail/13165); Flowers Recognition dataset (https://www.kaggle.com/alxmamaev/flowers-recognition); and Fruits 360 dataset (https://www.kaggle.com/moltean/fruits).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to acknowledge the National Research Foundation (NRF) of South Africa for funding this research (grant no. 138414 to MBC and grant nos. 112980 and 145396 to OJP). Furthermore, the authors acknowledge the South African Medical Research Council through its Division of Research Capacity Development under the Early Investigators Programme awarded to OJP.

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