

Assessment of Bioactive Components (Ergothioneine Contents) in Three Oyster Mushrooms for Antioxidant Potentials

Nmom, F. W., Orlu, H. and Agbagwa, S. S*

Department of Plant Science and Biotechnology,
 Rivers State University, Nkpolu-Oroworukwo,
 P.M.B. 5080, Port Harcourt, Nigeria

*Corresponding Author: samuelagbagwa@gmail.com

ABSTRACT

Fungi are sources of bioactive compounds, yet to be fully harnessed. Understanding bioactive components in macrofungi could serve as a lead for investigating their biological activities and medicinal potentials. The bioactive compounds in the chloroform extracts of *Pleurotus ostreatus*, *P. tuber-regium* and *Lentinus squarrosulus* were investigated, using Gas chromatography-mass spectrometry (GC-MS). Results revealed nine bioactive components present in *L. squarrosulus*. The highest component and quantity was 9, 12-octadecanoic acid ethyl ester (37.49%) followed by Hexadecanoic acid ethyl ester (14.49%) while the lowest (4.04%) as 3a, 6-methano-3AH-indene, 2, 3, 6, 7 tetrahydro. Other fatty acids and their ethyl esters as well as other compounds included; 2-Butenethioic acid; 3-(ethylthio)-S- (1-methylethyl ester (4.51%); Hexadecanoic acid (4.74%); 9, 12 – Octadecadienoic acid (Z-Z) – (11.14%); 9, 17-Octadecadienal, (Z) – (5.01%); 9, 12 – octadecadienoic acid (Z, Z) – Z hydroxyl – 1 – (hydroxymethyl) ethyl ester (12.68%) and Ethyl oleate (5.27%). Five bioactive compounds were harvested from *Pleurotus ostreatus* of which Linoleic acid was the highest (70.98%) and Cis-vecanic acid occurred as the lowest (2.46%). *P. tuber-regium* expressed 14 bioactive compounds with N-Hexadecanoic acid recording the highest quantity (28.83%); Ethane, 1, 1, 2-trichloro-Z-fluoro (9.15%) and the lowest in quantity was 2-Hexenal (E) - with (3.18%). These compounds are derivatives of Ergothioneine implicated in anti-microbial, antioxidant, hepatoprotective, hypocholesterolemic and in cancer prevention, amongst many other bioactivities. This study has shown bioactive components for therapeutic potentials in the oyster mushrooms, while creating a platform for screening, isolating and identifying many bioactive compounds which are thought to be useful in the treatment of various ailments, disorders and diseases of mankind in the nearest future.

Keywords: Ergothioneine, oyster mushrooms, *L. squarrosulus*, bioactive compounds, antioxidants, free radicals, cancer.

Introduction

Over the time, mushrooms have been relished by various cultures as an integral part of food and tastes in diets and as basic ingredients of ethnopharmacology and folklore medicines (Wi Young Lee *et al.*, 2009; Konstantinos *et al.*, 2021). Mushrooms are also relished because they contain digestible carbohydrates, less fat and higher protein contents than most vegetables. Certain mushrooms such as oyster mushroom have been reported to be loaded with fiber, vitamins, minerals and low in carbohydrate, improving human health and promoting quality life health (Valverde., 2015). Oyster mushrooms, otherwise known as *Pleurotus* species are a group of gilled mushrooms.

They include many types such as *Pleurotus ostreatus*, *Pleurotus tuber-regium*, *Lentinus squarrosulus* and many other *Pleurotus* species (Akanmu *et al.*, 1991). *Pleurotus ostreatus* is a common edible mushroom that was first cultivated in Germany as a subsistence measure during the World War I and commercially around the world for food (Dulay *et al.*, 2020).

Pleurotus tuber-regium, also known as king tuber mushroom is a tropical edible gilled mushroom native to Africa, Asia and Australia. *Lentinus squarrosulus* is also an edible oyster mushroom that belongs to the family polyporaceae. It is commonly found in the wild in Africa and Malaysia and has not yet been cultivated on a large scale (Bao *et al.*, 2010).

