

## Phytochemical constituents and antimicrobial evaluation of ethanolic extracts from *Pleurotus* spp. cultivated on substrate fortified with selenium

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### Abstract

Edible mushrooms have the ability to bio-accumulate minerals from the growth medium into the fruiting body. Selenium (Se) is an example of minerals that can be easily absorbed by mushroom. This study investigated the phytochemical constituents and antimicrobial properties of *Pleurotus* spp. fortified with selenium. *Pleurotus* spp. (fortified with selenium and non-fortified with selenium) were cultivated on corn cobs using standard procedures. The yield, biological efficiency, selenium absorption level, phytochemical constituents and antimicrobial properties were determined using standard methods. Non-fortified *Pleurotus pulmonarius* recorded the highest yield and biological efficiency, while fortified *Pleurotus ostreatus* had the lowest yields and biological efficiency. There was no significant difference ( $P < 0.05$ ) in the selenium concentration absorbed in *Pleurotus ostreatus* (51.79 µg/g) and *Pleurotus pulmonarius* (59.29 µg/g) but no selenium was detected in the non-fortified samples. The presence of 5 phytochemicals viz; saponins, alkaloids, phenols, tannins and flavonoids were detected in cultivated mushrooms. Saponin was highest (7.08 mg/g) in non-fortified *Pleurotus pulmonarius* while tannin was the least with the value of 0.02 mg/g in fortified *Pleurotus ostreatus*. The ethanolic extracts of *Pleurotus* spp. inhibited the growth of Gram positive and negative bacteria with *Pseudomonas aeruginosa* been the most susceptible (25.00 mm). The ethanolic extracts of fortified *Pleurotus* spp. inhibited *Aspergillus flavus*, *Candida albicans* and *Candida parapsilosis*. The non-fortified extracts however, had no inhibitory effects on the tested fungi. Based on the findings of this study, it can be concluded that Se-fortification increased the antibacterial activities of *Pleurotus* spp. These results therefore, suggest that Se-enriched mushrooms can be used as potential source of dietary supplements and nutraceuticals. Fortification of *Pleurotus* spp. with essential metals should be encouraged as this can improve its health promoting property.

**Key words** –Antimicrobial, phytochemical, *Pleurotus* species, selenium.

### Introduction

*Pleurotus* spp. is among the edible mushrooms consumed in Nigeria. It is used as spice in vegetable soups and also fried to serve as meat (Iwalokun *et al.* 2007). Taxa of the *Pleurotus* genus have an important place among the commercially employed basidiomycetes because they

have gastronomic, nutritional and medicinal properties. They can be easily cultivated on a wide range of substrates (Pauliuc and Botău 2013). Apart from their nutritional potentials, mushrooms are also sources of bioactive substances that possess health benefits (Oyetayo *et al.* 2009). These metabolites have been used as antimicrobials with fewer side effects and are a prolific resource for drugs because of their antitumor, antibacterial, antifungal and hypercholesterolemia activities (Selima 2012). Phenolic and tannin constituents of *P. ostreatus* may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesins (Filipic *et al.* 2002).

The yield and chemical composition of mushrooms depend on the substrate used for their cultivation (Shashirekha *et al.* 2005). The yield and chemical composition can be enhanced by adding micronutrients such as Selenium (Se) to the substrate (Zhao *et al.* 2004). Se is an essential micronutrient required for the biosynthesis of biologically significant selenoenzymes and selenoproteins. Its optimal intake could potentially prevent various types of cancer and diseases like diabetes, age-related immunosuppression and even problems related to fertility (Sharma *et al.* 2018). Se deficiency often occur in patients with severely compromised intestinal function, those undergoing parenteral nutrition, those who have had gastro intestinal bypass surgery persons of advanced age and HIV patients (Ravaglia *et al.* 2000). Se deficiency leads to infertility in men and women, muscle weakness, fatigue, mental fog, hair loss and weakened immune system (Olsen 2018). Life threatening diseases such as cancer, cardiovascular disease, susceptibility to viral infections, asthma, Kashan disease, thyroid dysfunction and osteoarthritis can also occur as a result of deficiency of Se in human blood (Papp *et al.* 2010). Considering the importance of Se in human, it can be incorporated into human diet through the process of bio-fortification. Due to mushrooms' ability to absorb inorganic Se forms and convert them to bioactive cell compounds, they could be used as food and dietary supplements and this represent an effective way to improve health (Milovanović *et al.* 2013).

