doi.org/10.1002/cbdv.202300111 Research Article CHEMISTRY & BIODIVERSITY

www.cb.wiley.com

Comparative Study on Biological Activities and Chemical Profiling of Mushroom (*Pleurotus pulmonarius* (Fr.) Quel.) Cultured on Lignocellulosic Agro Wastes

Nor Hisam Zamakshshari,*[a] Idris Adewale Ahmed,*[b, c] Muhammad Nazil Afiq Nasharuddin,[c] Nurul Syahira Zaharudin,[c] Najihah Mohd Hashim,[c, d] and Rozana Othman[c, d]

The relevance of the lignocellulosic substrate in the cultivation of mushrooms has lent support to the exploration of several lignocellulosic agro wastes. This study was, thus, aimed at the evaluation of durian peel as an alternative substrate for more sustainable mushroom cultivation and climate change mitigation. The secondary metabolites and biological activities of both aqueous and organic mushroom (*Pleurotus pulmonarius* (Fr.) Quel.) extract cultured on durian peel and rubberwood sawdust substrate were compared using GCMS, LCMS as well as various biological assays (cytotoxicity, antimicrobial and antioxidant activities). Mushroom extracts from durian peel substrates possess remarkable biological activities. The results showed that the aqueous extracts had poor antimicrobial activities. The organic extracts were more active against cancer cells than the aqueous extracts, while the aqueous extracts were more potent as antioxidants than the organic extracts. Overall, the mushroom extract from the durian substrate was the most effective except against A549 and SW948, while the aqueous extract from the durian substrate was the most effective against the A549 cancer cell lines with 29.53 ± 2.39% inhibition. On the other hand, the organic mushroom extract from the sawdust substrate was the most effective against SW948 with 60.24 ± 2.45% inhibition. Further studies, however, are needed to elucidate the molecular mechanism of action of *P. pulmonarius* extracts against cancer cell proliferation and the effect of the substrates on the nutritional composition, secondary metabolites, and other biological activities of *P. pulmonarius* extracts.

Introduction

Mushroom is categorized as a low-calorie food containing high protein, dietary fiber, minerals, vitamins, and low lipids content.[1–3] Edible mushrooms have become popular nowadays due to their excellent taste, richness in nutritional content, ease of purchase, medicinal value, and attraction as functional foods. The significant importance of mushrooms is attributed to their medical properties, organoleptic value, and economic importance.[1,4,5] Scientific research shows that mushroom contains metabolites with several biological activities such as antidiabetic, antioxidant, antitumor, and anti-inflammatory effect.[6,7] It is also helpful in preventing hypertension, hypercholesterolemia, and cancer.[8]

Pleurotus pulmonarius is one of the most popular edible mushrooms heavily grown in Malaysia. It is locally known as *'cendawan tiram'* and has become one of the commercialized foods in Malaysia. The genus *Pleurotus* (Class Basidiomycetes) belongs to the oyster mushrooms which are widely cultivated in several countries due to their texture, flavor, and health benefits. *P. pulmonarius*, like other mushrooms, obtain their nutrition by decomposing various agricultural by-products due to their saprophytic nature.[6,9] Presently, in Malaysia, most commercial mushroom producers use sawdust from the rubber tree and rice husk as the substrate to cultivate mushrooms. However, the relatively high cost, the environmental concern, and the decline in the availability of sawdust make it crucial to find other substrate sources for the cultivation of oyster mushrooms.[10] Moreover, sawdust production in Malaysia is declining since 1960 because rubber plantation has been replaced by oil palm plantation.[11] Thus, There is an urgent need to find another substrate that can grow mushrooms as a sustainable alternative to the commercialized substrate from rubber sawdust to internalize environmental and social costs.[12,13]

In mushroom cultivation, lignocellulosic materials such as sawdust and rice husk are required as a substrate to grow mushrooms.[14] The lignocellulosic material mainly contains cellulose and hemicellulose.[9] The digestion of both compo-

[a] *Assist. Prof. N. Hisam Zamakshshari*
Department of Chemistry, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Semarahan, Sarawak, Malaysia
E-mail: znhisam@unimas.my
norhisamzamakshshari@gmail.com

[b] *Assoc. Prof. I. Adewale Ahmed*
Department of Biotechnology, Faculty of Applied Science, Lincoln University, 47301, Petaling Jaya, Selangor, Malaysia
E-mail: idrisahmed@lincoln.edu.my
idrisahmed@um.edu.my
ahmedris1400@gmail.com