Another important reason why people consume oyster mushrooms is because they contain many bioactive chemical compounds that are of immense benefits to human health (Feng *et al.*, 2019). They are also good sources of antioxidants that help to reduce cellular damage in human body. Oyster mushrooms are reported to be beneficial to human heart health (Chen *et al.*, 2012). In 2021, it was found in the trading control study that eating food containing oyster mushroom for 21 days decreased triglycerides, total cholesterol and oxidized LDL (bad cholesterol) levels compared with other treatments (Chen *et al.*, 2013).

On the other hand, oyster mushrooms are known for impressive flavor, ease of cultivation and high nutritional values. They also contain many chemical compounds that are beneficial to human health; one of which is a rare amino acid compound known as Ergothioneine and lovastatin. These are strong antioxidants with established bioactive properties (Wudi *et al.*, 2020; Tsiantas *et al.*, 2021).

Ergothioneine is a naturally occurring amino acid; a derivative of histidine containing sulfur atom on the imidazole ring. It is a water-soluble compound, reported abundant in oyster mushrooms (Rathore *et al.*, 2019). Different transporters of tissues in the body are highly specific to ergothioneine. It is reported as readily absorbed into the blood after consumption of the mushrooms and stored in tissues for up to one month (Wi Young Lee *et al.*, 2009; Kalaras *et al.*, 2017; Windi *et al.*, 2020). Ergothioneine occurs in relatively few organisms, namely; Actinomycota, cyanobacteria and fungi, especially mushrooms (Fahey, 2001; Pfeiffer *et al.*, 2011). It is otherwise known as L-ergothioneine, (+) – ergothioneine, Ergo or thio-histidine betaine. Ergothioneine was reported to be discovered by Charles Tanret in 1909, while investigating the ergot fungus of *Claviceps purpurea* for which it was purified (Tanret, 1909).

In humans, ergothioneine is exclusively acquired through diet where it accumulates in erythrocytes, bone marrow, liver, kidney, brain, Red Blood Cells (RBCs), seminal fluids, the eyes and ocular tissues of human.

However, a specific function and role of this chemical still need to be clarified since a specific carrier is present in many tissues; ergothioneine may have some relevance in human health (Grandermn *et al.*, 2005; 2012; Rajakumar *et al.*, 2017; Robert, 2022).

Ergothioneine has been suggested to be an important biological anti-oxidative stress in mitochondrion (Paul and Synder, 2010). Cheah and Halliwell (2012) and Carta *et al.* (2017), suggested that it can protect against the damage to tissues due to oxidative stress and reduce the side effects of reactive oxygen; as well as protect the water-soluble protein from damage. Paul and Synder (1982), Kawano *et al.* (1982), Huang *et al.* (2010) and Apparoo (2023) reported that the scavenging of hypochlorous acid and deactivation of singlet oxygen are possible by ergothioneine. They added that Ergothioneine can rapidly be cleared from circulation and retained in cells and tissues.

However, Servillo, *et al.* (2015); Oyekunle (2017) and Gianfrance (2017) reported that, it is minimally metabolized, possibly by an oxidative degradation mechanism into hercynine and free sulfate before being excreted. Although, its effect *in vivo* is yet under preliminary study; its physiological roles in human is also yet unknown (Cheah and Halliwell, 2012; Phan *et al.*, 2013; Vanitha *et al.*, 2019; Bhalla *et al.*, 2021).

In the health sector, ergothioneine anti-oxidants nature are reported to be potential in preventing diseases such as cardiovascular disease, kidney disease, cancer and inhibit the growth of tumor cell (Valverde *et al.*, 2015). Additional reports by Lindequest *et al.* (2015); Mchanasundram *et al.* (2021); Roseline and Priya (2021) and Fernandes *et al.* (2013) suggested that ergothioneine can be used as supplement and medicine. It is based on the numerous benefits of ergothioneine in mushrooms consumptions for human health that this study is designed to investigate the presence and contents of Ergothioneine and its derivatives in three oyster mushrooms; *Pleurotus ostreatus*, *P. tuber-regium* and *Lentinus squarrosulus*; commonly used in Southern Nigeria and around the world, so as to ascertain the potentials of ergothioneine to human health and encourage their cultivation.

