Jurnal Teknologi

Full Paper

POTENTIAL ANTI-AGING AND PHENOLIC COMPOUNDS IN DIFFERENT PARTS OF *PLEUROTUS PULMONARIUS* MUSHROOM EXTRACTS CULTIVATED ON DIFFERENT SUBSTRATES

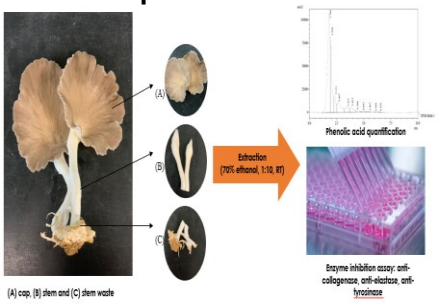
Nik Nur Azwanida Zakaria[a,b*], Norsyafiera Syuhada Fadzil[a], Zuharlida Tuan Harith[a,b], Kharul Azmi Mua'azam Abdul Rahman[a], Mohammad Feizal Daud[c], Siti Maryam Salamah Ab Rhaman[a]

[a]Faculty of Agrobased Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[b]Functional Agroproduct Research Group, Faculty of Agrobased Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[c]Field Crops Production and Product Quality Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM) Melaka, Jasin Campus, Melaka, Malaysia

Article history
Received
17 July 2024
Received in revised form
6 November 2024
Accepted
3 December 2024
Published Online
26 June 2025

*Corresponding author
azwanida@umk.edu.my

Graphical abstract



Abstract

Pleurotus mushrooms (*Pleurotus* spp.) are edible fungi known for their rich bioactive compounds and their ability to grow on various lignocellulosic substrates including agriculture byproducts. The unique attributes of *Pleurotus* spp. have gained popularity, thereby increasing the cultivation of *Pleurotus* mushroom among farmers. In this study, *Pleurotus pulmonarius* was cultivated using two different substrates sawdust substrate ((SS) and pineapple-waste substrate (PS)) and separated into caps, stems and stem waste after harvest. The mushrooms parts were extracted using 80% ethanol prior to analysis of the bioactivities and specific phenolic acids. Anti-aging activities of *P. pulmonarius* samples were evaluated using enzyme inhibition reactions against tyrosinase, elastase and collagenase. Quantification of phenolic acids was carried out using high-performance liquid chromatography coupled with a diode-array detector (HPLC-DAD). PS-cap samples demonstrated the highest potency against tyrosinase (IC_{50}: 21.22 ± 0.20 mg/mL) and collagenase (IC_{50}: 26.12 ± 1.17 mg/mL), while SS-cap exhibited the highest elastase inhibition atIC50: 36.39 ± 1.93 mg/mL. Additionally, p-coumaric acid was higher in the stem waste parts with 7.37 ± 0.88 mg/g in SS-stem waste and 5.99 ± 0.78 mg/g in PS-stem waste. This study presents the first report on the anti-elastase and anti-collagenase activities of *Pleurotus* mushroom extract and the potential bioactivities and phenolics in the stem waste extracts. In conclusion, this study highlights the bioactivities and phenolic acid compounds of *Pleurotus* mushroom extracts cultivated on different substrates, indicating their promising applications in various industries.

Keywords: *Pleurotus pulmonarius*, bioactivities, phenolics, agriculture sustainability, pineapple waste

Abstrak

Cendawan *Pleurotus* (*Pleurotus* spp.) ialah kulat boleh dimakan yang terkenal, kaya dengan sebatian bioaktif dan keupayaannya untuk tumbuh pada pelbagai substrat lignoselulosa termasuk hasil sampingan pertanian. Ciri-ciri unik *Pleurotus* spp. telah mendapat populariti, sekali gus meningkatkan penanaman cendawan *Pleurotus* di kalangan petani. Dalam kajian ini, *Pleurotus pulmonarius* ditanam menggunakan dua substrat berbeza (substrat habuk kayu (SS) dan substrat sisa nanas (PS)) dan diasingkan kepada penutup, batang dan sisa batang selepas dituai. Bahagian cendawan diekstrak menggunakan 80% etanol sebelum analisis bioaktiviti dan asid fenolik tertentu. Aktiviti anti-penuaan sampel *P. pulmonarius* dinilai menggunakan tindak balas perencatan enzim terhadap tyrosinase, elastase dan kolagenase. Pengukuran asid fenolik telah dijalankan menggunakan kromatografi cecair berprestasi tinggi ditambah dengan pengesan tatasusunan diod (HPLC-DAD). Sampel PS-cap menunjukkan potensi tertinggi terhadap tyrosinase (IC_{50}: 21.22 ± 0.20 mg/mL) dan kolagenase (IC_{50}: 26.12 ± 1.17 mg/mL), manakala SS-cap mempamerkan perencatan elastase tertinggi pada IC_{50}: 36.39 ± 1.93 mg/mL. Selain itu, asid p-coumaric adalah lebih tinggi dalam bahagian sisa batang dengan 7.37 ± 0.88 mg/g dalam sisa batang-SS dan 5.99 ± 0.78 mg/g dalam sisa batang-PS. Kajian ini membentangkan laporan pertama mengenai aktiviti anti-elastase dan anti-kolagenase ekstrak cendawan *Pleurotus* dan potensi bioaktiviti dan fenolik dalam ekstrak sisa batang. Kesimpulannya, kajian ini menekankan bioaktiviti dan sebatian asid fenolik ekstrak cendawan *Pleurotus* yang ditanam pada substrat yang berbeza, menunjukkan keterjaminan aplikasinya dalam pelbagai industri.

Kata kunci: *Pleurotus pulmonarius*, bioaktiviti, fenolik, kelestarian pertanian, sisa nenas

1.0 INTRODUCTION

Mushrooms can be classified into four main groups; edible, therapeutic, toxic mushrooms, and a diverse category of unclassified mushrooms [1]. *Pleurotus* mushrooms (*Pleurotus* spp.) or known as oyster mushroom has been widely cultivated all around the world due to their edibility and safe to consume as well as their ability to grow naturally on different substrates including woody materials, primarily due to their ligninolytic properties [2]. Since 1997, there has been a significant increase in the cultivation and consumption of *Pleurotus* mushrooms, with per capita consumption rising from 1.0 to 4.7 kg in 2013 worldwide [2, 3]. The increasing cultivation of mushrooms particularly in Malaysia has generated a significant amount of stem waste, which is the leftover material from the mushroom body and is typically discarded before selling to retailers. Previous studies have reported that the quantity of residual parts, including stem waste, can range from 5 to 20% of the production capacity of a mushroom farm [4]. Additionally, commercial oyster mushroom farms have been observed to generate approximately 50 g of stem waste from a harvest of 300 g [5].