[c] *Assoc. Prof. I. Adewale Ahmed, M. Sc. M. Nazil Afiq Nasharuddin, M. Sc. N. Syahira Zaharudin, Assoc. Prof. N. Mohd Hashim, Assoc. Prof. R. Othman*
Center for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia

[d] *Assoc. Prof. N. Mohd Hashim, Assoc. Prof. R. Othman*
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202300111

nents typically produces cellobiose, glucose, xylose, and other sugars such as arabinose galactose, glucuronic acid, galacturonic acid, mannose, and pyranose. Most of these compounds are secondary products that are essential for the mycelium to grow. Mycelium, which appeared as a frost-liked thread, is a fiber of fungi that grows beneath bark and leaves. It then grows into a dense network for sprouting mushrooms. Sawdust is often not nutritious enough. Thus, it needs to be enriched with a nitrogen supplement such as rice husk and coco peat. Mixing two different substrates enhances mushroom growth, thus, increasing the production yield of mushrooms compared to using plain sawdust alone.[10]

Locally, *Durio zibethinus Murr* known as durian is an edible fruit that grows heavily in Malaysia. It is called the king of fruit due to its size. However, only one-third of the whole durian fruit is edible while the seeds (20–25%) including the shells are mostly thrown away.[15] Due to the heavy consumption of durian fruits, large amounts of the peels are disposed of, leading to severe community and environmental problems. Numerous kinds of research have, thus, been carried out owing to the environment, to utilize this agricultural waste for possible conversion into value-added and more useful materials. For example, durian peel is used in the removal of dye in the textile industry as a low-cost bio-sorbent.[16] The durian peel has also been phytochemically investigated for pectin production. Pectin is found in fruits as fiber and is used to make medicine.[17] The analysis of the volatile compounds of durian peel using GCMS has shown that durian peel contains ketones, hydrocarbons, ester, and acid.[18] Durian peel is also very rich in protein and mineral elements such as magnesium and iron as well as low-fat content.[19] Durian peel is an agricultural waste containing lignin, cellulose, and hemicelluloses as a significant component, making it a suitable substrate for mushroom cultivation.[20] Therefore, this research evaluated durian peel as an alternative substrate for mushroom cultivation to protect the environment from excess solid waste, thus developing more sustainable mushroom cultivation and as a climate change mitigation approach. Mushroom (*P. pulmonarius*) was cultured in two main substrates: durian peel and rubberwood sawdust. The extracts of both mushrooms were subjected to phytochemical analysis using GCMS and LCMS as well as biological and pharmacological assays.

Results

Mushroom extracts' yield and metabolite profiling

The mushroom samples were extracted accordingly. The extracts' yields of mushroom from durian substrate were 6.1870 g and 1.5785 g for aqueous (Daq) and organic (Dorg), respectively, while the mushroom from sawdust substrate gave extract yields of 5.5215 g and 0.94857 g for aqueous (Caq) and organic (Corg), respectively. Figure 1 shows the mushroom from both substrates.

Both aqueous extracts containing non-volatile compounds were further screened for their chemical profiling using LCMS. Meanwhile, the screening for their volatile compounds was done using GCMS for both organic extracts. Figure 2 and 3 show LCMS and GCMS chromatograms for aqueous and organic extracts, respectively. Corg and Dorg extracts' chemical content was assigned with a compound name, while others were labeled as 'unknown' since the SI of these compounds did not achieve more than 70% when the mass spectrum was compared with the NIST library. Both organic extracts consist of alkanes, alcohol, fatty acid, and other organic compounds. The profile of Corg with GCMS revealed that there are 32 compounds. Of 32 compounds, 31 were assigned a name, while one was labeled as unknown. Meanwhile, Dorg contains 35 compounds of which 32 compounds were identified and three compounds labeled unknown. There are seven different minor compounds found only in Dorg including 11,14-eicosadienoic acid, *Z,Z*-8,10-hexadecadien-1-ol, bis(tridecyl) phthalate, dotriacontane, 7-hexadecenal, pregnan-20-one, and ergosterol. How-

Figure 1. a) Mushroom harvest from durian substrate b) mushroom harvest from sawdust substrate.

