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AMINO ACID COMPOSITION AND ANTI-TYROSINASE ACTIVITY OF METABOLITES FROM EDIBLE *Pleurotus* SPECIES FOR THEIR NUTRITIONAL AND THERAPEUTIC POTENTIAL

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KEYWORDS

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Health-promoting properties

Anti-tyrosinase inhibition

Amino acid quantity

Crude extract metabolite

ABSTRACT

Mushrooms are being extensively researched due to their nutritional value and medicinal importance. The genus *Pleurotus* is the second most cultivated mushroom and is known for its high nutritional value, therapeutic properties, taste, flavor, as well as their application in biotechnology and environmental study. Also, tyrosinase is prevalent in most living organisms. The enzyme catalyzes the oxidation of monophenols to ortho-quinones in a two-step reaction process. This study was aimed to assess the amino acid composition and anti-tyrosinase activity of metabolites obtained from edible *Pleurotus* species. Assessment of the nutritional content and inhibitory studies of mushroom tyrosinase produced from four *Pleurotus* strains was carried out using high-performance liquid chromatography (RP-HPLC). The results of the study showed that seventeen different amino acids were identified in the crude and partially purified protein metabolites. Also, the crude extract metabolite had the highest quantity of amino acids than the partially purified. The highest and lowest amino acids value Glutamic acid (1343.26 µmol/mL) and valine (0.34 µmol/mL). The anti-tyrosinase inhibition studies of the four *Pleurotus* strains showed varying results from significantly inhibitory effects to slightly inhibitory effects on mushroom tyrosinase. The highest inhibition was 14.86% (Pleu-W), while the lowest inhibition was 51.42% (Plof-30) respectively. The high point of this study is that the *Pleurotus* species contains a significant number of amino acids and also, they possess good anti-tyrosinase activity. Therefore, these are a good source of nutritional and therapeutic metabolites and these can be explored further for their nutritional and medicinal importance to man.

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1 Introduction

A lot of people in the world live on grains, tubers, meat, and small quantities of pulses and vegetables; consequently, some human health problems have been attributed to the food consumption lifestyle. The increase in obesity and cardiovascular diseases among the growing human population due to the food consumption pattern is becoming an issue of great concern.

Mushroom has been appreciated for centuries as an important food and medicinal source. These are known as higher fungi or macro-fungi; having both reproductive and vegetative phases (reproductive fruiting bodies and vegetative mycelia). Mushroom is lately considered as a goldmine of bio-actives nutrient and a key functional food with immense therapeutic potentials (Deepalakshmi & Mirunalini, 2014; Ozturk et al., 2015; Sekan et al., 2019; Ianni et al., 2021).

Oyster mushrooms are well known as a very high nutritional/biological food that has an enhanced content of dietary fiber, amino acids, protein, vitamins, and unsaturated fatty acids (Michael et al., 2011; Kalac, 2013; Bach et al., 2017; Carrasco-González et al., 2017). The rapid increase/expansion of both cultivation/consumption of oyster mushrooms (*Pleurotus* species) is due to their excellent taste, enhanced value in terms of nutrition and therapeutic properties. They are a vital source of nutritional calories (proteins, dietary fiber, carbohydrates, among others). More so, they are cheaper to produce and possess medicinal properties such as anti-tumorigenic, antioxidant, antimicrobial properties, modulation of cholesterol (Corrêa et al., 2016; Dicks & Ellinger, 2020). Importantly, the quick rate of growth of oyster mushrooms makes it an excellent resource for prospecting bioactive metabolites unlike plants for biotechnological applications (Chandra et al. 2020).

Tyrosinase (EC 1.14.18.1) is an enzyme of great significance in melanogenesis. This vital multi-copper enzyme sequentially catalyzes oxidation steps with various phenolic substrates. The first reactive step involves aromatic ring substitution with a hydroxyl group; this is known as “monooxygenase” or “cresolase” activity. While the second reaction step involves oxidizing the *o*-dihydroxy compound produced in the first reaction to an *o*-dopaquinone, this is “diphenolase” or “catecholase” activity. Molecular oxygen is essential for both reactions (Haghbeen & Tan, 2003). These activities can be used as the basis for several biotechnological applications (Halaouili et al., 2006). Although tyrosinase is prevalent in most living things, but the emphasis is more on mushroom tyrosinase biotechnological potentials.

Tyrosinase is responsible for hyper-pigmentation / browning of vegetables and fruits, as well as melanogenesis in mammals (Dembitsky & Kilimnik, 2016). Due to the key role of tyrosinase in the melanin pathway and its impact; the scientific community is focusing on characterizing newer anti-tyrosinase agents/inhibitors for different applications in agri-food industries, medicinal and cosmetic industries (Halaouli et al., 2005; Te-Cheng, 2009; Sahu et al., 2014). Moreover, with increasing biotechnological demands and applications of tyrosinase inhibitors, there is a need for improving the screening and development methods for these key enzyme inhibitory agents (Halaouili et al., 2006). An easier, eco-friendly, and sustainable means of production of anti-tyrosinase agents, as well as a rapid, sensitive, and accurate assay method, is highly necessary.

Therefore, this study was conceptualized because there is an ever-increasing demand for functional/nutritional food with immense potentials as a goldmine of bioactive therapeutic metabolites. Also, there is a demand for new tyrosinase inhibitors to be applied in the food, cosmetics, and medicinal industries, using simple, rapid, and sensitive assay procedures.

2 Materials and Methods

2.1 Mushrooms and liquid culture fermentation conditions

The oyster mushrooms utilized in this investigation were provided/obtained from a mushroom germplasm culture collection, Plant Science Division, CSIR-NEIST, Jorhat, Assam-state, India. The strains designated as Pleu-W, Pleu-90, Plof-W, and Plof-30 were refrigerated at 4^{o}C on (PDA) and re-culture bimonthly. All inoculations were carried out inside a laminar flow to ensure aseptic inoculation conditions. Wheat waste formulated liquid medium which was used in this study consist of Glucose (50g/liter), Peptone water (2.5g/liter), KH_2PO4 (2g/liter), $MgSO_4.7H_2O$ (1g/liter), $CaCl_2.2H_2O$ (1g/liter), Yeast extract (2.5g/liter), and pH 5.8. The fermentation medium was sterilized in an autoclave at 121^{o}C for 15 minutes and 200ml was aseptically distributed into 250ml conical flasks. Inoculation into flasks was done by using three agar plugs of (6mm diameter) an actively growing *Pleurotus* strain. Inoculated flasks were incubated for 7 days using a shaker incubator at 150 rpm at a temperature of 28 ℃.