Materials and Methods

Source of Oyster mushroom Samples and Sample Preparation

The Oyster mushroom samples used for this study are; *Lentinus squarrosulus*, *Pleurotus tuber-regium* and *Pleurotus ostreatus*. *P. ostreatus* was procured from Dilomat Farms and Services, located in Rivers State University; while *Pleurotus tuber-regium* was raised in the screen house of Plant Science and Biotechnology, Rivers State University, Port Harcourt. *Lentinus squarrosulus* was obtained from the wild, in Omuanwa in Ikwerre Local Government Area of Rivers State that lies roughly within the coordinates of 4^o: 50N 5^o: 15N 6^o: 3 DE 7^o: 15E. The geology and geomorphy of the area are intimately associated with that of the Niger Delta. The Oyster mushroom samples were air dried at room temperature for 5 days and ground to powder form for GC-MS analysis.

Analysis of Bioactive Components of Mushrooms

The procedure was carried out using Gas chromatography – mass spectrometry (GC-MS), according to the analysis of Mcclafferty and Gowke (1950). It employed the use of GC-MS characterization of 2µl each of the samples; *Lentinus squarrosulus*, *Pleurotus tuber-regium* and *P. ostreatus*. These were separately injected into the GC column for analysis. The GC (Agilent, 6890N and MS 5973 MSD) were equipped with DB – 5MS capillary column (30M x 0.25MM; film thickness, 0.25µm). The United temperature was set at 40°C which was increased to 150°C at the rate of 10°C/min. The temperature was further increased to 230°C at the rate of 5°C/min. The process continued until the temperature reached for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C at this point. Helium was used as the carrier gas with a flow rate of 1 min/min.

Split ratio and ionization voltage were 110: 1 and 70ev respectively. To identify the unknown components present in the samples, their individual mass spectral peak value were compared each with the database of National Institute of Science and Technology (NIST, 2014). Then, the components were identified after comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram; peak value from the NIST library database. Subsequently, the details about the samples molecular formula, molecular weight, retention time and percentage contents were obtained.

Results

The result of the GC-MS analysis for bioactive components of chloroform extract of *P. ostreatus* is as presented in Table1. The result revealed the presence of five (5) bioactive components which included: Linoleic acid (70.98%) as the highest in quantity. This was followed by n-Hexadecanoic acid (Palmitic acid) (21.76%); other fatty acids, their ethyl ether and other compounds identified included cis-veccanic acid (2.46%), Anthuaergostan (1.70%) and Ergosterol (3.10%) respectively.

The result of the GC-MS analysis for bioactive components of chloroform extract of *Pleurotus tuber-regium* is as presented in Table 2. It revealed the presence of fourteen (14) bioactive components which included: n-Hexadecanoic acid (28.83%) as the highest; followed by ethane 1, 1, 2-trichloro-2-fluoro (9.15%). Other fatty acids and their ether recovered included Acetic acid (6.25%), Ethane, 1, 1, 2, 2-tetrachloro (6.22%); 2-methyl – Z; 2 – 3, 13 – octadecadionol (6.18%), Ethane, 1, 1, 2, 2-tetrachloro (5.28%); Cyclododecanol, 1-aminomethyl (4.65%); Bicyclo (3.1.1) heptanes – 2,3-diol, 2,6,6-trimethyl (4.57%); 1-methyl bicycle (3.3.0) octane -3,7-dione, (4.87%); undec-10 ynoic acid (3.93%); carbazic acid (4.07); 2 Hexenal (3.81%) and Taitaric acid (3.92%).

Table 1: Bioactive Components of Chloroform Extract of *Pleurotus ostreatus*

S/N	RT/min	Bioactive Component	Formula	Molecular Weight (g/mol)	Percentage (%)
1	2.090	Cis-veccanic acid	C ₁₈ H ₃₄ O ₂	282	2.46
2	24.094	Palmitic acid	C ₂ OH ₃₅ F ₃ O ₂	256	21.76
3	27.283	Linoleic acid	C ₁₈ H ₃₄ O ₂	280	70.98
4	39.617	Authiaergostan	C ₁₈ H ₃₄ O ₂	436	1.70
5	43.143	Ergosterol	C ₂₄ H ₄₄ O	396	3.10