Keeping in view the health benefits of mushrooms and selenium, this study was conducted to determine the effects of selenium-biofortification on the phytochemical constituents and antimicrobial activities of two *Pleurotus* spp. (*P. ostreatus* and *P. pulmonarius*)

## **Materials and Methods**

### **Collection of Corn Cobs**

The corn cobs used as substrates for this research work were procured at the King's market, Akure, Ondo State.

### **Source of *Pleurotus* spp. spawn**

Spawns of *Pleurotus ostreatus* and *Pleurotus pulmonarius* were obtained from Federal Institute of Industrial Research (FIRO), Oshodi Lagos, Nigeria. The spawns were taken to the Department of Microbiology Federal University of Technology, Akure for confirmation.

### **Test microorganisms**

All tested bacteria and fungi were obtained from the stock cultures maintained in the Microbiology Laboratory, Federal Medical Centre (FMC), Owo, Ondo State. The cultures were maintained on slants of Nutrient Agar and Potato Dextrose Agar and kept at 4 °C until when required for use. A total of 11 bacteria and 4 fungi were used. They include: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus cereus*, *Enterobacter aerogenes*, *Salmonella dysenteriae*, *Serratia marcescens*, *Streptococcus viridans*, *Streptococcus oralis*, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Candida parapsilosis*.

### **Substrate preparation**

Corn cobs were sundried and milled into smaller pieces using mortar and pestle and further pulverized into fine powdered using commercial milling machine. The substrate was moistened with water to a concentration of 60%. Approximately 1000 g of the substrate was packed into polypropylene bags and sealed with paper with the aid of polyvinyl rings. The bags were then autoclaved for 2 hours at 121°C

### **Mushroom preparation**

The substrates were allowed to cool down and then inoculated with 30g of *Pleurotus* spawn. Sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>) of 50mg/Kg was injected into each package. A control treatment with no sodium selenium was also prepared. The bags were then kept in spawn room with 75% relative humidity until formation of primordia. The bags were then uncapped and transferred to the fruiting room to allow the normal development of the fruit bodies. The bags were kept in bed position on the rack.

### **Harvesting of basidiomata**

As soon as the fruiting bodies developed and attained their full size, they were harvested by twisting to uproot from the base. The harvesting was done in three flushes of 10 days intervals.

### **Calculation of total yield**

Mean fresh weight of the flushes of each bag was calculated for total yield. The whole cluster of fruiting bodies was considered for biological yield measurement. Biological efficiency (B.E) was calculated according to Ahmed et al. (2013) as follows :

$$B.E = \frac{\text{grams of fresh weight of mushroom}}{\text{dry weight of substrate}} \times 100$$

### **Preparation of mushroom extracts**

The mushroom samples were cut into small pieces and air dried. The dried sample was ground into fine powder. Mushroom (250g) was soaked in 1000 ml of ethanol and kept for three days with occasional shaking. Each portion was then filtered using Whatman filter paper No 1. The filtrates were evaporated to dryness and the extracts (residues) were stored at 4 °C in a sterile container for further use.