2.2 Extraction of intracellular protein

The mycelia mat was homogenized in a blender with 0.1M sodium phosphate buffer (pH 7.0). This was now centrifuged at 10,000 rpm at 4℃ for 10 minutes; the supernatant after centrifugation was used in the extraction of the crude protein by ammonium sulfate precipitation.

2.3 Assay for tyrosinase inhibitory activities of *Pleurotus* strains

Tyrosinase inhibitory assay was done by using Arung et al. (2006) protocol. Briefly, the inhibitory assay protocol consists of using 59

µl of 0.1M phosphate buffer (pH 6.5), 47 µl of L-DOPA (2.5 mM), and 70 µl of each *Pleurotus* metabolite sample, this was followed by the addition of 24 µl tyrosinase. Five minutes of incubation at 25 °C was done and the reading was reported at 475 nm. The activity was monitored using UV/VIS, Sigma Spectrophotometer.

$$\text{The inhibition rate} = 100 - \{A - B \div C - D\} \times 100$$

A represent value for the complete reaction, B and C are the obtained values without tyrosinase and the *Pleurotus* metabolite solution, D represents value without *Pleurotus* metabolite and tyrosinase.

2.4 Determination and quantification of individual amino acids in crude and purified protein by (HPLC)

Individual amino acids in the *Pleurotus* strains, both crude and partially purified protein samples were quantified by a kit method using RP-HPLC column (HPLC, Waters, U.S.A.).

2.5 Sample preparation and HPLC amino acids analysis experimental procedure

The crude and partially purified protein metabolites of the *Pleurotus* strains were freeze-dried at -20°C for 4 hours to powdery form. The lyophilized samples were transferred into vial bottles and kept in the freezers. From this, 0.05 grams of each sample were weighed and put separately in open-mouthed test tubes and 5ml of 6N HCl containing 1% phenol was added. The mouth of all the test tubes was sealed and digested for 24 hours at 100°C in a boiling water bath. After digestion, each sample was filtered using a 0.45µm pore size membrane filter (Tarsons). Then 1.5ml of the filtrate was taken and to it, 300µl of Ethanol: Water: Triethylamine (2:2:1) was added. All samples vortexed and then boiled in a water bath for 2 hours. This was followed by the addition of 60 µl of derivatizing solution (Ethanol: Water: Triethylamine: Phenyl-isothiocyanate (PITC) = (7:1:1:1). This reaction mixture was mixed by vortexing and boiled at the hot water bath to remove traces of PITC. The samples were filtered using membrane filters (0.45µm). The filtrates were centrifuged at 6000rpm for 5minutes to precipitate any impurities if present and the supernatant were collected.

The eluent was used for the analysis of free amino acid by a C-18 column HPLC (Waters, 2489 UV visible detector). The flow rate of solvent is 0.8ml/min; Wavelength 280nm; Chart speed 1cm/min; Injected sample of 20µl; pressure is of 1,986psi. From the prepared sample solutions, 20µl of each sample was directly injected into a reverse phase of the C-18 column. Each sample was run for 20 minutes, eluted with gradient elution of water and 35% of Acetonitrile containing 0.1% TFA, and the graph was recorded.

2.6 Preparation of standard (amino acids)

5µl of each amino acid standard solution was taken and re-dried, derivatized, and then diluted with 200µl of sample diluent solution. The average of the triplicate sample was calculated, then compared with standard amino acids peak area and retention time on the column for estimation of amino acid present within the sample.

2.7 Tyrosinase inhibition studies

2.7.1 Effects of DL-aspartic acid on tyrosinase inhibition

The inhibitory effect of DL-aspartic acid on the activity of tyrosinase from *Pleurotus* strains (Pleu-W, Pleu-90, Plof-W, and Plof-30) were investigated using L-DOPA as substrate. The inhibitory effects of using different concentrations of (2mg/ml - 10mg/ml) of DL-aspartic acid were used following standard assay procedure.

2.7.2 Effects of Ascorbic acid and EDTA on Tyrosinase inhibition

The inhibitory effects of EDTA and ascorbic acid were investigated on tyrosinases from the four *Pleurotus* strains (Pleu-W, Pleu-90, Plof-W, and Plof-30) using Kubo et al. (2003) protocol. Their concentrations were varied from (2mg/ml - 10mg/ml) using standard assay procedure.

2.7.3 Effects of Gallic acid

Gallic acid (3, 4, 5-trihydroxybenzoate) were tested for their inhibitory effect on the tyrosinases from the four *Pleurotus* strains (Pleu-W, Pleu-90, Plof-W, and Plof-30). Varying concentrations (2mg/ml - 10mg/ml) of gallic acid were used following the standard assay procedure.

2.8 Statistical data evaluation

The data evaluation was carried out by summing the means of three replicate and a one-way (ANOVA) of data was done. Differences at $p < 0.05$ were considered to be significant. Origin Lab 8 statistical software was deployed.

3 Results

3.1 Determination of amino acids composition

Amino acid quantification was done to evaluate the nutritional composition of the protein metabolites in the crude and partially purified mushroom metabolite from the four *Pleurotus* strains. Different amino acid standards were used for comparisons. The chromatograms obtained with an RP-HPLC column are shown in Figures 1 - 9. The names, peaks, and quantity of each free amino acid are also reflected in Tables 1 - 8. Free amino acids detected in

the metabolites are Aspartic acid, Glutamic acid, Asparagine, Serine, Glycine, Histidine, Glutamine, Threonine, Alanine, Proline, Tyrosine, Valine, Methionine, Cystine, Isoleucine, Hydroxy-proline, and Leucine. Generally, the crude protein metabolite had a higher quantity of amino acids than the partially purified one. Also, the amino acid quantities in the strains Pleu-90 and Plof-30 are higher than the quantities estimated in Pleu-W and Plof-W. With regards to results of the partially purified protein metabolite of *Pleurotus* strains, (Pleu-W) had the highest amino acid value in Glutamic acid 1343.26 µmol/mL, while the lowest amino acid value was reported for the valine 0.34 µmol/mL. Also, the results of crude protein metabolite of *Pleurotus* strains indicated that (Plof-30) alanine concentration was highest 803.34 µmol/mL while valine was lowest having 0.34 µmol/mL.