Legend: RT min = Retention time per minute

Table 2: Bioactive components of chloroform extract of *Pleurotus tuber-regium*

S/N	RT/min	Bioactive Component	Formula	Molecular Weight (g/mol)	Percentage (%)
1	7.629	Tartaric acid	C ₄ H ₆ O ₆	150	3.92
2	8.737	Ethane, 1, 1, 2-trichloro-2-fluoro	C ₂ H ₂ Cl ₃ F	150	9.15
3	8.972	Bycyclo (3.1.1) heptanes-2, 3, -diol, 2, 6, 6-trimethyl	C ₁₀ H ₁₈ O ₂	170	4.57
4	9.046	Bicycle (3.1.1) heptanes-2, 3-diol, 2, 6, 6-trimethyl	C ₁₃ H ₂₇ NO	213	4.65
5	10.395	1-methylbicyclo (3.3.0) Octane-3,7-dione	C ₉ H ₁₂ O ₂	152	4.87
6	11.075	Undec-10-ynoic acid	C ₁₁ H ₁₈ O ₂	182	3.93
7	11.206	Ethane, 1,1,2,2-tetrachloro or Tetrachloroethane	C ₂ H ₂ Cl ₄	166	6.22
8	12.075	Undec-10-ynoic acid	C ₁₁ H ₁₈ O ₂	182	3.93
9	11.772	Hydrazinecarbodithioic acidl-methyl-methyl ester or carbuzic acid	C ₃ H ₈ N ₂ S ₂	136	4.07
10	12.544	2 Hexenal (E)	C ₆ H ₁₀ O	98	3.81
11	14.818	Trifluoroacetic acid or Acetic acid	C ₂ HF ₃ O ₂	114	6.25
12	15.761	Ethane, 1,1,2,2-tetrachloro	C ₂ H ₂ Cl ₄	166	5.28
13	17.988	2-methyle – 2,2-3 Octadecadienol	C ₁₉ H ₃₆ O	280	6.18
14	24.369	N-Hexadecanoic acid acid	C ₁₆ H ₃₂ O ₂	256	28.83

Legend: RT/min = Retention time per minute

The GC-MS analysis of the chloroform extract of *Lentinus squarrosulus* (Table 3) revealed the presence of nine (9) bioactive components; namely; 9, 12-Octadecanoic acid ethyl ester (37.39%) as the highest component quantity. This was followed by Hexadecanoic acid ethyl ester (14.49%). The other fatty acids and their ethl esters as well as other components identified included: 2-Butanethioic acid, 3-(ethylthio) – S (1-Methylethyl) ester (4.51%); n-Hexadecanoic acid (4.74%); 9, 12-Octadecadienoic acid (2,2) (11.88%); 9, 17-octadecadienal, (Z) – (5.01%); ethyl oleate (5.27%), 3a, 6-methano-3a H-indene, 2, 3, 6, 7 tetrahydro (4.04%); 9, 120 Octadecadienoic acid (z,z)-, 2 hydroxy-1 (hydroxymethyl) ethyl ester (12.68%).

Table 3: Bioactive Components of Chloroform Extract of *Lentinus squarrosulus*

S/N	RT/min	Bioactive Component	Formula	Molecular Weight (g/mol)	Percentage (%)
1	15.36	2-Butenethioic acid, 3-(ethylthio) –S (1-methyl) ester	C ₇ H ₁₀ O ₄	158.15	4.51
2	36.18	Hexadecanoic acid	C ₁₀ H ₈	128	4.74
3	36.54	Hexadecanoic acid ethyl ester	C ₁₇ H ₂₈ O ₂	364	14.49
4	38.01	9,12-Octadecanoic acid	C ₁₀ H ₈	128	37.49
5	39.13	9,12-Octadecanoic acid (Z-Z)	C ₁₆ H ₃₂	224	11.14
6	39.48	9,17-Octadecadienal (Z)	C ₁₈ H ₃₂ O ₂	264.5	5.01
7	39.89	Ethyl oleate	C ₂₀ H ₃₈ O ₂	310.5	5.27
8	48.32	3a, 6-Methano-3AH-indene, 2, 3, 6, 7 tetrahydro	C ₂₀ H ₄₀ O	296	4.04
9	48.68	9, 12 – Octadecadienoic acid (Z, Z) – 2 hydroxyl – 1 – (hydroxymethyl ethyl ester	C ₂₂ H ₄₆	310	12.68