### **Determination of Selenium Concentration in Cultivated *Pleurotus* spp.**

The digestion of the dried mushroom fruit bodies was carried out by mixing the 0.1 g of sample with 8 ml of 65 % HNO<sub>3</sub>, suprapur (Sigma Aldrich, St. Louis, MO, USA). The prepared sample was then digested under a closed-vessel microwave digester (Anton Paar India Pvt. Ltd., India) at a high temperature (180 °C) with maximum pressure increase rate (0.5 bar/sec) and power (1200 W) for 30 minutes. After digestion, the solution obtained was filtered using a Millipore 45 mm filters (qualitative filter papers, Grade 595: 4-7 mm, Whatman, UK) and was made up to a final volume of 50 ml using deionised water (Milli-Q Advanced A10 Water Purification Systems, Merck, Darmstadt, Germany). The total Se content was determined using an inductively coupled plasma mass spectrometry (Agilent ICP-MS 7900, Agilent Technologies, Santa Clara, CA, USA). The commercial Se standard (Sigma Aldrich) in 1 g/L concentration was used as the appropriate diluted standard for preparation of the calibration curve. The linearity of the calibration curve was 0.9996; the detection limit for the samples was 0.0007 mg/L. The method for determination of the Se was validated using the pure Se standards (Rathore *et al.* 2018).

### Phytochemical analysis of cultivated *Pleurotus* spp.

Phytochemicals screenings were carried out on the powdered samples to identify their major constituents such as alkaloids, saponins, tannins, flavonoid, steroid, phytate, phenols and oxalate using standard qualitative methods as described by Parihar *et al.* (2015).

### Antimicrobial Analysis of *Pleurotus* spp.

Antibacterial activity of the *pleurotus* spp. extract was tested using agar well diffusion method (Balakumar *et al.* 2011) with modification. Petri dishes with nutrient agar were inoculated with a 100 µl suspension of each bacterial culture. Wells were made on the agar surface with 6mm cork borer. The bacteria seeded plates were placed in the refrigerator for 8 hours at 4 °C. Extracts was reconstituted in 3.0% dimethyl sulfoxide (DMSO) and filtered through 0.22 µm before introduced into the well. Chloramphenicol was used as standard antibiotics (control) for bacteria while ketoconazole was used as control for fungi. The plates were then incubated at 37±2 °C for 24 h and 26 °C for 48 to 72 h for bacteria and fungi respectively. The plates were observed for the inhibited zones around the wells and measured in millimeter. The minimum inhibition concentration of each extracts were determined by ranging the concentration of the extracts from 10 to 200 µg. Antifungal activity of ethanolic extract of *Pleurotus* spp. was determined by the agar well diffusion modified method of Oyetayo *et al.* (2009).

### Statistical Analysis

All the treatments were carried out in triplicates and the data obtained were analyzed using analysis of variance (ANOVA). Means were separated using Duncan's New Multiple Range Test at 95% confidence level using Statistical Packages for the Social Sciences (SPSS) version 17.0.

### Results

Table 1 shows the total yield and biological efficiency of the cultivated *Pleurotus* spp. The *Pleurotus* spp. were harvested in three flushes. Non-fortified *Pleurotus* spp. (PPN) at the second flush had the highest yield (196.00 g) while selenium fortified *Pleurotus ostreatus* (POS) had the lowest yield at the third flush (95.00 g). Non-fortified *Pleurotus pulmonarius* (PPN) had the highest biological efficiency (177.73 %) while fortified *Pleurotus ostreatus* (POS) had the lowest biological efficiency (113.73%).

**Table 1** Yield and biological efficiency (B.E) of cultivated *Pleurotus* spp.

Samples	1 <sup>st</sup> flush (g)	2 <sup>nd</sup> flush (g)	3 <sup>rd</sup> flush (g)	B.E (%)
PON	161.00±2.00 <sup>c</sup>	176.50±2.50 <sup>b</sup>	120±10.00 <sup>b</sup>	152.50 <sup>b</sup>
POS	121.20±7.50 <sup>a</sup>	125.00±15.00 <sup>a</sup>	95.00±10.00 <sup>a</sup>	113.73 <sup>a</sup>
PPS	137.00±4.00 <sup>b</sup>	141.20±2.50 <sup>a</sup>	121.20±7.50 <sup>b</sup>	133.13 <sup>a</sup>
PPN	190.00±5.00 <sup>d</sup>	196.00±7.50 <sup>b</sup>	147.20±5.00 <sup>c</sup>	177.73 <sup>c</sup>

Values are means of triplicates ±SD. Values carrying the same alphabets in the same column are not significantly different at (p>0.05). Values are means of triplicates ±SD. Where : PPN= *Pleurotus pulmonarius* (non-fortified with selenium), PPS=*P. pulmonarius* (fortified with selenium), PON= *P. ostreatus* (non-fortified with selenium) and POS= *P. ostreatus* (fortified with selenium).