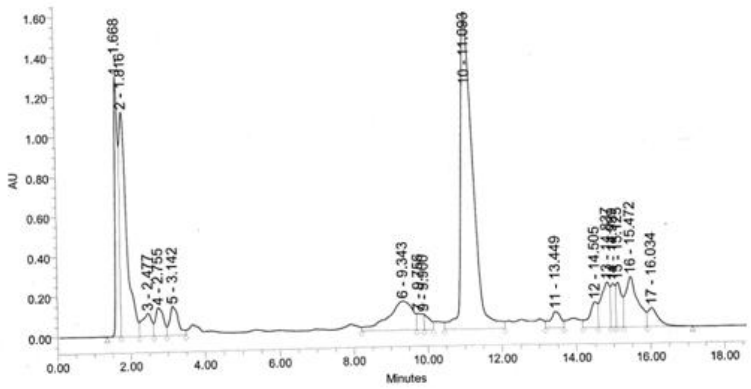

Figure 1 Chromatogram of amino acid composition present in crude protein metabolite of *Pleurotus* strain (Plof-30) analyzed by HPLC.

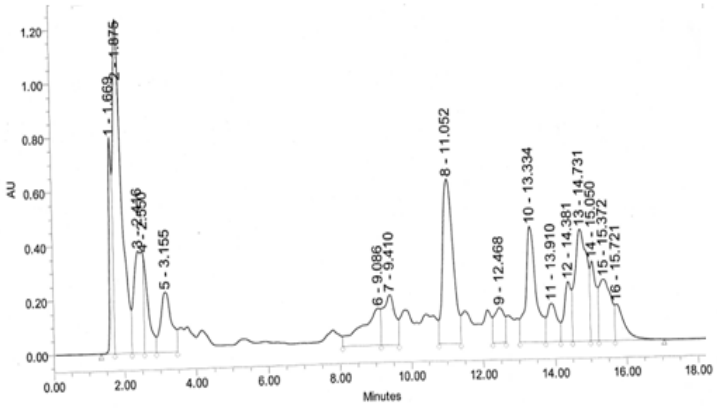

Figure 2 Chromatogram of amino acid composition present in crude protein metabolite of *Pleurotus* strain (Plof-W) analyzed by HPLC.

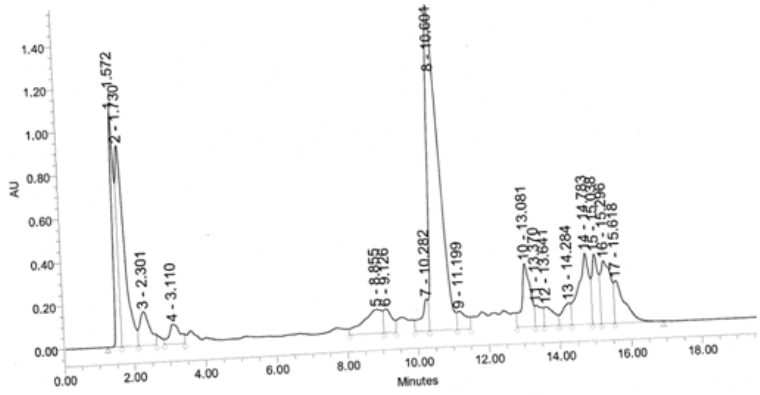

Figure 3 Chromatogram of amino acid composition present in crude protein metabolite of *Pleurotus* strain (Pleu-90) analyzed by HPLC.

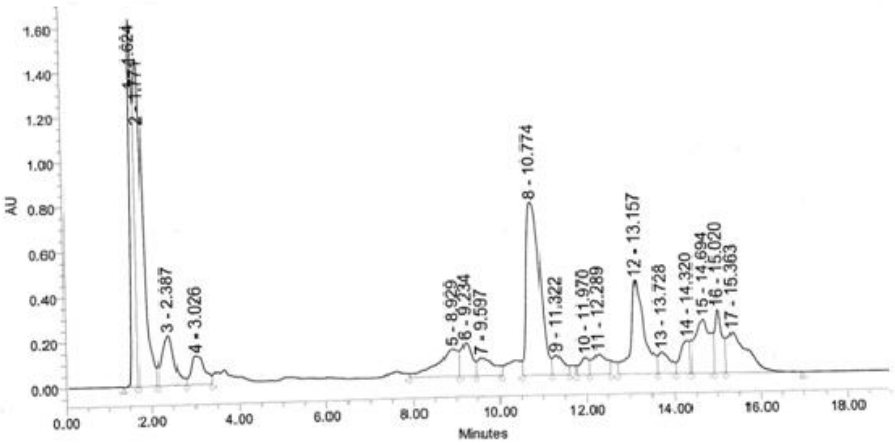

Figure 4 Chromatogram of amino acid composition present in crude protein metabolite of *Pleurotus* strain (Pleu-W) analyzed by HPLC.

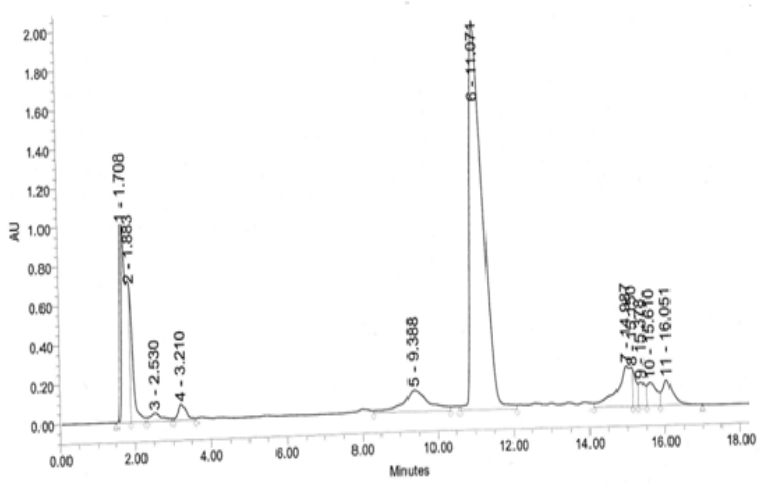

Figure 5 Chromatogram of amino acid composition present in partially purified protein metabolite of *Pleurotus* strain (Plof-30) tyrosinase analyzed by HPLC.