Pleurotus spp. exhibits notable bioactivity due to their rich content of bioactive compounds, including polysaccharides, peptides, proteins, terpenes, fatty acids, and polyphenols [6]. The bioactive compounds found in *Pleurotus* spp. have been shown to possess various health-promoting properties, including antioxidant, anti-aging, antimutagenic, anti-inflammatory, and antibacterial activities [7]. Several factors has been reported able to influence the presence of bioactive compounds in mushrooms including different mushroom species, genetic variation within species, growth conditions (such as substrate type, temperature, humidity, and light exposure), maturity stage, post-harvest handling, and processing techniques [8]. Additionally, environmental factors, including geographical location and climate, can also impact the composition and concentration of bioactive compounds in mushrooms [9]. Despite the substantial research conducted on *Pleurotus* spp., there is limited evidence available regarding their anti-aging properties [10].

Substrate that are used to provide a nourishing environment for the growth and development of *Pleurotus* spp. include agricultural waste materials such as sawdust, straw, corn cobs, cottonseed hulls, and composted mixtures [11]. Wood-based substrates, such as sawdust and wood chips, are commonly used as these substrates offer a rich source of nutrients and mimic the natural environment where these mushrooms thrive [12]. Additionally, it is readily available and relatively cheap. However, different substrates can significantly impact the bioactivity and nutrient content of mushroom extracts [13, 14, 15].

Sawdust is the byproducts of wood processing and timber industry and commonly used material in

various industries including agriculture, construction and furniture industry. It is known as one of the main substrates for the cultivation of mushroom due to its rich in cellulose and hemicellulose content as well as it provides good moisture retention to support mushroom growth. In Malaysia, the plantation of pineapple has increase yearly. This has resulted in accumulation of agriculture waste from pineapple that holds potential to further utilized as it is rich in nutrients, including carbohydrates, proteins, and minerals able to support the growth and development of mushrooms [16]. Parts of pineapple waste including peels, crown, and leaves has been explored as a potential substrate for mushroom cultivation [17, 16]. The utilization of pineapple waste as an alternative to sawdust substrate may have impacts on the bioactive compounds present in mushrooms (*Pleurotus* spp.) and their corresponding biological activities. Therefore, this study was conducted as a comparison study to identify the potential anti-aging properties and phenolic profiles of *P. pulmonarius* parts that were cultivated using pineapple waste and sawdust substrates.

2.0 METHODOLOGY

Sample Preparation

P. pulmonarius blocks from pineapple waste (PS) and sawdust substrates (SS) were obtained from Saifulam Agro Farm, Pontian, Johor (1.682659° N, 103.208170° E). Approximately 500 g of substrate medium was filled into each block. The SS block contained 90% sawdust and a 10% mixture of rice bran powder and agricultural lime. In contrast, the PS block consisted of 90% pineapple residue (leaves and stems) and sawdust in a 6:4 ratio (pineapple waste to sawdust), along with a 10% mixture of rice bran powder and agricultural lime. After 50-60 days of fermentation, the mushrooms were harvested, cleaned, divided into cap, stem, and stem waste parts, and dried at 40 °C for 48 hours. The dried samples were ground and stored in airtight containers until further use.

Sample Extraction

The grounded mushroom samples (cap, stem and stem waste) were extracted using 80% ethanol at a ratio of 1:10 (sample to solvent) by stirring at room temperature with a magnetic stirrer. After 24 hours, the extract was filtered, partially evaporated at 70 °C using a rotary evaporator, and further dried using a freeze-drier. The dried extract was then stored at -80 °C for subsequent analysis.

Anti-tyrosinase Activity

The tyrosinase activity was measured using a modified method as described by Zakaria *et al*., (2019) [18], where 100 µL of the sample at concentrations ranging from 5 to 40 mg/mL were pre-incubated with 100 µL of tyrosinase (300 units/mL) for 15 minutes at 25 °C. Subsequently, 800 µL of L-tyrosine (2 mM) was added to the test tubes, and the tubes were further incubated for 60 minutes. The absorbance of the reaction mixture was measured at 450 nm using a spectrophotometer (UV-1900 Shimadzu). The experiment was conducted in triplicates, and kojic acid at concentrations of 100-500 µg/mL was used as a positive control. Percentage inhibition against the tyrosinase was calculated using Equation 1.

$$\text{Enzyme inhibition (\%)} = (A_0 - A_1)/A_0 \times 100 \quad (1)$$

Where A_0 was the absorbance of the control and A1 was the absorbance of the mushroom extracts. The potency of the extract to inhibit the enzyme was expressed as the IC_{50} value (mg/mL).

Anti-elastase Activity

The elastase activity was determined using a modified method outlined by [18]. In a 96-well microtiter plate, 20 µL of samples at concentrations ranging from 0.5 to 25 mg/mL were mixed with 20 µL of elastase enzyme (0.34 unit/mL) and incubated at 25 °C for 15 minutes. Following this, 240 µL of Tris-HCl buffer (100 mM) and 20 µL of SucAla3-pNA (SANA) substrate (4 mM) were added to the mixture. The plate was then incubated for 60 minutes. The absorbance of the reaction mixture was measured at 410 nm using a microplate reader (iMark). The experiment was conducted in triplicates, and Epigallocatechin gallate (EGCG) at concentrations of 100-500 µg/mL was employed as the positive control. Percentage inhibition against the collagenase was calculated using Equation 1.

Anti-collagenase Activity

The anti-collagenase activity was assessed using a modified version of the method described by Shirzad *et al*. (2018) [19] and Azwanida *et al*. (2020) [20]. In a 96-well microtiter plate, 20 µl of Tricine buffer (50 mM) and Calcium Chloride (10 mM) were combined. To this mixture, 20 µl of samples at concentrations ranging from 0.5 to 25 mg/mL and 20 µl of collagenase enzyme (0.8 unit/mL) were added. The plate was then incubated for 20 minutes at 37 °C under dark conditions. After the initial incubation, 40 µl of N-[3-(2-Furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) substrate (2 mM) was added to each well. The plate was further incubated for 30 minutes at 37 °C in the absence of light. Following the incubation period, the absorbance of the reaction mixture was measured at 410 nm using a microplate reader (iMark). The experiment was conducted in triplicates, and Epigallocatechin gallate (EGCG) was utilized as the positive control. Percentage inhibition against the collagenase was calculated using Equation 1. The

potency of the extract to inhibit the enzyme was expressed as the IC_{50} value (mg/mL).