Table 2 shows the concentration of selenium (Se) in the cultivated *Pleurotus* spp. Non-fortified *Pleurotus ostreatus* (PON) and non-fortified *Pleurotus pulmonarius* (PPN) had no selenium. Fortified *Pleurotus ostreatus* (POS) and *Pleurotus pulmonarius* (PPS) recorded a Se concentration of 51.79 µg/g and 59.29 µg/g respectively and there was no significant differences (P<0.05) in their Se concentration.

**Table 2** Selenium content of *Pleurotus* spp. after fortification ( $\mu\text{g/g}$ ).

Sample	Concentration
PON	0.00 $\pm$ 0.00 <sup>a</sup>
POS	51.79 $\pm$ 2.60 <sup>b</sup>
PPS	59.29 $\pm$ 1.52 <sup>b</sup>
PPN	0.00 $\pm$ 0.00 <sup>a</sup>

Values are means of triplicates  $\pm$ SD. Values carrying the same alphabets in the same column are not significantly different at ( $p>0.05$ ). Values are means of triplicates  $\pm$ SD. Where : PPN= *Pleurotus pulmonarius* (non-fortified with selenium), PPS=*P. pulmonarius* (fortified with selenium), PON= *P. ostreatus* (non-fortified with selenium) and POS= *P. ostreatus* (fortified with selenium).

Table 3 shows the quantitative phytochemicals of the cultivated *Pleurotus* spp. Saponins had the highest value in all cultivated *Pleurotus* spp.: fortified *Pleurotus ostreatus* (4.29 mg/g), fortified *Pleurotus pulmonarius* (5.34 mg/g), non-fortified *Pleurotus pulmonarius* (5.58 mg/g), and non-fortified *Pleurotus ostreatus* (7.08 mg/g) while tanins had the least value in all cultivated *Pleurotus* spp.: fortified *Pleurotus ostreatus* (0.02 mg/g), Non-fortified *Pleurotus ostreatus* (0.03 mg/g), non-fortified *Pleurotus pulmonarius* (0.03 mg/g) and fortified *Pleurotus pulmonarius* (0.03 mg/g). Alkaloids, phenols and flavonoids ranged from 1.38-4.37 mg/g, 0.08-0.39 mg/g and 0.26-1.23 mg/g respectively.

**Table 3** Phytochemical composition (mg/g) of *Pleurotus* spp. fortified with selenium.

Composition	PPN	PPS	POS	PON
Alkaloids	4.37 $\pm$ 0.13 <sup>d</sup>	1.23 $\pm$ 0.07 <sup>b</sup>	3.40 $\pm$ 0.12 <sup>c</sup>	1.38 $\pm$ 0.06 <sup>a</sup>
Phenols	0.23 $\pm$ 0.13 <sup>a</sup>	0.23 $\pm$ 0.13 <sup>a</sup>	0.08 $\pm$ 0.01 <sup>a</sup>	0.39 $\pm$ 0.51 <sup>a</sup>
Tannins	0.03 $\pm$ 0.01 <sup>a</sup>	0.03 $\pm$ 0.00 <sup>a</sup>	0.02 $\pm$ 0.00 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>a</sup>
Saponins	5.58 $\pm$ 0.13 <sup>c</sup>	5.34 $\pm$ 0.02 <sup>b</sup>	4.29 $\pm$ 0.06 <sup>a</sup>	7.08 $\pm$ 0.08 <sup>d</sup>
Flavonoids	1.23 $\pm$ 0.07 <sup>b</sup>	0.74 $\pm$ 0.58 <sup>b</sup>	0.26 $\pm$ 0.04 <sup>a</sup>	0.44 $\pm$ 0.02 <sup>a</sup>

Values are means of triplicates  $\pm$ SD. Values carrying the same alphabets in the same column are not significantly different at ( $p>0.05$ ). Values are means of triplicates  $\pm$ SD. Where : PPN= *Pleurotus pulmonarius* (non-fortified with selenium), PPS=*P. pulmonarius* (fortified with selenium), PON= *P. ostreatus* (non-fortified with selenium) and POS= *P. ostreatus* (fortified with selenium).