Legend: RT/min = Retention time per minute

Discussion

The findings of this study indicated that the bioactive compounds extracted from the samples are derivatives of ergothioneine. Cis – vacanic acid (Cis – 9 octadecenoic acid), also known as oleic acid is an omega 7 fatty acid known for various biological effects, such as antimicrobial and hypolipidemic effects (Oyekunle 2017). It has multiple modes of actions such as antioxidant activity which involves neutralization of free – radicals and reduction of oxidative stress. It also functions as anti-inflammatory agent by decreasing the production of cytokines that promote inflammation. This result on the bioactive components of oleic acid contained in *Pleurotus ostreatus* is in line with the report of Cheah and Halliwell (2012) and Carta et al. (2017) who suggested that any substance where ergothioneine occurs, it exerts important biological anti-oxidant against oxidative stress in the mitochondrion. Paul and Synder (2010) also reported that it protects against the damage of oxidative stress and reduce the side effects of reactive oxygen as well as protect the water soluble protein from damage. Those are essentials; hence oleic acid is a derivative of ergothioneine. The findings of this present study also well align with the suggestions of Kawano et al. (1982) and Huang et al. (2010) as well as Apparo (2023) who reported on the important biological antioxidants of ergothioneine in oyster mushrooms and that the scavenging of hypochlorous acid and deaction of singlet oxygen are possible by ergothioneine.

Another important bioactive compound derived from this study is palmitic acid, also known as n-hexadecanoic acid. It is the most common saturated fatty acid found in human body and can be provided on diet or synthesized from other fatty acids, carbohydrate or amino acid. It supports cellular membrane functions and helps to create sphingolipids found in cell membrane that helps protect the brain and nerve cells. It is also needed to form other beneficial fatty acids and supports skin health in healing some skin diseases such as dryness and rashes etc. These are evident with the report of Grundemman et al. (2005, 2012); Rajakumar et al. (2017) and Robert (2022) who submitted that in humans, ergothioneine is exclusively acquired through diet and accumulates in erythrocytes, bone marrow, liver, kidneys, brain RBC (s), seminal fluid, the eye and human ocular tissues where it possibly supports cellular functions.

The findings align with the report of Servile et al. (2015); Oyekunle (2017) and Gianafra (2017). These had reported that ergothioneine can rapidly be cleared from circulation and retained in cells and tissues where it is minimally metabolized, possibly by an oxidative degradation mechanism into mercynine and free sulfate before being excreted.

Linoleic acid, also known as 9, 12, 15 – octadecatrienoic acid is an essential omega-3 fatty acid found majorly in nut and now in oyster mushroom. It is essential for the synthesis of various hormones, such as prostaglandins, thromboxanes and lenkotrienes which are used for the regulation of many physiological processes. It is thought to decrease the risk of heart disease by helping to maintain normal heart rhythm and pumping; in line with the report of Chen et al. (2012) who suggested that oyster mushrooms are beneficial to human heart functions.

Infact, randomized clinical trials have also shown that replacing saturated fat with inioaic acid reduces total and LDL cholesterol, improves insulin sensitivity and blood pressure. If these are the room, it becomes a booster to the users as this report agrees with the suggestions of Chen et al. (2012); who reported that in 2021, it was found in the trading control study that eating food containing oyster mushroom for 21 days decreased triglycerides, total cholesterol and oxidized LDL (bad cholesterol) levels compared with other treatments.

An important bioactive compound found in this study having strong bearing as a derivative of ergothioneine is ergosterol. The occurrence of ergosterol as ergothioneine derivatives in the chloroform extract as the predominant storoids could be due to the occurrence of ergosterols in yeast and fungal cell membranes (in which oyster mushroom is among) functioning in same manner with the cholesterol in animal cell. It had been shown that in oyster mushrooms (e.g. *P. ostreatus*); ergosterol may exhibit some degree of anti-tumor effects; in line with the report of Yazawa et al. (2000) and Takaku et al. (2001). Also in line with the report of Ramakumar et al. (2007), ergosterol are known to act as biological precursors of vitamin D₂; hence they are classified as provitamins. In line with their sugestions which reported that this bioactive compound is an important biological antioxidant against oxidative stress posed on mitochondria.