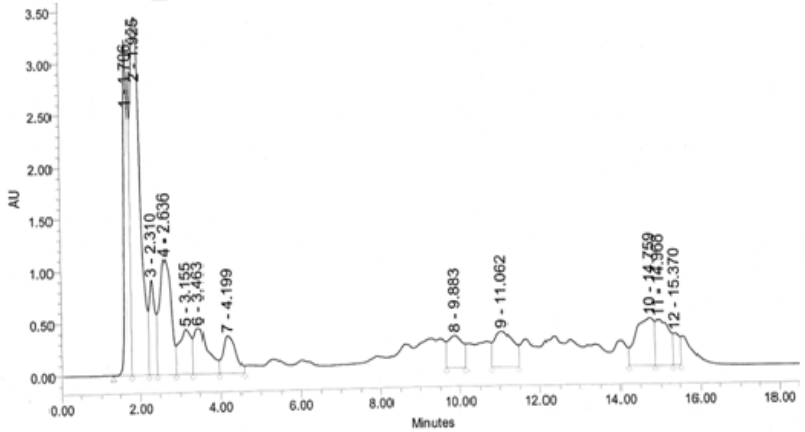

Figure 6 Chromatogram of amino acid composition present in partially purified protein metabolite of *Pleurotus* strain (Plof-W) analyzed by HPLC.

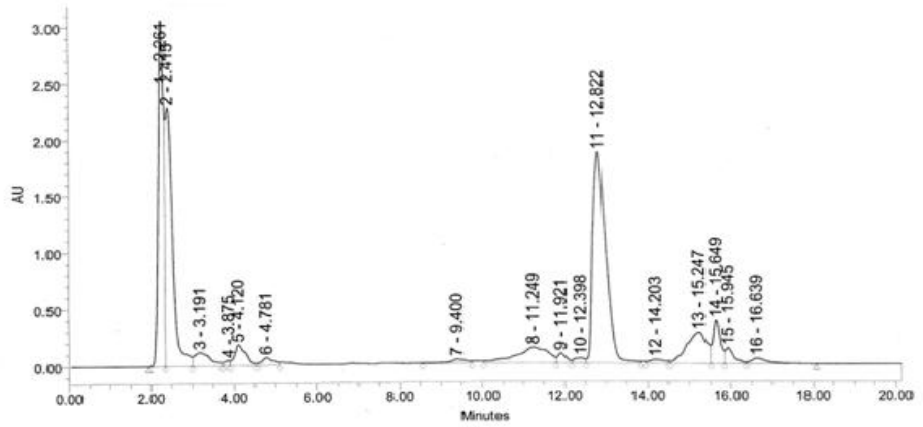

Figure 7 Chromatogram of amino acid composition present in partially purified protein metabolite of *Pleurotus* strain (Pleu-90) analyzed by HPLC

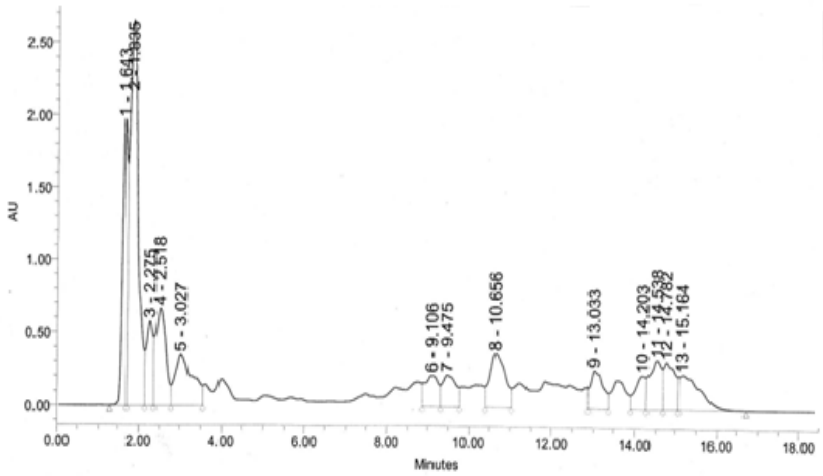

Figure 8 Chromatogram of amino acid composition present in partially purified protein metabolite of *Pleurotus* strain (Pleu-W) analyzed by HPLC

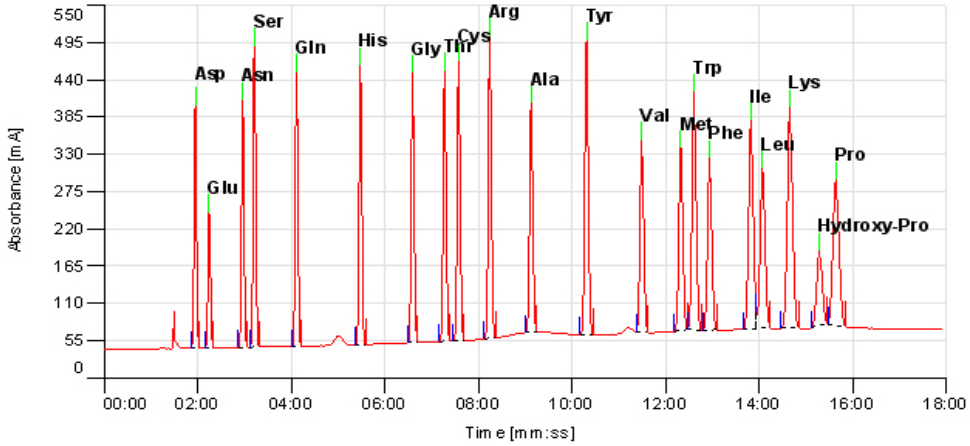

Figure 9 Amino acids standard chromatogram using RP-HPLC

Table 1 Quantitative analysis of individual amino acid present in crude protein metabolite of *Pleurotus* strain (Plof-30).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	196.84
2	Glutamic acid	327.46
3	Asparagine	45.76
4	Serine	47.05
5	Glycine	52.89
6	Histidine	148.12
7	Glutamine	21.19
8	Threonine	18.93
9	Alanine	803.34
10	Proline	35.40
11	Tyrosine	22.45
12	Valine	0.34
13	Methionine	42.12
14	Cysteine	51.13
15	Isoleucine	130.48
16	Leucine	40.97
17	Hydroxy-Proline	4.45