Table 4 shows the *in\_vitro* antibacterial activities of ethanolic extracts of the cultivated *Pleurotus* spp. against eleven (11) bacteria. All extracts exhibited antibacterial activities against all the tested bacteria. Extracts from fortified *Pleurotus pulmonarius* (PPS) had the highest zones of inhibition (21.00-25.30 mm) against tested organisms. The zones of inhibition displayed by PPS (21.00-25.30 mm) compared favourably with the conventional antibiotic; chloramphenicol (12.70-25.00 mm). Extracts from non-fortified *Pleurotus ostreatus* (PON) had the lowest zones of inhibition (9.00-18.00 mm).

Table 5 shows the minimum inhibitory concentration of ethanolic extracts of the cultivated *Pleurotus*. The minimum inhibitory concentration displayed by extracts of fortified *Pleurotus* species against all test bacteria ranged from 50  $\mu\text{g/g}$  to 150  $\mu\text{g/g}$  while the minimum inhibitory concentration displayed by extracts of non-fortified *Pleurotus* species ranged from 50-200  $\mu\text{g/g}$  against all test bacteria.

Table 6 shows the antifungal activities of ethanolic extracts of cultivated *Pleurotus* spp. Ethanolic extracts of non-fortified *Pleurotus* spp. showed no zones of inhibition against all tested fungi at a concentration of 200  $\mu\text{g/g}$ . Extracts of fortified *Pleurotus* spp. however displayed zones of inhibitions against *Aspergillus flavus* (13.00 mm), *Candida albicans* (11.00-13.00 mm) and *Candida parapsilosis* (13.00 mm) at a concentration of 200  $\mu\text{g/g}$ .

**Table 4** Zones of inhibition(mm) displayed by ethanolic extracts of cultivated *Pleurotus* spp. against bacteria at 200 µg/g.

Isolates	PON	PPN	PPS	POS	CHL
<i>Pseudomonas aeruginosa</i>	15.30±1.54 <sup>a</sup>	14.00±1.00 <sup>a</sup>	25.00±1.00 <sup>c</sup>	21.70±1.15 <sup>b</sup>	23.30±0.58 <sup>b</sup>
<i>Staphylococcus aureus</i>	15.70±0.58 <sup>a</sup>	13.70±0.58 <sup>a</sup>	21.30±2.89 <sup>c</sup>	16.70±1.52 <sup>b</sup>	24.00±1.00 <sup>c</sup>
<i>Klebsiella pneumoniae</i>	15.30±0.58 <sup>a</sup>	15.00±1.73 <sup>a</sup>	21.30±2.89 <sup>c</sup>	17.70±1.15 <sup>a</sup>	21.70±0.58 <sup>b</sup>
<i>Proteus mirabalis</i>	13.00±1.00 <sup>a</sup>	15.00±2.00 <sup>a</sup>	22.30±5.00 <sup>b</sup>	22.00±1.00 <sup>b</sup>	12.30±1.15 <sup>a</sup>
<i>Escherichia coli</i>	14.00±1.00 <sup>a</sup>	15.70±3.06 <sup>a</sup>	24.00±1.73 <sup>c</sup>	19.70±2.08 <sup>b</sup>	24.00±1.73 <sup>c</sup>
<i>Bacillus cereus</i>	9.00±2.00 <sup>a</sup>	14.30±3.21 <sup>b</sup>	23.70±2.08 <sup>c</sup>	17.00±2.00 <sup>b</sup>	23.70±1.15 <sup>c</sup>
<i>Enterococcus aerogenes</i>	10.00±1.00 <sup>a</sup>	16.70±3.51 <sup>b</sup>	24.00±1.00 <sup>d</sup>	19.30±2.08 <sup>c</sup>	21.30±0.58 <sup>c</sup>
<i>Streptococcus viridans</i>	13.00±1.00 <sup>a</sup>	11.30±1.53 <sup>a</sup>	21.00±2.00 <sup>b</sup>	20.00±1.73 <sup>b</sup>	12.70±1.15 <sup>a</sup>
<i>Salmonella dysenteriae</i>	11.30±1.52 <sup>a</sup>	12.30±0.58 <sup>a</sup>	23.70±2.08 <sup>c</sup>	17.30±1.53 <sup>b</sup>	24.00±1.00 <sup>c</sup>
<i>Streptococcus oralis</i>	18.00±2.00 <sup>a</sup>	14.70±2.08 <sup>a</sup>	25.30±1.53 <sup>b</sup>	19.00±2.00 <sup>a</sup>	25.00±1.73 <sup>b</sup>
<i>Seratia marcescens</i>	14.70±2.53 <sup>a</sup>	15.00±1.00 <sup>a</sup>	24.30±1.00 <sup>c</sup>	18.30±1.15 <sup>b</sup>	22.30±2.52 <sup>c</sup>