2-Butenethioic acid, 3-(ethylthio)-S-(1-methylethyl) oyster is a thioester of butane. Thioesters are organosulfur compounds, a functional group R-S-C (=O) – R, analogues to carboxylate esters (R-O-C(=O) – R) with the Sulphur in the thioester playing the role of linking oxygen in carboxylate ester. They are precursors and intermediate compounds in biosynthesis of other compounds in living oysters. Typically, acetyl co-enzyme A; a thioester and intermediate in the biosynthesis and degradation of fatty acids. 2-Butenethioic acid, 3-(ethylthio)-S-(1-methylethyl) ester had also been found in *Moringa oleifera* and *Tylophora indica*.

Esters are dietary supplements important to human body in line with the finding of Bhalla Vanitha et al. (2019) and Bhalla et al. (2021) who reported that inclusion of oyster mushrooms to diet reduce tryglycerides, total cholesterol and oxidized LDL. And that they are used to improve digestion, reduce inflammation and boost immune system, the exact report of this study on enteric compounds.

Hexadecenoic acid ethyl ester, also known as palmitic acid ester are triterpens esters found in most edible plants. They express strong antimicrobial activity against most oral microbes in accordance with the report of Huang et al. (2010) and Fernandes et al. (2013) as well as Mohanasunderam et al. (2021) and Roseline and Priya (2021); all of whom accredited ergothioneine as supplement and medicine for healthy living.

9, 12 – octadecanoic acid ethyl ester is a linoleic acid ethyl ester which have other derivatives such 9, 12 – Octadecadienoic acid (Z, Z) – and 9, 12 octadecadienoic acid (z,z) -, 2 hydroxy-1-1 (hydroxymethyl) ethyl ester contained in *Lentinus squarrosulus* exhibiting similar biological activities. They are antimicrobial and are found in several plants. This finding also agreed with the suggestions of Roseline and Priya (2021); Shyamala and Manikandan (2019); Chandra et al. (2015); Younis et al. (2019) and Mahanasundaram et al. (2021); who altogether reported that these compounds are also found in *Sarcotemma acidum*, *Ziziphus oenoplia*, *Ipomen aquatic*. Additionally, their report aligns with the findings of this study as they report that the compounds are well found in *Lactiporus sulphurous*, a mushroom where they are implicated for anti-inflammation and anti-cancer properties.

Ethyl oleate is a long chain fatty acid ethyl ester which function is similar to oleic acid in line with the report of Akin-Osanaye (2011), Abcha et al. (2019), Plaan et al. (2013). Castillo et al. (2012) and Dijkstra (1972) who reported that compound as antimicrobial and antioxidant and also found in other mushrooms such as *Agaricus bisporus* and *Plaurotus giganteus*. 3a, 6-methano-3aH – indene, 2, 3, 6, 7 tetrahydro is a derivative of indene; known for anti-microbial and antioxidant activities which agrees with the submission of fabres et al. (2022) who noted that this could be a contribution to the antioxidant activity exhibited by the oyster mushrooms investigated in this study.

In conclusion, the findings from this study showed that ergothioneine is indeed present in the three oyster mushrooms studied, though occurred as its derivatives implicating its presence in the samples studied. This shows that the chloroform extract of the mushrooms can successfully be applied in the development of more potent and efficient antioxidant, anti-inflammatory and antimicrobial agents for human health enhancement.

References

- Akanmu, D., Cecchins, R., Okezie, I. Aruoma & Halliwell, B. (1991). The antioxidant action of ergothioneine *Archives of Biochemistry and Biophysics*, 288 (1), 10 – 16.
- Apparao, Y., Phan, C. W., Kuppusamu, U. R. & Chang, E. C. W. (2023). Potential role of ergothioneine rich mushrooms as anti-aging candidate through elimination of neuronal senescent cells. *Brain Research*, 1824 (23), 148693.
- Bao, H. N. D., Osako, K. & Ohshima, T. (2010). Value-added use of mushroom ergothioneine as a colour stabilizer in processed fish and meats. *Journal of the Science of Food and Agriculture*, 90, 1634 – 1641.
- Bhalla, N., Ingle, N., Patri, S. V. & Havanath, D. (2021). Phytochemical analysis of *Moringa oleifera* leaves extract by GC-MS and free radicals scavenging potency for industrial applications. *Saudi Journal of Biological Science*, 28(12), 6915 - 6928