Table 2 Quantitative analysis of individual amino acid present in crude protein metabolite of *Pleurotus* strain (Plof-W).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	110.96
2	Glutamic acid	434.31
3	Asparagine	148.25
4	Serine	72.20
5	Glutamine	106.09
6	Histidine	119.90
7	Alanine	97.04
8	Tyrosine	280.10
9	Valine	56.22
10	Methionine	190.52
11	Tryptophan	61.73
12	Leucine	70.88
13	Cysteine	216.71
14	Isoleucine	78.08
15	Hydroxy-proline	5.14
16	Leucine	56.19

Table 3 Quantitative analysis of individual amino acid present in crude protein metabolite of *Pleurotus* strain (Pleu-90).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	174.19
2	Glutamic acid	268.28
3	Serine	69.26
4	Glycine	44.55
5	Threonine	93.07
6	Arginine	41.05
7	Alanine	47.55
8	Tyrosine	786.94
9	Valine	36.01
10	Phenylalanine	109.91
11	Tryptophan	30.51
12	Methionine	39.69
13	Isoleucine	37.04
14	Leucine	156.44
15	Lysine	88.88
16	Proline	128.81
17	Hydroxy-proline	91.69

Table 4 Quantitative analysis of individual amino acid present in crude protein metabolite of *Pleurotus* strain (Pleu-W).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	240.81
2	Glutamic acid	395.77
3	Asparagine	103.02
4	Serine	60.64
5	Cysteine	95.12
6	Arginine	57.96
7	Alanine	47.54
8	Tyrosine	388.60
9	Valine	34.67
10	Methionine	25.69
11	Tryptophan	47.50
12	Phenylalanine	193.43
13	Isoleucine	41.64
14	Leucine	50.21
15	Lysine	136.96
16	Hydroxy-proline	78.88
17	Proline	129.67

Table 5 Quantitative analysis of individual amino acid present in partially purified protein metabolite of *Pleurotus* strain (Plof-30).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	640.24
2	Glutamic acid	665.72
3	Serine	72.15
4	Glycine	9.27
5	Histidine	77.95
6	Arginine	29.88
7	Threonine	21.58
8	Alanine	6.47
9	Proline	26.50
10	Tyrosine	17.61
11	Valine	894.61
12	Methionine	18.81
13	Cysteine	202.68
14	Isoleucine	104.95
15	Leucine	44.88
16	Phenylalanine	29.40

Table 6 Quantitative analysis of individual amino acid present in partially purified protein metabolite of *Pleurotus* strain (Plof-W)

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	298.20
2	Glutamic acid	914.26
3	Serine	143.56
4	Glycine	254.57
5	Histidine	230.32
6	Arginine	104.20
7	Threonine	115.01
8	Alanine	208.60
9	Proline	126.10
10	Tyrosine	89.59
11	Valine	151.87
12	Methionine	145.90
13	Cysteine	193.55

Table 7 Quantitative analysis of individual amino acid present in partially purified protein metabolite of *Pleurotus* strain (Pleu-90)

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	261.13
2	Glutamic acid	88.33
3	Serine	22.53
4	Glycine	30.37
5	Threonine	119.78
6	Alanine	1084.61
7	Proline	127.83
8	Tyrosine	29.34
9	Valine	30.67
10	Methionine	49.71
11	Cysteine	55.75

Table 8 Quantitative analysis of individual amino acid present in partially purified protein metabolite of *Pleurotus* strain (Pleu-W).

Peak numbers	Amino acid	Quantity of Amino acids (µmol/mL)
1	Aspartic acid	513.20
2	Glutamic acid	1343.26
3	Asparagine	215.01
4	Serine	466.23
5	Glycine	208.16
6	Histidine	241.79
7	Arginine	181.32
8	Threonine	184.53
9	Alanine	225.11
10	Proline	334.29
11	Tyrosine	22.45
12	Valine	0.34

3.1.1 Essential and non-essential amino acids percentage composition

The results presented in Figures 10 – 13 showed the percentages of essential and non-essential amino acids found in crude and partially purified protein metabolite samples for the four *Pleurotus* strains. The results indicated that the percentage distribution of non-essential amino acids was 27.23 % for Plof-W and Plof-30, while it was 25.73 % for Pleu-W and 19.81 % for Pleu-90 in the crude protein sample analyzed. For the essential amino acids composition in the crude metabolite analyzed, Pleu-90 had the highest percentage of 31.82 %, followed by Pleu-W with 27.27 %. Furthermore, the analysis of partially purified protein metabolite from the fungal strains indicated

that (Plof-W) had the highest percentage of non-essential amino acids 27.78 % and for the essential amino acids; among the tested compositions, Plof-30 had the highest percentage composition with 42.86%.

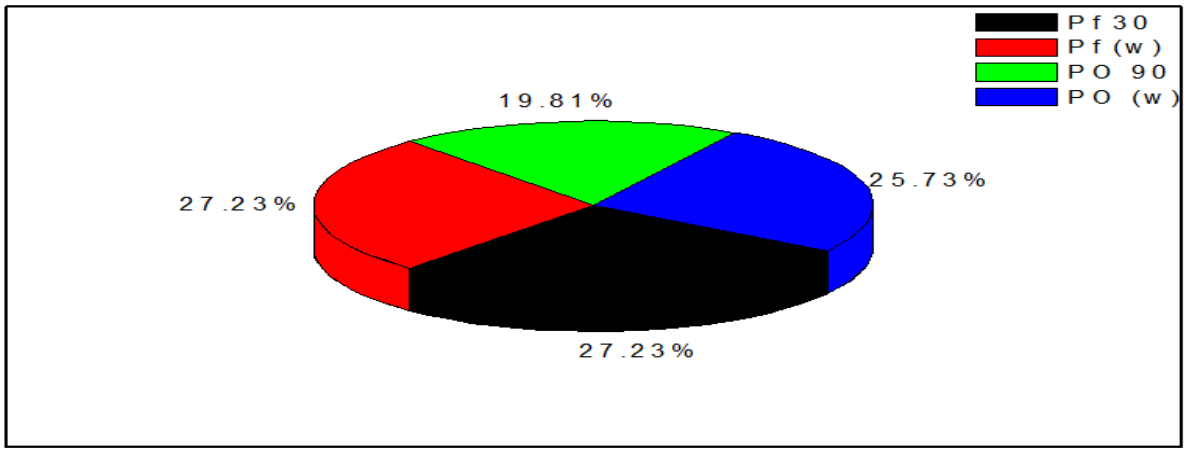

Figure 10 Percentages of non-essential amino acids in crude protein metabolite of edible *Pleurotus* strains