High-performance Liquid Chromatography (HPLC) Analysis of Phenolic Compounds

The quantification of phenolic compounds was performed using a modified HPLC method by [21] on a Shimadzu UFLC system equipped with an LC-20AT gradient pump, SPD-M20A PDA diode array detector, and Spinchrom LC Solution software. A Phenomenex Luna C18 column (4.6 x 150mm, 5µm particle size) served as stationary phase. The mobile phase consisted of low-pressure gradient of solvents; methanol: 0.1% phosphoric acid in water with the ratio of 70:30 (cinnamic acid and caffeic acid), 20:80 (gallic acid) and 30:70 (p-coumaric acid). Detection was conducted at 272 nm with an injection volume of 20 µL and a flow rate of 1.0 mL/min. The total run time for the analysis was 10 minutes. Sample extraction was diluted to 1 mg/mL, while standard (cinnamic acid, caffeic acid, gallic acid and p-coumaric acid) stock solutions that ranged from 1 to 300 µg/mL, prepared using HPLC-grade methanol. The peaks of different samples and their retention times were compared with those of the standard.

Statistical Analysis

The data obtained in the study was analyzed using GraphPad Prism v.7 software. Analysis of variance (ANOVA) was employed for comparison with $p \leq 0.05$ considered as significant.

3.0 RESULTS AND DISCUSSION

Anti-tyrosinase Activity

Skin pigmentation, primarily regulated by the enzyme tyrosinase, plays a crucial role in determining an individual's skin color and protection against harmful UV radiation [22]. Mushroom extracts have emerged as promising candidates for anti-pigmentation agents due to their bioactive components and potential to modulate tyrosinase activity [23], including *P. pulmonarius* [24]. This current study evaluates the inhibitory of the mushroom extracts cultivated with PS and SS blocks against tyrosinase enzyme. The highest tyrosinase inhibition was observed in PS-cap (77.01 ± 1.20 %), followed by SS-cap (68.5 ± 1.27), PS-stem waste (67.3 ± 1.42 %), PS-stem (65.4 ± 2.30 %), SS-stem (64.2 ± 1.27 %) and SS-stem waste (60.6 ± 1.74 %), as shown in Figure 1. PS-cap (21.22 ± 0.20 mg/mL) also had the highest potency of anti-tyrosinase activity of all the samples (Table 1).

Previous studies have reported the anti-tyrosinase activity of mushroom extract, showing inhibition percentages ranging from 6.90 to 49.6% (hot water extraction) at concentrations of 0.125 to 1.0 mg/mL [24]. In comparison, our IC_{50} values were higher than those reported by [25], who found IC_{50} values ranging from 1.125 to 2.350 mg/mL for anti-tyrosinase activity in their study. The disparity in our results compared to the previous study could be attributed to variations in growing conditions, substrates utilized, solvent selection, and extraction techniques [26]. Additionally, it is worth noting that the previous study examined the entire mushroom, whereas our study focused on a specific part of the mushroom. The anti-tyrosinase activity observed in the extracts is likely due to the presence of phenolic compounds [27]. Phenolics, such as hydroxamic can bind to the active site of the enzyme, causing structural changes and reducing its enzymatic activity [28]. P-coumaric acid, a secondary metabolite found in mushrooms, has been found to exhibit stronger inhibition of melanin production in murine melanoma cells compared to similar compounds [29].

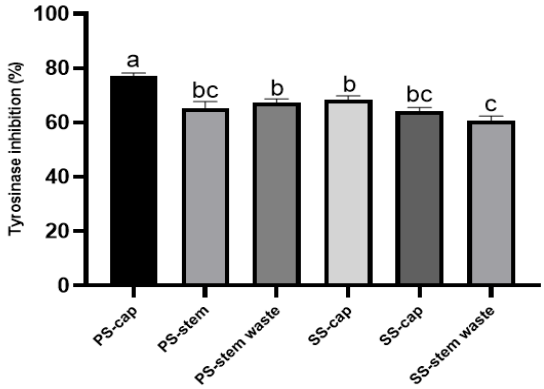


Figure 1 Tyrosinase inhibition at 40 mg/mL of different mushroom extract. Different superscript letters indicate a statistical difference at $p < 0.05$ using ANOVA. (PS=Pineapple waste substrate and SS=Sawdust substrate)

Anti-elastase Activity

Elastase is a proteinase that plays a significant role in the breakdown of elastin, a key protein involves in maintaining the flexibility of the skin. Elastin, in conjunction with other protein constituents, forms an integral part of connective tissue. However, excessive activity of elastase can lead to the degradation of elastin, which in turn can contribute to degenerative diseases and accelerate skin aging. SS-cap extract showed the highest elastase inhibition (34.47 ± 1.14 %), followed by PS-cap (31.52 ± 0.62 %), PS-stem waste (28.25 ± 0.08 %), SS-stem waste (28.43 ± 0.30 %), SS-stem (25.34 ± 1.85 %) and PS-stem (24.81 ± 0.74 %), as shown in Figure 2. It can be observed that the caps in both substrates (SS-cap and PS-cap) have significantly higher anti-elastase potency than other part with IC_{50} values 36.39 and 39.77 mg/mL (Table 1).

While the evidence for *Pleurotus* mushrooms as elastase inhibitors is limited, a study investigating *Tricholoma matsutake* mycelium demonstrated notable elastase inhibition at a dosage of 100 µg/mL,, with inhibition rate of 81.4 ± 3.92 % [30]. The

observed lower inhibitory activities in our study compared to previous studies could potentially be attributed to factors such as the type of sample, species used, and the employed extraction technique. In this study, the detected elastase activity might be linked to the presence of polyphenolic compounds, specifically ellagic acid and tannic acid. These compounds have been previously demonstrated to degrade the proteolytic activity of dermal elastic fibers and enhance skin elasticity [31].

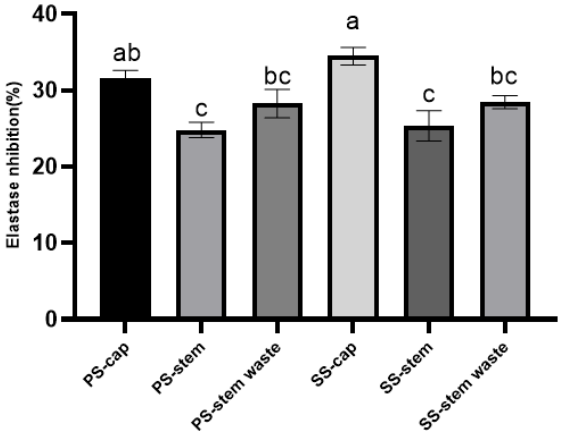


Figure 2 Elastase inhibition of different mushroom parts at 25 mg/mL. Different superscript letters indicate a statistical difference at $p < 0.05$ using ANOVA. (PS=Pineapple waste substrate and SS=Sawdust substrate)

Anti-collagenase Activity

Collagenase is an enzyme with the ability to degrade native collagen under specific conditions [32, 33]. Collagen plays a critical role in skin cell regeneration and contributes to the structural integrity, strength, and elasticity of the skin. It forms fibrous structures in different areas of the human body, particularly in the skin, joints, and bones [34]. Type I collagen is a prominent structural protein, while major fibrillar collagens, including Types I, II, and III, contribute to the formation of the structural framework essential for tissue organization and functionality [35]. Studies have demonstrated that mushroom extracts possess collagenase inhibitory properties, which may contribute to preserving the integrity and stability of collagen in various tissues [10].