Values are means of triplicates ±SD. Values carrying the same alphabets in the same row are not significantly different at (p>0.05). Where PPN=*Pleurotus pulmonarius* (non-fortified with selenium), PPS=*P. pulmonarius* (fortified with selenium), PON =*P. ostreatus* (non-fortified with selenium), POS=*P. ostreatus* (fortified with selenium), CHL: Chloramphenicol.

**Table 5** Minimum inhibitory concentration (µg/g) of ethanolic extracts of *Pleurotus* spp. against test bacteria.

Isolates	PPN	PPS	POS	PON
<i>Pseudomonas aeruginosa</i>	100	50	100	100
<i>Staphylococcus aureus</i>	100	50	150	150
<i>Klebsiella pneumoniae</i>	100	50	150	150
<i>Proteus mirabalis</i>	150	50	150	150
<i>Escherichia coli</i>	150	50	50	100
<i>Bacillus cereus</i>	150	50	150	200
<i>Enterobacter aerogenes</i>	100	50	50	250
<i>Streptococcus viridans</i>	150	50	100	150
<i>Salmonella dysenteriae</i>	100	50	100	200
<i>Streptococcus oralis</i>	100	50	150	150
<i>Seratia. Marcescens</i>	150	50	150	100

Where PPN = *Pleurotus pulmonarius* (non-fortified with selenium), PPS = *P. pulmonarius* (fortified with selenium), PON = *P. ostreatus* (non-fortified with selenium) and POS = *P. ostreatus* (fortified with selenium).

**Table 6** Zones of inhibition (mm) displayed by ethanolic extracts of cultivated *Pleurotus* spp. against fungi at 200µg/g.

Isolates	PON	PPN	PPS	POS	KCZ
<i>Aspergillus flavus</i>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	13.00±1.00 <sup>b</sup>	13.00±1.00 <sup>b</sup>	23.30±0.58 <sup>c</sup>
<i>Aspergillus niger</i>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	24.00±1.73 <sup>b</sup>
<i>Candida albicans</i>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	11.00±1.00 <sup>b</sup>	13.00±1.00 <sup>b</sup>	24.00±1.00 <sup>c</sup>
<i>Candida parapsilosis</i>	0.00±0.00 <sup>a</sup>	0.00±0.00 <sup>a</sup>	13.00±1.00 <sup>b</sup>	0.00±0.00 <sup>a</sup>	25.00±1.73 <sup>c</sup>

Values are means of triplicates ±SD. Values carrying the same alphabets in the same row are not significantly different at (p>0.05). Where PPN = *Pleurotus pulmonarius* (non-fortified with selenium), PPS = *P. pulmonarius* (fortified with selenium), PON = *P. ostreatus* (non-fortified with selenium) , POS = *P. ostreatus* (fortified with selenium) and KCZ = Ketoconazole.