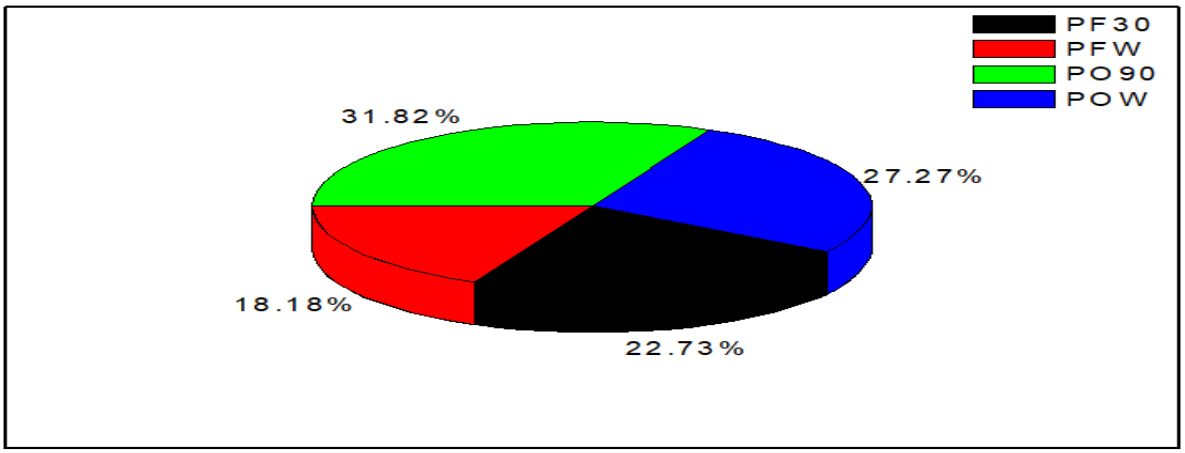

Figure 11 Percentages of essential amino acids in crude protein metabolite of edible Pleurotus strains.

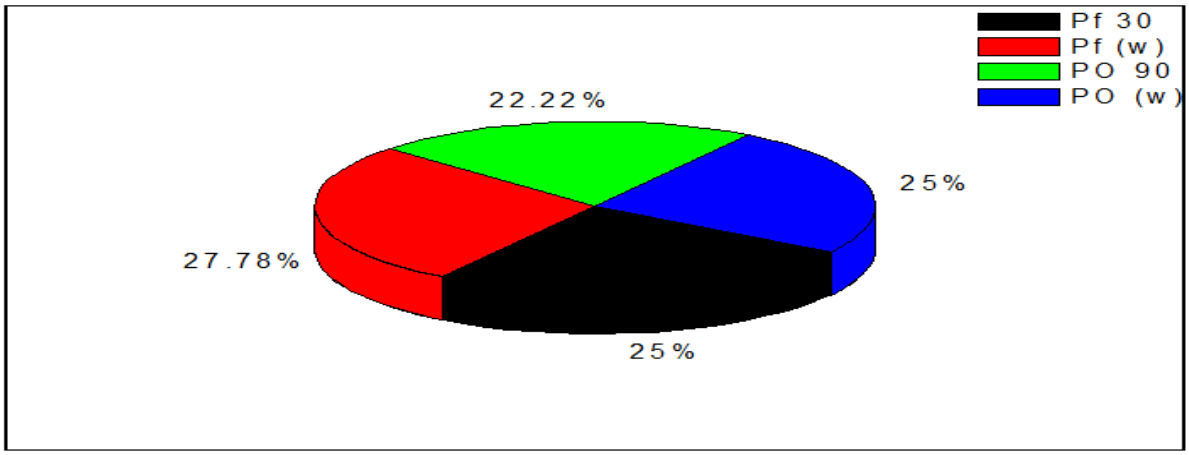

Figure 12 Percentages of non-essential amino acids in partially purified protein metabolite of edible *Pleurotus* strains

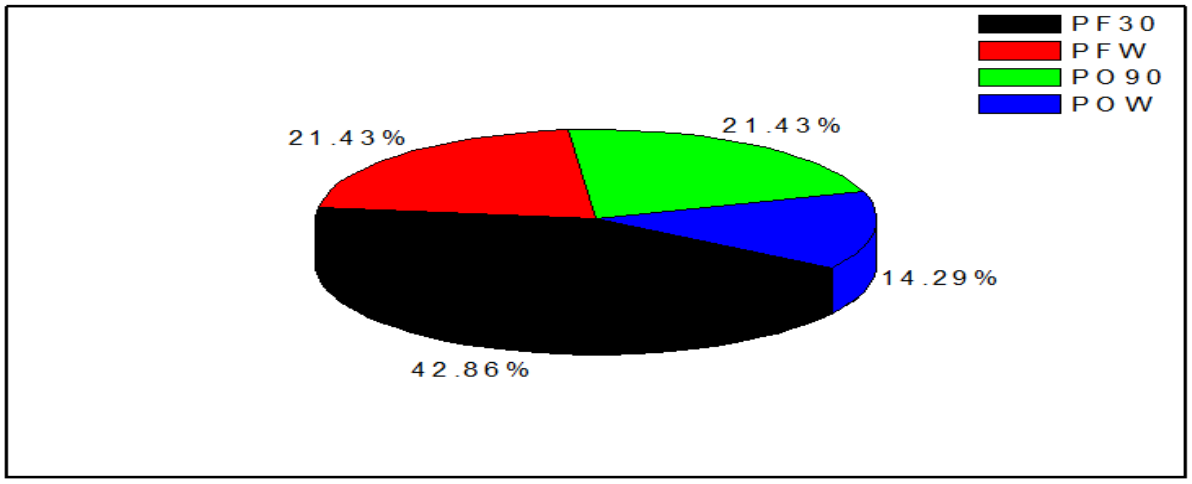

Figure 13 Percentages of essential amino acids in partially purified protein metabolite of edible *Pleurotus* strains.