Figure 3 shows that PS-cap had the highest collagenase inhibition (48.02 ± 1.08 %), followed by SS-cap (45.07 ± 1.01 %), SS-stem waste (37.22 ± 1.70 %), PS-stem waste (35.95 ± 0.10 %), PS-stem (28.36 ± 0.78 %), and SS-stem (27.79 ± 0.48 %). The assay showed that the caps from both substrates (PS and SS) showed higher potency with IC_{50} values of 26.12 ± 1.17 and 29.13 ± 0.97 mg/mL respectively. Both stem wastes samples (PS and SS) showed higher potency than the stems (PS and SS).

Studies investigating the potential of *Pleurotus* mushrooms as collagenase inhibitors are currently limited. Kim *et al*., (2007) [36] reported that the use of isolated extracellular polysaccharides (EPS) and mycelium extract from *Grifola* mushroom as a collagenase inhibitor resulted in 20% and 40% inhibition at a minimum concentration of 100 µg/mL. Laccaridiones A and B, which were isolated from *Laccaria amethystina*, demonstrated significant collagenase inhibitory activity with IC_{50} values of 7.2 and 5.7 µg/mL, respectively [37]. The variation in outcomes as compared to ours could be attributed to the distinct mushroom species used, as well as the specific type of extracts employed.

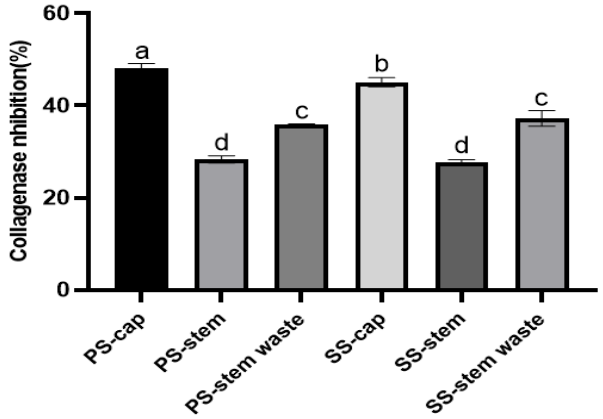


Figure 3 Collagenase inhibition of different mushroom parts at 25 mg/mL. Different superscript letters indicate a statistical difference at $p < 0.05$ using ANOVA. (PS=Pineapple waste substrate and SS=Sawdust substrate)

Table 1 The IC_{50} value of anti-tyrosinase, anti-elastase and anti-collagenase activities

Sample		IC_{50} value (mg/mL)		
		Anti-tyrosinase	Anti-elastase	Anti-collagenase
PS-	cap	21.22 ± 0.20^{a}	39.77 ± 1.95^{a}	26.12 ± 1.17^{a}
	stem	27.88 ± 0.53^{b}	58.28 ± 3.69^{c}	45.53 ± 0.90^{c}
	stem waste	25.92 ± 0.27^{ab}	46.82 ± 3.80^{b}	36.88 ± 0.21^{b}
SS-	cap	25.93 ± 0.66^{ab}	36.39 ± 1.93^{a}	29.13 ± 0.97^{a}
	stem	29.34 ± 0.10^{b}	54.42 ± 3.70^{c}	48.64 ± 1.34^{c}
	stem waste	26.71 ± 0.85^{ab}	47.58 ± 2.77^{b}	34.69 ± 2.43^{b}
Standard		**IC_{50} value (µg/mL)**		
Kojic acid		279.51 ± 0.12	-	-
EGCG		-	9.03 ± 0.27	5.51 ± 0.14

Data are the mean ± SD of triplicates. Different superscript letters indicate a statistical mean difference at $p < 0.05$ using ANOVA within the same column. (PS=Pineapple waste substrate and SS=Sawdust substrate)

Table 1 shows the comparison of the IC_{50} values for the samples and the standards in anti-tyrosinase, anti-elastase and anti-collagenase assays. PS-cap displayed the lowest IC_{50} value against tyrosinase enzyme, suggesting higher potency compared to other samples. However, the result was not as potent as kojic acid standard, a well-known inhibitor of tyrosinase [38]. The inhibition of tyrosinase causes reduction in melanin production and preventing hyperpigmentation, thus contributes to its skin-lightening properties [39]. Some anti-tyrosinase inhibitors also could acts as a UV protector and exhibits antioxidant properties, enhancing its role in skincare [40]. Meanwhile, EGCG standard, a catechin usually found in green tea are known as potent elastase and collagenase inhibitors. The mechanisms of actions of EGCG as anti-elastase and anti-collagenase have been associated with its antioxidant and metal chelating properties, ability to downregulate matrix metalloproteinases (MMPs) expression and interfering with signaling pathways of elastase and collagenase activation [41, 42]. The mushroom extracts show lower potency compared to EGCG, but SS-cap and PS-cap have promising activities against elastase and collagenase. Purification and isolation of compounds from the extract could further enhance the activities [43].

Phenolic Acids Content

Phenolic acids consist of a phenolic ring and a carboxyl functional group. They are categorized into two groups: hydroxy derivatives of benzoic acid (p-hydroxybenzoic, protocatechuic, gallic, gentisic, vanillic, and syringic acids) and the trans-cinnamic acid group (p-coumaric, caffeic, and ferulic acids) [44]. Hydroxybenzoic acid derivatives are typically conjugated and found in complex structures like lignin and hydrolyzable tannins. Meanwhile, hydroxycinnamic acid derivatives are primarily present in conjugated form, bound to cell wall components such as cellulose, lignin, proteins, and organic acids like tartaric or quinic acid through ester linkage [45].