- Carta, G., Murru, E., Banni, S. & Manca, C. (2017). Palmitic acid: Physiological role, metabolism and nutritional implication. *Front physiol*, 8, 902.
- Cheah, I. K. & Halliwell, B. (2012). Ergothioneine; antioxidant potential, physiological function and role in disease. *Biochem. Biophysica Acta-Molecular Basis of Disease*, 1822, 784 – 793.
- Chen, J., Yong, Y., Xing, M., Zhang, Z., Zhang, S. & Lu, L. (2013). Characterization of Polysaccharides with marked inhibitory effect on lipids accumulation in *Pleurotus eryngii*. *Carbohydrates Polym*, 97, 604 – 613.
- Chen, S. Y., Ho, K. J., Hsieh, Y. J., Wang, L. T. & Mau, J. L. (2020). Contents of Lovastatin, γ -amino butyric acid and ergothioneine in Mushroom fruiting bodies and mycelia. *LWT. Food Sci. Technology*, 47, 274 – 278.
- Coyekunle, B. T. (2017). Analysis of the chemical composition of the essential oil extracted from *T. peruviana* seeds using gas, chromatography analysis. *American Journal of Engineering Research*, 6(10), 51 – 55.
- Dulay, R. M. R., Vicente, J. J. A., Cruz, A. D. & Gagarin, J. M. (2016). Antioxidant activity and Total phenolic content of *Volvanella volvacca* and *Schizo phylum commune* mycolia culturck in indigenous liquid media. *Mycosphere*, Doi:10.5943/mycosphere/7/2/4.
- Fahey, R. C. (2001). Novel thiols of Prokaryotes. *Annual Review Microbiology*, 55, 333 – 356.
- Feng, L., Cheah, I. K., Ng, M. M., Li, J., Chan, S. M., Lim, S. L., Mahendran, R., Kua, E. H. & Halliwell, B. (2019). The association between mushroom consumption and mild cognitive impairment: a community-based cross – sectional study in Singapore. *J. Alzheimers Dis*, 68(1), 197 – 203.
- Fernandas, G. M., Possenti, R. A., & De Mattos, W. T. S. (2013). *Arch. Anim. Nutr*, 67(5), 393 – 405.
- Gianafrica, C., Elisabattamm Sabastiano, B. & Claudia, M. (2017). Palmitic acid physiological role, metabolism and nutritional implications. *Frantiers in Physiology*, 8, 1 – 14.
- Gundermn, D. (2012). The ergothioneine transporter controls and indicates ergothioneine activity – A review. *Preventive Medicine (Baltim)*, 54, 571 – 574.
- Gundermn, D. Harlfinger, S., Golz, S., Geerts, A., Lazar, A., Berkels, R., Jung, N., Rubbert, A. & Sch “Onug, E. (2005). Discovery of the ergothioneine transporter. *In: the Proceedings of the National Academy of Sciences*, 102, 5256 – 5261.
- Huang, C. B., George, B. & Ebersole, J. L. (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archivas of Oral Biology*, 55(8), 555 – 560.
- Kalaras, M. D., Richie, J. P., Calcagnotto, A. & Beelman, R. B. (2017). Mushrooms: A rich source of the antioxidants ergothioneine and glutathione. *Food Chemistry*. 233, 429 – 433.
- Kawano, H., Higudi, F., Mayumi, T. & Hama, T. (1982). Studies on Ergothioeieine, viii. Some effects on orgothioeieine on glycolytic metabolism in red blood cells from rats. *Chem. Pharm. Bull (Tokyo)*, 30, 2611 – 2613.
- Konstantinos, K., Tsaloglidou, A., Kourkouta, L. & Papatthanasiou, L. U. (2021). Simulation in clinical nursing education. *Pubmed National Library of Medicine*.
- Kumosani, T. A. (2001). L-ergothioneine level in red blood cells of healthy human males in the Western Province of Saudi Arabia. *Exp. Mol. Med*, 33, 20 – 22.
- Lindequest, K. A., Maccormack, J. & Shablack, H. (2015). The role of language in emotions. Predictions from psychological constructionism. *Frontiers in Psychology*, 6.
- Mclafferty, F & Gohlke, R. (1950). Procedure for gas chromatography. Dow Circle, ACS. 1155, Sixteenth Street NW, Washington DC, 20036 USA.
- Mohanasundaram, D., Bhaskar, R., Sankarganesh, M., Nehru, K. G. & Kumar, G. (2021). A simple pyridine based flurescent chemosensors for copper ion. *Spectrochim Acta A Mol Bionol Spectrosc*.
- NIST (2014). National Institute of Science and Technology. *US Department of Commerce*. 100