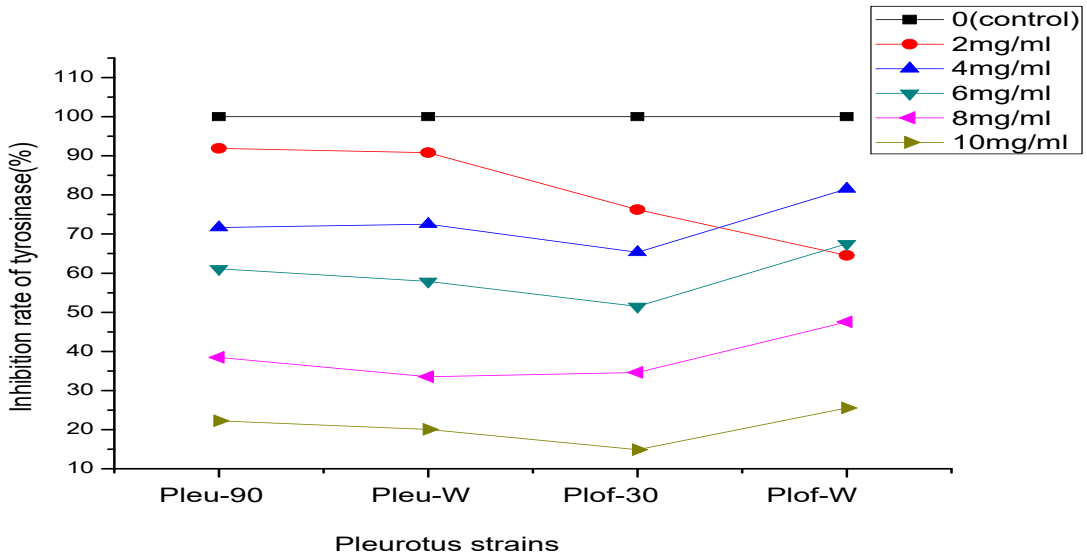

Figure 14 Inhibitory effects of different concentrations of DL-Aspartic acid on tyrosinase activity of four *Pleurotus* strains.

3.2 Anti-tyrosinase inhibitory studies

The results obtained with the effect of various concentrations of DL-Aspartic acid on the activity of tyrosinase are presented in figure 14. Tyrosinase activity was markedly inhibited by the concentration of 10mg/ml of DL-Aspartic acid in all four fungal strains. DL-Aspartic acid at 10mg/ml resulted in the various inhibition rate from 14.86% (Plof-W), to 20.06 (Pleu-90), 22.27% (Pleu-W), and lastly 25.54% for Plof-30.

In addition, the tyrosinase inhibitory effects of Ascorbic acid and EDTA on the activity of fungal tyrosinase are also shown in Figures 15 and 16. These results confirmed that all four fungal strains were inhibited at 10mg/ml concentration considerably. The ascorbic acid inhibitory results indicated that the highest inactivation percentage of 30.36 % was attained with *Pleurotus strain* Pleu-90, while *Pleurotus* strain Plof-W had the highest inactivation percentage of 50.91 % when EDTA was used in combination.

Also, the inhibitory effect of different concentrations of Gallic acid on fungal tyrosinase activity is shown in Figure 17. The result showed high inhibitory activity when a 10mg/ml concentration of gallic acid was also estimated and reported different inhibition rates for different strains and this was reported 24.55%, 32.7%, 44.78%, and 51.42% for Plof-W, Plof-30, Pleu-W, and Pleu-90 respectively.

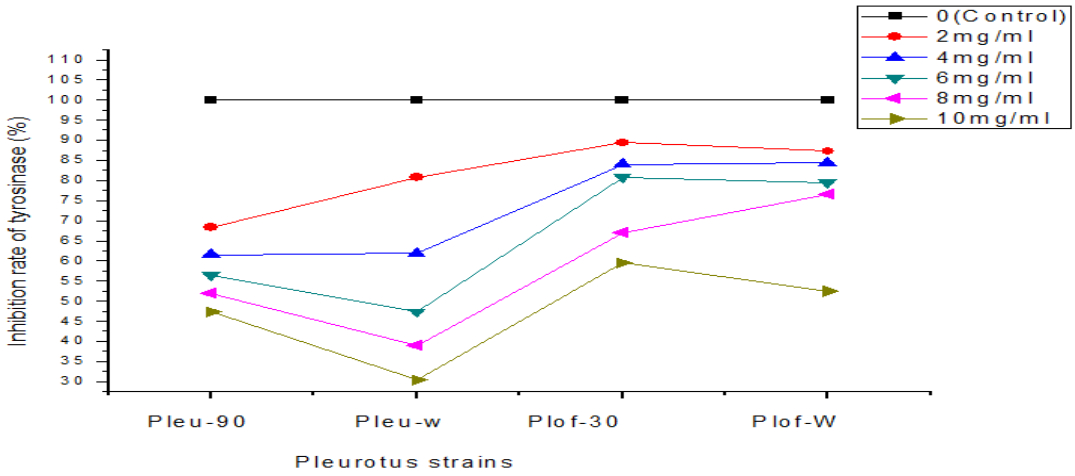

Figure 15 Inhibitory effects of different concentrations of Ascorbic acid on tyrosinase activity of four *Pleurotus* strains.

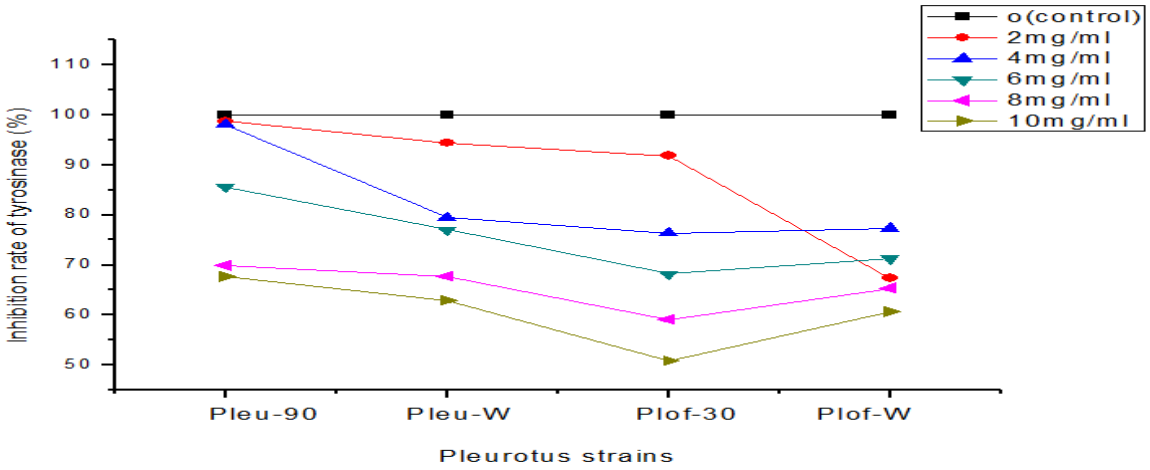

Figure 16 Inhibitory effect of different concentrations of EDTA on tyrosinase activity of four *Pleurotus* strains.