Table 2 shows the LOD and LOQ values for the standards, indicating the method's sensitivity. Meanwhile, the regression equations show strong correlations (R^2=0.99) between concentration and retention time, suggesting the reliability of the HPLC method. The retention times (rt) for the standards; gallic acid (5.692), caffeic acid (2.160), cinnamic acid (3.218) and p-coumaric acid (1.923) were compared with those of samples for identification and quantification. Table 3 shows the retention times of all samples, while Figure 4, 5 and 6 represent chromatograms for gallic, caffeic, cinnamic and p-coumaric acids identification in the samples. p-coumaric acid was found in the highest amount with 7.37 ± 0.88 mg/g in SS-stem waste and lowest found in SS-cap (3.30 ± 0.34 mg/g), as shown in Table 4. However, it was absent in the stem samples of both PS and SS substrates. While, cinnamic acid was found in the range of 0.99-1.46 mg/g. Additionally, gallic acid was detected in all samples (PS and SS) within the range of 2.55 ± 0.05 to 3.16 ± 0.12 mg/g. Caffeic acid content was found to be highest in SS-cap (2.84 ± 0.18 mg/g) and SS-stem (2.71 ± 0.28 mg/g) samples. It was also present in PS-cap (1.99 ± 0.20 mg/g) and PS-stem (1.84 ± 0.12 mg/g) samples. However, no detectable levels of caffeic acid were observed in both stem waste samples (PS and SS).

Table 2 Limit of Detection (LOD) and Limit of Quantification (LOQ) of the HPLC method

Standard	Retention time (rt)	Conc. (µg/mL)	LOD	LOQ	Regression eq.	R^2
Gallic acid	5.692	20-100	0.098	0.298	y=191919x-878099	0.993
Caffeic acid	2.160	50-250	0.033	0.102	y=48104 x+174692	0.992
Cinnamic acid	3.218	10-50	0.070	0.213	y=166997x +43782	0.996
p-coumaric acid	1.923	20-100	0.104	0.317	y=58239 x+16665	0.992

Table 3 Retention times (rt) of *Pleurotus* mushroom extracts for identification of gallic, caffeic cinnamic and p-coumaric acids

Compounds	Pineapple waste substrate (PS)			Sawdust substrate (SS)		
	Cap	Stem	Stem waste	Cap	Stem	Stem waste
Gallic acid	5.688	5.503	5.622	5.683	5.546	5.705
Caffeic acid	2.184	2.188	ND	2.182	2.180	ND
Cinnamic acid	3.248	3.183	3.208	3.126	3.189	3.188
p-coumaric acid	1.963	ND	1.954	1.960	ND	1.958

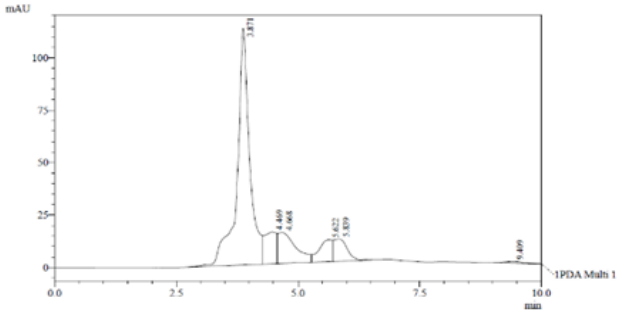


Figure 4 Representative chromatograms for gallic acid identification in sample (SS-stem waste) with peak observed at retention time 5.622

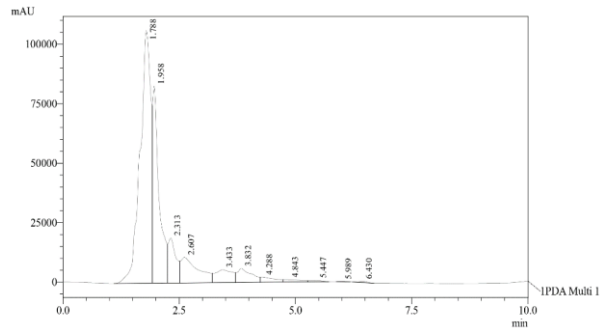


Figure 5 Representative chromatograms for p-coumaric acid identification in sample (SS-stem waste) with peak observed at retention time 1.958

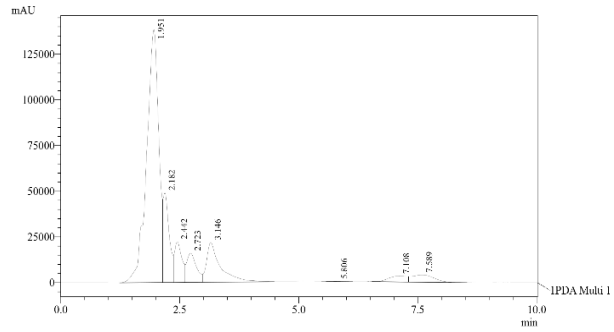


Figure 6 Representative chromatograms for caffeic and cinnamic acids identification in sample (SS-cap) with peak observed at retention times 2.182 and 3.126 respectively

Table 4 Phenolic acid profiles in different parts of *Pleurotus* spp extracts

Phenolics (mg/g)	Pineapple waste substrate (PS)			Sawdust substrate (SS)		
	Cap	Stem	Stem waste	Cap	Stem	Stem waste
Gallic acid	3.16 ± 0.12^{a}	2.79 ± 0.06^{a}	2.92 ± 0.18^{a}	2.76 ± 0.26^{a}	2.55 ± 0.05^{a}	2.71 ± 0.26^{a}
Caffeic acid	1.99 ± 0.20^{b}	1.84 ± 0.12^{b}	ND	2.84 ± 0.18^{a}	2.71 ± 0.28^{a}	ND
Cinnamic acid	1.10 ± 0.33^{a}	0.99 ± 0.29^{a}	1.15 ± 0.13^{a}	1.46 ± 0.02^{a}	1.25 ± 0.27^{a}	1.12 ± 0.07^{a}
p-coumaric acid	3.82 ± 0.46^{c}	ND	5.99 ± 0.78^{b}	3.30 ± 0.34^{c}	ND	7.37 ± 0.88^{a}

Data are the mean ± SD of triplicates. Different superscript letters indicate a statistical mean difference at $p < 0.05$ using ANOVA for each of the compounds.

Phenolic acids, including gallic, caffeic, cinnamic and p-coumarics, have been shown to plays a significant role in contributing to the bioactivity and potential health benefits of *P. ostreatus*, such as antioxidant, anti-inflammatory, and antimicrobial activities [46]. Our findings demonstrate higher level of p-coumaric compared to a study conducted by Dundar *et al.*, (2015) [47], where they were unable to detect any in their *Pleurotus* mushroom. Additionally, Palacios *et al.*, (2011) [48] reported the presence of 11.15 µg/g of p-coumaric acid and 290.34 µg/g of gallic acid in their *Pleurotus* mushroom, while caffeic acid could not be detected. Since p-coumaric and gallic acids are the highest in SS-and PS-stem waste, they could play a role in the observed activities by neutralizing harmful free radicals, protecting against oxidative stress, modifying the bacterial membrane, as well as inhibiting enzymes activities. However, it is also possible that these compounds exert the activities synergistically as the interaction of phenolic compounds can lead to increase effectiveness [49]. Phenolic extracts from some mushroom species, like *Brasiliensis*, *Ganoderma lucidum*, *Boletus edulis* and *Marasmius oreades*, have shown significant enhancement for both antioxidant and antibacterial activities, indicating a synergistic effect [50, 51, 52].