## Discussion

Edible fungi especially *Pleurotus* spp. have been used as dietary supplements when there are deficiencies of certain constituents in natural foods. This is because of their ability to absorb micro and macro nutrients from the substrates used for their cultivation (Ling *et al.* 2015). The enrichment of foods with micronutrients such as Selenium (Se) is very desirable (Finley 2006). This enrichment presents the opportunity to use bio-fortified foods as natural sources of antimicrobials and antioxidants. This present study emphasizes on the effect of Se biofortification on the phytochemical constituents and antimicrobial activities of *Pleurotus* spp.

The results obtained from the yield and biological efficiency (BE) of *Pleurotus* spp. show that the second flush produced the highest yield of mushrooms. The addition of Se to the substrates however caused a reduced yield. This result correlates with the observations of Da Silva *et al.* (2012) and Ogidi *et al.* (2017). The highest BE (177.73%) was obtained from non-fortified *Pleurotus pulmonarius* while fortified *Pleurotus ostreatus* (POS) had the least BE. These results confirm that mushroom yield and BE varies according to the substrates used (Dias *et al.* 2003, Curvetto *et al.* 2002). Se, though important to human health can become poisonous if ingested in high quantity (Hartikainen 2005). The recommended dose for an adult (male or female) is 55-65 µg/day.

Selenium (Se) has been found to be part of the biomass of *Pleurotus* spp. and also found to be associated with the membrane and cell wall (Munoz *et al.* 2006). It is recognized as an essential micronutrient for humans and animals and has an important role in biological functions that is mediated primarily by selenoproteins. *Pleurotus* spp. cultivated with Sodium selenite were observed to possess Se while the one not cultivated with Sodium selenite recorded no trace of Se. There was no significant difference ( $P < 0.05$ ) in the Se- level of both fortified *P. ostreatus* and *P. pulmonarius*. This shows the high capability of these mushrooms to absorb inorganic Se and accumulate it into the fruit body of the mushroom.

Edible mushrooms display variable concentrations of Se depending on the species, compost and growth substrate conditions, such as the nutrient content and availability (Maseko *et al.* 2013). The results obtained from this present study (51.79 and 59.39 µg/g) conforms with Da Silva *et al.* (2012) who reported that the level of Se found in *P. ostreatus* cultivated on substrate supplemented with 3.2 mg/kg of sodium selenite was 57.6 mg/g. The extent of accumulation in this study was notably higher than the Se concentrations of 4.6 and 9.3 mg/g reported for *P. eryngii* cultivated on substrates supplemented with 5.0 and 10.0 mg/kg of sodium selenite, respectively (Estrada *et al.* 2009). The differences in these results may in part be explained by different methods used in the extraction and quantification of the selenium and also by the oxidative degradation of the selenocompounds (Amoako *et al.* 2009). The cultivation process of the fungus, the concentration and form of Se added during enrichment and the physiology of the fungus all influence the forms of Se absorption and also the capability for biotransformation (Da Silva *et al.* 2012). The mechanisms for the Se uptake by fruiting bodies have been reported to be due to the methylation of  $\text{Na}_2\text{SeO}_3$  to seleno compounds viz., methylselenol, dimethylselenide, and trimethylselenonium (Rathore *et al.* 2018). This approach of incorporating element could serve as another nutritional source for meeting the daily dietary recommended allowances for Se (Rathore *et al.* 2018).

Phytochemical analyses conducted on the Selenium enriched *Pleurotus* spp revealed the presence of alkaloids, phenol, tannins, saponins and flavonoid. It is known that Saponins inhibit  $\text{Na}^+$  efflux by blockage of the influx of concentration in the cells, activating a  $\text{Na}^+ - \text{Ca}^{2+}$  antiporter in cardiac muscles. The increase in  $\text{Ca}^{2+}$  influx through this antiporter strengthens the contraction of heart muscles (Edeoga and Enata 2001). Omojate *et al.* (2014) advocated that the consumption of tannin containing beverages especially green teas and red wine, can prevent or

cure a variety of illness. Karou *et al.* (2011) also stated that many physiological activities such as stimulation of phagocytic cells and wide range of anti-infective actions have been attributed to tannins because of its molecular actions of forming complexes with proteins. Tannins were able to inhibit the growth of insect and disrupt the digestive activities in ruminant animals (Karou *et al.* 2011). Tanins have been reported to be responsible for the ability of plant to inactivate microbial adhesions, enzymes and cell envelope transport proteins (Omojate *et al.* 2014).