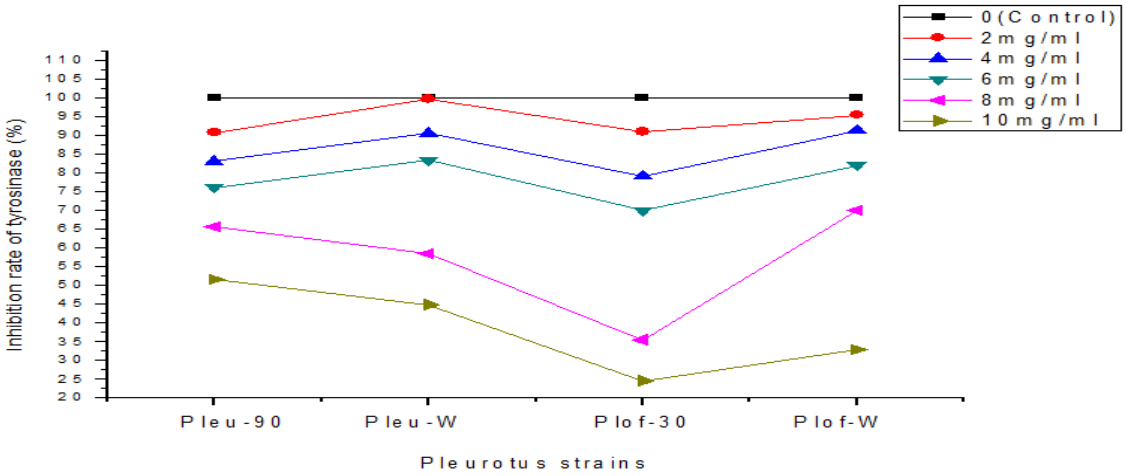

Figure 17 Inhibitory effect of different concentrations of Gallic acid on tyrosinase activity of four *Pleurotus* strains.

4 Discussion

Mushrooms are appreciated globally because they are the source of immense benefits to humans and the environment. They are nutritionally and functionally beneficial, and useful in tackling a lot of waste problems in society. Mushrooms are a goldmine of potential scientific and commercial bioactive metabolites like important enzymes, enzyme inhibitors, and nutraceuticals that can be biotechnological applied for useful purposes. This study focused on determining the amino acid composition and assessing the anti-tyrosinase inhibitory activities of metabolites from four selected oyster mushrooms that were cultivated under liquid fermentation to authenticate their nutritional and therapeutic potency.

Results obtained from the analysis of crude and partially purified protein metabolites from the strains of the *Pleurotus* species used in the investigation indicated that it is composed of 17 different amino acids. The amino acids not detected in this study could be explained as enough quantities of the amino acids could not be fluorescence because of variations in experimental parameters.

Taken together, the profiles of the amino acid of the four oyster mushroom strains are comparable because they all are from the *Pleurotaceae* family. Generally, the crude tyrosinase had a higher quantity of amino acids than the partially purified type. Also, the result showed varying percentages of essential and non-essential amino acids are found in crude and partially purified protein metabolite samples for all the mushroom strains. Concerning abundance, glutamic acid, alanine, and valine are the topmost amino acids found in mushroom strains.

These findings are nearly in tandem with results obtained in the work done by various researchers (Manzi et al., 1999; Mattila et al., 2002; Akindahunsi & Oyetayo, 2006; Pornariya & Kanok-Orn, 2009; Kayode et al., 2015; Maninen et al., 2018; Jaures et al., 2019; Yin et al., 2019) on different mushroom species. They reported that an appreciable amount of total amino acids was present in different mushrooms that included *Pleurotus* species and glutamic acid, aspartic acid and arginine were more in terms of their abundance.

Changes observed in the quantity and composition of amino acids found in this similar (*Pleurotus* strains) could be due to diverse production/ cultivation parameters and also due to genetic shift/variation as explained in earlier studies (Chiu et al. 1998; Mendez et al. 2005; Zhang et al. 2013).

The findings indicated that tryptophan was minute relative to other amino acids abundance in the four *Pleurotus* strains which are in agreement with the reports of Manzi et al. (1999) and Mdachi et al. (2004). Of importance, mushroom metabolites are known vital resources that possess nutritional and therapeutic benefits, which have a complete amino acids production profile (essential and non-essential).

Some of these amino acids present in mushrooms like arginine are effective as functional supplements in most cancer patients by boosting their immunity, delaying the rapid and uncontrollable growth of cancerous cells, enhancing their life expectancy among other benefits (Novaes et al., 2011). Furthermore, tyrosinase and its inhibitors have received increasing attention lately as biotechnologically vital biometabolite worthy of further investigations. In the current study also moderate to higher anti-tyrosinase inhibitory activity was reported and this is concentration dependent. The results reported by Fattahifar et al. (2018), Li et al. (2019), and Selinheimo et al. (2006) in their different investigations are in agreement with the results of the present findings. Also, the studies on different tyrosinase inhibitors from diverse synthetic and natural sources were comprehensively reported an appreciable tyrosinase inhibitory activity and these results are correlated with the findings of the current study (Wang et al. 2015; Mukherjee et al., 2018; Zolghadri et al., 2019).

The mechanisms attributable/behind the inhibitory effect were reported to be substrate permission of access to active site through structural modifications; also, the inhibitors copper chelating capability (Baek et al., 2008; Hu et al. 2016), which ultimately reduces the enzymatic activity. More so, the antioxidant potency of these inhibitors is also a possible mechanism for anti-tyrosinase actions (Momtaz et al. 2008).

However, further research studies are required to be carried out to elucidate the mechanisms behind the inhibitory activities of these oyster mushroom metabolite extracts. The catalytic studies and kinetics of the inhibitory actions are also needed to be determined in a future study.

Conclusion

In conclusion, our results from this investigation affirmed that metabolites from edible *Pleurotus* strains are a repository of bioactive metabolites that can be explored for their biotechnological potentials in terms of their nutritious functional food status and therapeutic application potentials for the benefits of man and the society at large.

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