Bereaue Drive Gaithersburg MD, 20899-301-975-2000.

Oyekunle, B. T. (2017). Analysis of the chemical composition of the essential oil extracted from *T. peruviana* seeds using gas, chromatography analysis. *American Journal of Engineering Research*, 6(10), 51 – 55.

Paul, B. D. & Synder, S. H. (2010). The unusual amino acid L-ergothioneine is a physiologic cytoprotectant. *Cell Death and Differentiation*, 17, 1134 – 1140.

Pfeiffer, C., Bauer, T., Surek, B., Schomig, E. & Grundermann, D. (2011). Cyanobacteria produce high levels of ergothioneine. *Food Chem*, 129(4), 1766 – 1769.

Phan, C. W., Lee, G. S., Macreadie, I. G., Malek, S. N. A., Pamda, D. & Sabaratnam, V. (2013). Lipid constituents of the edible mushrooms *Pleurotus giganteus* demonstrate anti candida activity. *Natural Product Communications*, 8(12), 1934518x1300801.

Rajakumar, S. V., Dianopoulos, M. A., Palumbo, A., Blade, J., Merlini, G. & Matros, M. V. (2017). *Int. Psycloma Working Group Updated Criteria for the Diagnosis of Multiple Myeloma. The Lancet Oncology* 15(12), C538-C548.

Rathore, H., Prasad, S., Kapri, M., Tiwari, A. & Shaorma, S. (2019). Medicinal importance of mushroom mycelium: mechanisms and applications. *Journal of Functional Foods*, 56, 182 – 193.

Robert, W. C. (2022). The Robert W. Cahn best paper award. *J. Mater. Sci.* (2023), 58, 3375-3376.

Roseline, P. V. & Priya, V. (2021). *Sarcostemma acidum* stem extract. *Int. Journal of Botany Studies*, 6(3), 422 – 426.

Servillo, L., Castaldo, D., Casale, R., Donotrio, N., Giovane, A., Cantela, D. & Balestrieri, M. L. (2015). An uncommon redox behaviour sheds light on the cellular antioxidant properties of ergothioneine. *Free radical. Biol, Med.* 79, 228 – 236.

Tanret, C. (1909). Sur une base nouvelle retirée du seigle ergote, l'ergothioneine. *Compt. Rende, Acad. Sci.*, 149, 222 – 224.

Tsiantas, K., Tsiaka, T., Koutrotsios; Siapi, E., Z. Vasskis, G. I., Kalogeropoulos, N. & Zoumpoulakis, P. (2021). On the identification and Quantification of Ergothioneine and Lovastatin in various mushroom species; Assets and challenges of different analytical approaches. *Molecules*, 26(7), 1832.

Valverde, M. E., Perez, T. H. & Paredes-Lopez, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *Journal of Microbiology*, 7(3), 207-220.

Vanitha, A., Vijayakumar, S., Ranjitha, V. & Kalimuthus, K. (2019). Phytochemical screening and antimicrobial activity of wild and tissue culture plant extracts of *Tylophora indica*. *Asian Journal of Pharmacy and Pharmacology*, 5(1), 21 – 32.

Wi Young, L., Park, E., Alin, J. K. & Kang-Hyeon K. (2009). Ergothioneine contents in fruiting bodies and their enhancement in mycelial cultures by the addition of methionine. *Mycobiology*, 37(1), 43 – 47.

Windi, R., Offending F., Oonaah A., Estiningtyas Q., Advani S., Ramadan K. & Wedad M. A. (2020). Determinants of acute respiratory infection among children under five years in Indonesia. *Journal of Pediatric Nursing*, 60, e54 – e59.