Phenols may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesins (Cowan 1999). Various researchers have earlier defined role of selenium in inducing antioxidant capacity by facilitating increase in the amounts of tocopherol and phenolic compounds in plants (Xie *et al.* 2003). The valuable pharmaceutical properties of mushroom may also be attributed to the presence of alkaloids which has been reported to have a stimulating effect, act as topical anaesthetic in ophthalmology, powerful pain reliever, antipuretic action among other uses (Edeoga *et al.* 2001). The presence of flavonoids in *Pleurotus* spp. may help provide protection against oxidative stress induced diseases by contributing along with other antioxidant, vitamins, and enzyme to the total antioxidative defense system of the human body.

The saponin, alkaloid, phenols, flavonoids and tannin contents differed from one species to another. This demonstrates the fact that, every species has its own unique mechanisms in the synthesis of these phytochemicals. *Pleurotus* spp. may be used as potential sources of phytochemicals and for designing drugs that can prove to be of keen interest in the treatment and prevention of diseases like cancer, tumor and heart diseases (Ashok and Shabudeen 2015).

Mushrooms have been appreciated as sources of food nutrients and especially used for medicinal purposes in the orients for centuries (Lindequist *et al.* 2005). Antibiotics provide the main basis for the therapy of bacterial infections. However, the high genetic variability of bacteria enables them to rapidly evade the action of antibiotics by developing antibiotic resistance (Nehra *et al.* 2012).

Ethanol extract of selenium enriched *Pleurotus* species was found to exhibit good antibacterial effect against the test bacteria used in this study (Table 4). The extracts inhibited both Gram positive and negative bacteria. This suggested that Se fortified *P.* species has a broad-spectrum antibacterial activity. However, Selenium enriched *Pleurotus* species were more active against the test bacteria than the non-selenium fortified *P.* species. The maximum antibacterial effect of aqueous extract of Se enriched *Pleurotus* species was found against *Streptococcus oralis* (25.30 mm), *Pseudomonas aeruginosa* (25.00 mm) and minimum against *Streptococcus viridians* (21.00 mm). This is more or less similar with the results of Akyuz and Kirbag (2009) who reported that methanolic extract of *Pleurotus* species showed activity against Gram positive and negative bacteria at different concentrations. The result is also similar to Iwalokun *et al.* (2007) who reported that macrofungus extracted with petroleum ether and acetone, inhibited Gram positive and Gram negative bacterial tested *in vitro*. Similarly, organic extracts (methanol and chloroform) obtained from *P. ostreatus* has earlier been reported to be effective against Gram-positive bacteria (Karaman *et al.* 2010). The mechanism of the active compounds is still to be studied; nevertheless, membrane disruption could be suggested as one of the likely mechanisms of action of the compounds belonging to the terpenoids. Based on the results of this study, it can be concluded that Se enriched *Pleurotus* spp. possess a broad-spectrum antibacterial activity. Moreover, fortification of *P.* spp. further enhanced its antibacterial potency. The Se-fortified *Pleurotus* spp. had mild inhibitory effects against *Candida albicans*, *Aspergillus flavus* and *Candida parapsilosis*. The non-fortified *Pleurotus* spp. were however not active against fungi. Drugs derived from macrofungi are effective, readily available, less expensive and rarely have side effects (Parihar *et al.* 2015).

Conclusively, Se-fortification in *Pleurotus* spp. increased the antimicrobial activities of *Pleurotus* spp. These results therefore suggest that Se-enriched mushrooms can be used as potential source of dietary supplements and nutraceuticals.

### Conflict of Interest

The authors do not have any conflicts of interest.

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