4.0 CONCLUSION

In conclusion, this study highlights the effect of PS substrates in modulating the bioactivities of the *Pleurotus* mushroom extract, depending on the parts of mushrooms. For example, the PS-cap showed the highest inhibitory activity against tyrosinase, elastase and collagenase, and to our knowledge this is the first study reported on the anti-elastase and anti-collagenase of *Pleurotus* mushroom extract. Furthermore, the stem waste extracts obtained from both substrates, PS and SS, show promising potential as a valuable source of bioactive compounds and phenolic acids, with particularly high levels of p-coumaric acid observed. The presence of p-coumaric, gallic, caffeic, and cinnamic acids may contribute to the antimicrobial and antiaging activities exhibited by the *Pleurotus* mushroom samples. Future investigations should explore the impact of varied solvents and extraction methods to optimize the observed bioactivities. Furthermore, it is recommended to assess additional bioactivity parameters using isolated compounds to enhance our understanding and uncover novel applications.

Acknowledgement

This research is funded by the Ministry of Higher Education (MOHE), Malaysia under the Fundamental Research Grant Scheme, (FRGS/1/2021/STG03/UMK/02/3).

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

References

[1] Noor, M., Sivasamugham, L. A., and Subramaniam, G. 2021. A Review on the Antibacterial Activity of Edible Mushrooms. *INTI Journal*. 2021(009). https://doi.org/10.9734/jpri/2021/v33i48a33251.

[2] Iwalokun, B. A., Usen, U. A., Otunba, A. A., & Olukoya, D. K. 2007. Comparative Phytochemical Evaluation, Antimicrobial and Antioxidant Properties of *Pleurotus ostreatus*. *African Journal of Biotechnology*. 6(15): 1732–1739. https://doi.org/10.5897/AJB2007.000-2254.

[3] Koutrotsios, G., Tagkouli, D., Bekiaris, G., Kaliora, A., Tsiaka, T., Tsiantas, K., Chatzipavlidis, I., Zoumpoulakis, P., Kalogeropoulos, N., & Zervakis, G. I. 2022. Enhancing the Nutritional and Functional Properties of *Pleurotus* citrinopileatus Mushrooms through the Exploitation of Winery and Olive Mill Wastes. *Food Chemistry*. 370: 1–15. https://doi.org/10.1016/j.foodchem.2021.131022.

[4] Antunes, F., Marçal, S., Taofiq, O., Morais, A. M. M. B., Freitas, A. C., Ferreira, I. C. F. R., & Pintado, M. 2020. Valorization of Mushroom By-products as a Source of Value-added Compounds and Potential Applications. *Molecules*. 25(11). https://doi.org/10.3390/molecules25112672.

[5] Wan-Mohtar, W. A. A. Q. I., Halim-Lim, S. A., Kamarudin, N. Z., Rukayadi, Y., Abd Rahim, M. H., Jamaludin, A. A., & Ilham, Z. 2020. Fruiting-body-base Flour from an Oyster Mushroom Waste in the Development of Antioxidative Chicken Patty. *Journal of Food Science*. 85(10): 3124–3133. https://doi.org/10.1111/1750-3841.15402.

[6] Patel, S., & Goyal, A. 2012. Recent Developments in Mushrooms as Anti-cancer Therapeutics: A Review. *3 Biotech*. 2(1): 1–15. https://doi.org/10.1007/s13205-011-0036-2.

[7] Albayrak, S., Aksoy, A., Sagdic, O., & Hamzaoglu, E. 2010. Compositions, Antioxidant and Antimicrobial Activities of *Helichrysum* (Asteraceae) Species Collected from Turkey. *Food Chemistry*. 119(1): 114–122. https://doi.org/10.1016/j.foodchem.2009.06.003.

[8] Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Ávila, S., Hornung, P. S., Júnior, A. M., & Ribani, R. H. 2019. Factors Affecting Mushroom *Pleurotus* spp. *Saudi Journal of Biological Sciences*. 26(4): 633–646. https://doi.org/10.1016/j.sjbs.2016.12.005.

[9] Erbiai, E. H., Pinto da Silva, L., Saidi, R., Lamrani, Z., Esteves da Silva, J. C. G., & Maouni, A. 2021. Chemical Composition, Bioactive Compounds and Antioxidant Activity of Two Wild Edible Mushrooms *Armillaria mellea* and *Macrolepiota procera* from Two Countries (Morocco and Portugal). *Biomolecules*. 11(4): 1–11. https://doi.org/10.3390/biom11040575.

[10] Sujarit, K., Suwannarach, N., Kumla, J., & Lomthong, T. 2021. Mushrooms: Splendid Gifts for the Cosmetic Industry. *Chiang Mai Journal of Science*. 48(3): 699–725.

[11] Girmay, Z., Gorems, W., Birhanu, G., & Zewdie, S. 2016. Growth and Yield Performance of *Pleurotus ostreatus* (acq. Fr) Kumm (Oyster Mushroom) on Different Substrates. *AMB Express*. 6(87): 1–7. https://doi.org/10.1186/s13568-016-0265-1.

[12] Paul, R. K., Bhattacharjya, D. K., Kabir, A. K. L., Harun-Or-Rashid, M., Rahaman, M. S., Rahaman, M. S., Miah, M. N., & Ahmed, K. U. 2015. Effect of Different Saw Dust Substrates on the Nutritional Composition of Oyster Mushroom (*Pleurotus florida*) and Its Applications in Human Health. *Dhaka University Journal of Pharmaceutical Sciences*. 14(2): 215–223. https://doi.org/10.3329/dujps.v14i2.28513.

[13] Banik, S., & Nandi, R. 2004. Effect of Supplementation of Rice Straw with Biogas Residual Slurry Manure on the Yield, Protein and Mineral Contents of Oyster Mushroom. *Industrial Crops and Products*. 20: 311–319. https://doi.org/10.1016/j.indcrop.2003.11.003.

[14] Radzi, M. P. M. F., Azizah, M., Maininah, T., & Sumaiyah, A. (2021). Growth, Yield and Antioxidant Activity of Grey Oyster Mushroom (*Pleurotus pulmonarius*) Grown in Sawdust Substrate with the Supplementation of Alkaline Materials. *Journal of Animal and Plant Sciences*. 31(6): 1699–1711. https://doi.org/10.36899/JAPS.2021.6.0377.

[15] Yohannes, B., Abraham, M., Bikila, G., Robel, D., Getahun, T., Jale, M., Malesu, A., Tsehaynesh, F., & Lalise, D. 2020. Selection of Appropriate Substrate for Production of Oyster Mushroom (*Pleurotus ostreatus*). *Journal of Yeast and Fungal Research*. 11(1): 15–25. https://doi.org/10.5897/jyfr2019.0187.

[16] Upadhyay, A., Lama, J. P., & Tawata, S. 2005. Utilization of Pineapple Waste: A Review. *Journal of Food Science and Technology Nepal*. 6(1): 10–18. https://www.nepjol.info/index.php/JFSTN/article/view/8255/6722.

[17] Hikal, W. M., Mahmoud, A. A., Said-Al Ahl, H. A. H., Bratovcic, A., Tkachenko, K. G., Kačániová, M., & Rodriguez, R. M. 2021. Pineapple (*Ananas comosus* L. Merr.), Waste Streams, Characterisation and Valorisation: An Overview. *Open Journal of Ecology*. 11(09): 610–634. https://doi.org/10.4236/oje.2021.119039.

[18] Zakaria, N. N. A., Okello, E. J., & Birch-machin, M. 2019. Evaluation of Anti-ageing Properties of *Moringa oliefera* Lam., *Centella asiatica* (L.) Urban, *Clitoria ternatea* L. and *Cosmos caudatus* Kunth. for Potential Application as Cosmeceuticals (Issue June). Newcastle University.

[19] Shirzad, M., Hamedi, J., Motevaseli, E., & Modarressi, M. H. 2018. Anti-elastase and Anti-collagenase Potential of Lactobacilli Exopolysaccharides on Human Fibroblast. *Artificial Cells, Nanomedicine and Biotechnology*. 46(1): 1051–1061. https://doi.org/10.1080/21691401.2018.1443274.

[20] Azwanida, Z. N., Jonathan, O. E., & Melanie-Jaynes, H. 2020. Antioxidant, Anti-Collagenase, Anti-Elastase and Anti-Tyrosinase Activities of an Aqueous *Cosmos caudatus* Kunth (Asteraceae) Leaf Extract. *Tropical Journal of Natural Product Research*. 4(12): 1124–1130. https://doi.org/10.26538/tjnpr/v4i12.15.

[21] Pal Singh, M., Gupta, A., & Singh Sisodia, S. 2019. Qualitative Analysis of Gallic Acid by HPLC Method in Different Extracts of *Terminalia bellerica* Roxb. Fruit. *Fabad Journal of Pharmaceutical Sciences*. 44(2): 101–106.

[22] Ebanks, J. P., Wickett, R. R., & Boissy, R. E. 2009. Mechanisms Regulating Skin Pigmentation: The Rise and Fall of Complexion Coloration. *International Journal of Molecular Sciences*. 10(9): 4066–4087. https://doi.org/10.3390/ijms10094066.

[23] Abd Razak, D. L., Mohd Fadzil, N. H., Jamaluddin, A., Abd Rashid, N. Y., Sani, N. A., & Abdul Manan, M. 2019. Effects of Different Extracting Conditions on Anti-tyrosinase and Antioxidant Activities of *Schizophyllum commune* Fruit Bodies. *Biocatalysis and Agricultural Biotechnology*. 19(11): 101116. https://doi.org/10.1016/j.bcab.2019.101116.

[24] Alam, N., Yoon, K. N., Lee, K. R., Shin, P. G., Cheong, J. C., Yoo, Y. B., Shim, J. M., Lee, M. W., Lee, U. Y., & Lee, T. S. 2010. Antioxidant Activities and Tyrosinase Inhibitory Effects of Different Extracts from *Pleurotus ostreatus* Fruiting Bodies. *Mycobiology*. 38(4): 295. https://doi.org/10.4489/myco.2010.38.4.295.

[25] Hapsari, R., Elya, B., & Amin, J. 2012. Formulation and Evaluation of Antioxidant and Tyrosinase Inhibitory Effect from Gel Containing the 70% Ethanolic *Pleurotus ostreatus* Extract. *International Journal of Medicinal and Aromatic Plants*. 2(1): 135–140.

[26] Gregori, A., Vagelj, M., & Pohleven, J. 2007. Cultivation Techniques and Medicinal Properties of *Pleurotus* spp. *Food Technology & Biotechnology*. 45(3): 238–249.

[27] Azieana, J., Zainon, M.N., Noriham, A., & Rohana, M. N. 2017. Total Phenolic and Flavonoid Content and Antioxidant Activities of Ten Malaysian Wild Mushrooms. *Open Access Library Journal*. 4: 1–10. Doi: 10.4236/oalib.1103987.

[28] Heung, S. B., Ho, S. R., Jae, W. Y., Soo, M. A., Jin, Y. L., Jeonga-Lee, Kim, M. K., Duck, H. K., & Ih, S. C. 2008. The Inhibitory Effect of New Hydroxamic Acid Derivatives on Melanogenesis. *Bulletin of the Korean Chemical Society*, 29(1): 43–46. https://doi.org/10.5012/bkcs.2008.29.1.043.

[29] An, S. M., Koh, J. S., & Boo, Y. C. 2010. p-coumaric Acid Not Only Inhibits Human Tyrosinase Activity in Vitro But Also Melanogenesis in Cells Exposed to UVB. *Phytotherapy Research*. 24(8): 1175–1180. https://doi.org/10.1002/ptr.3095.

[30] Kim, S. Y., Go, K. C., Song, Y. S., Jeong, Y. S., Kim, E. J., & Kim, B. J. 2014. Extract of the Mycelium of *T. matsutake* Inhibits Elastase Activity and TPA-induced MMP-1 Expression in Human

Fibroblasts. *International Journal of Molecular Medicine*. 34(6): 1613–1621. https://doi.org/10.3892/ijmm.2014.1969.
[31] Jimenez, F., Mitts, T. F., Liu, K., Wang, Y., & Hinek, A. 2006. Ellagic and Tannic Acids Protect Newly Synthesized Elastic Fibers from Premature Enzymatic Degradation in Dermal Fibroblast Cultures. *Journal of Investigative Dermatology*. 126(6): 1272–1280. https://doi.org/10.1038/sj.jid.5700285.
[32] Holmbeck, K., & Birkedal-Hansen, H. 2013. Collagenases. Encyclopedia of Biological Chemistry: Second Edition. 1: 542–544. https://doi.org/10.1016/B978-0-12-378630-2.00008-.
[33] Quan, T., Qin, Z., Xia, W., Shao, Y., Voorhees, J., & Fisher, G. J. 2009. Matrix-degrading Metalloproteinases in Photoaging. *Journal of Investigative Dermatology*. 14(1): 20–24. https://doi.org/10.1007/s11103-011-9767-z.Plastid.
[34] Rodríguez, M. I. V., Barroso, L. G. R., & Sánchez, M. L. 2018. Collagen: A Review of Its Sources and Potential Cosmetic Applications. *Journal of Cosmetic Dermatology*. 17(1): 1–7. https://doi.org/10.1111/jocd.12450.
[35] Holmbeck, K., & Birkedal-Hansen, H. 2013. Collagenases. *Encyclopedia of Biological Chemistry: Second Edition*. 1: 542–544. https://doi.org/10.1016/B978-0-12-378630-2.00008-.
[36] Kim, S. W., Hwang, H. J., Lee, B. C., & Yun, J. W. 2007. Submerged Production and Characterization of *Grifola frondosa* Polysaccharides-A New Application to Cosmeceuticals. *Food Technology and Biotechnology*. 45(3): 295–305.
[37] Berg, A., Reiber, K., Dorfelt, H., Walther, G., Schlegel, B., & Grafe, U. 2000. Laccaridiones A and B, New Protease Inhibitors from *Laccaria amethystea*. *The Journal of Antibioics*. 53(11): 1313–1316.
[38] Wang, W., Gao, Y., Wang, W., Zhang, J., Yin, J., Le, T., Xue, J., Engelhardt, U.H., & Jiang, H. 2022. Kojic Acid Showed Consistent Inhibitory Activity on Tyrosinase from Mushroom and in Cultured B16F10 Cells Compared with Arbutins. *Antioxidants (Basel)*. 11(3): 502. Doi: 10.3390/antiox11030502.
[39] Phasha, V.; Senabe, J.; Ndzotoyi, P.; Okole, B.; Fouche, G.; Chuturgoon, A. 2022. Review on the Use of Kojic Acid - A Skin-lightening Ingredient. *Cosmetics*. 9: 64. https://doi.org/10.3390/cosmetics9030064.
[40] Saeedi, M.,Eslamifar, M. ad Khezri K. 2019. Kojic Acid Applications in Cosmetic and Pharmaceutical Preparations. *Biomedicine and Phamacotheraphy*. 110(2): 582–593. https://doi.org/10.1016/j.biopha.2018.12.006.
[41] Thring, T. S., Hili, P. & Naughton, D. P. 2009. Anti-collagenase, Anti-elastase and Anti-oxidant Activities of Extracts from 21 Plants. *BMC Complementary Alternative Medicine*. 9: 27. https://doi.org/10.1186/1472-6882-9-27.
[42] Yücel, Ç., Karatoprak, G. S., Yalçıntaş, S., Böncü, T. E. 2022. Ethosomal (−)-epigallocatechin-3-gallate as a Novel Approach to Enhance Antioxidant, Anti-collagenase and Anti-elastase Effects. *Beilstein Journal of Nanotechnology*. 13: 491–502. Doi:10.3762/bjnano.13.41.
[43] Xia, Y., Wang, D., Li, J., Chen, M., Wang, D., Jiang, Z., &Liu B. 2022. Compounds Purified from Edible Fungi Fight against Chronic Inflammation through Oxidative Stress Regulation. *Frontiers Pharmacology*. 13: 974794. Doi: 10.3389/fphar.2022.974794
[44] Heleno, S. A., Martins, A., Queiroz, M. J. R. P., & Ferreira, I. C. F. R. 2015. Bioactivity of Phenolic Acids: Metabolites Versus Parent Compounds: A Review. *Food Chemistry*. 173: 501–513. https://doi.org/https://doi.org/10.1016/j.foodchem.2014.10.057.
[45] Ferreira, I. C. F. R., Barros, L., & Abreu, R. M. V. 2009. Antioxidants in Wild Mushrooms. *Current Medicinal Chemistry*. 16(12): 1543–1560. https://doi.org/10.2174/092986709787909587.
[46] Torrez-Martinez, B.D.M., Vargas-Sanchez, R.D., Torrescano-Urrutia, G.R., Esqueda, M., Rodriguez-Carpena, J.G., Fernandez-Lopez, J., Perez-Alvarez, J.A., & Sanchez-Escalante, A. 2022. *Pleurotus* Genus as a Potential Ingredient for Meat Products. *Foods*. 11(6): 1–3. https://doi.org/10.3390/foods11060779.
[47] Dundar, A., Okumus, V., & Ozdemir, S. 2015. Antioxidant, Antimicrobial, Cytotoxic and Anticholinesterase Activities of Seven Mushroom Species with their Phenolic Acid Composition. *Journal of Horticulture*. 02(04). https://doi.org/10.4172/2376-0354.1000161.
[48] Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M. A., Martínez, J. A., García-Lafuente, A., Guillamón, E., & Villares, A. 2011. Antioxidant Properties of Phenolic Compounds Occurring in Edible Mushrooms. *Food Chemistry*. 128(3): 674–678. https://doi.org/10.1016/j.foodchem.2011.03.085.
[49] Butkhup L., Samappito W., Jorjong S. 2018. Evaluation of Bioactivities and Phenolic Contents of Wild Edible Mushrooms from Northeastern Thailand. *Food Science and Biotechnology*. 27(1): 193–202. Doi: 10.1007/s10068-017-0237-5.
[50] Bach, F., Zielinski, A. A. F., Helm, C. V., Maciel, G. M., Pedro, A. C., Stafussa, A. P., Ávila, S., & Haminiuk, C. W. I. 2019. Bio Compounds of Edible Mushrooms: In Vitro Antioxidant and Antimicrobial Activities. *Journal Food and Technology*. 107(March): 214–220. https://doi.org/10.1016/j.lwt.2019.03.017.
[51] Veljović S., Veljović M., Nikićević N., Despotović S., Radulović S., Nikšić M., Filipović L. 2017. Chemical Composition, Antiproliferative and Antioxidant Activity of Differently Processed *Ganoderma Lucidum* Ethanol Extracts. *Journal of Food Science and Technology*. 54(5): 1312–1320. Doi: 10.1007/s13197-017-2559-y.
[52] Viera, V., Marques A., Barreira, J. & Ferreira, I. C. F. R. 2012. Insights in the Antioxidant Synergistic Effects of Combined Edible Mushrooms: Phenolic and Polysaccharidic Extracts of *Boletus edulis* and *Marasmius oreades*, *Journal of Food and Nutrition Research*. 51(2): 109–116.