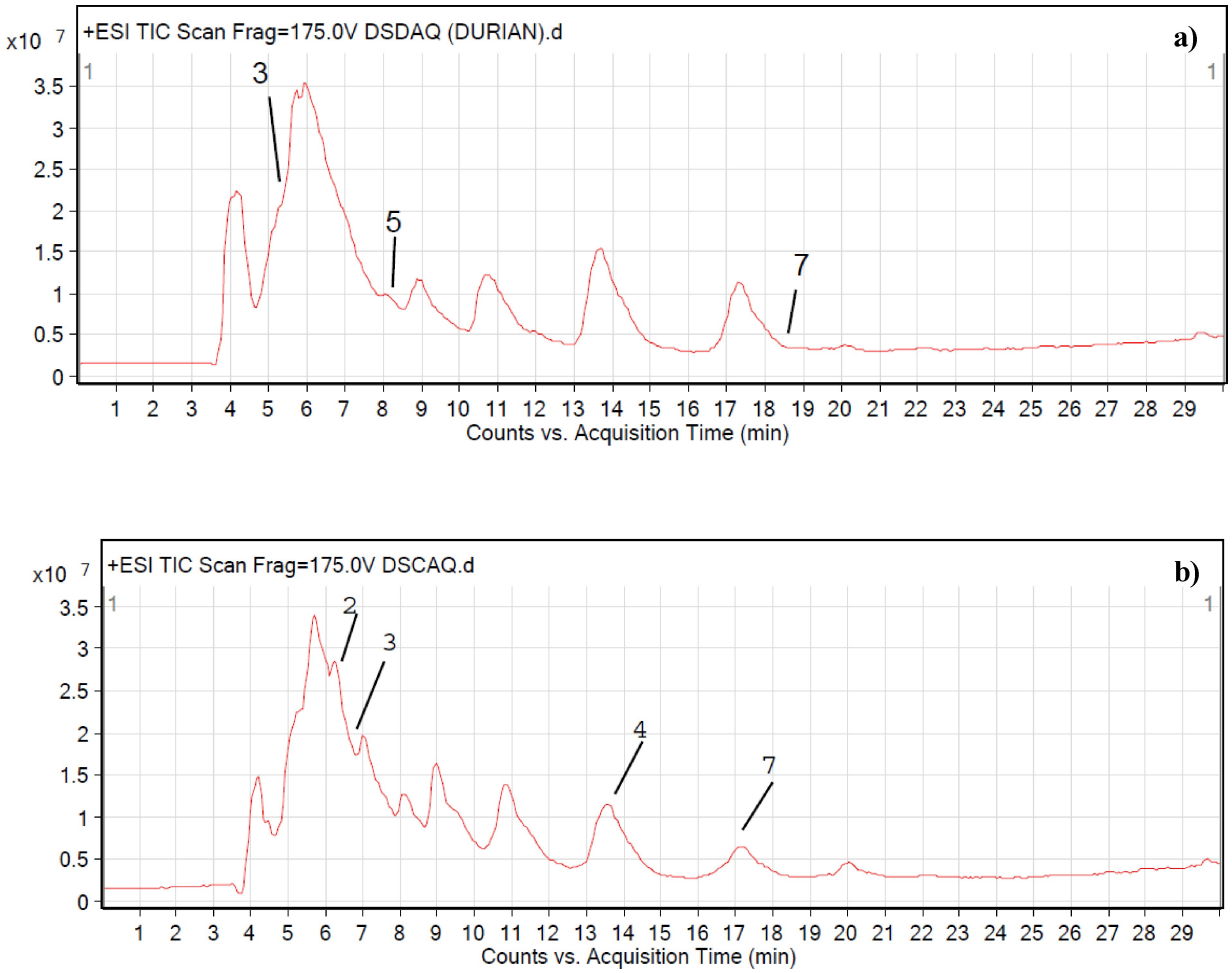


Figure 2. LCMS chromatogram for a) Daq and b) Caq.

ever, both extracts contain the same major volatile compounds, such as 2-pentanone, 4-hydroxy-4-methyl-9,12-octadecadienoic acid, based on the percentage of peak area. The Metlin database identified seven compounds from Daq extract, while nine were successfully recognized from the Caq chromatogram. Daq extract contains hydroxymethyl phosphonate, betaxolol, zidovudine, dehydrofalcarinone, 3-methylbutyraldehyde oxime, 10β-epoxynardosinane, and 5-aminopentanoic acid. Meanwhile, Caq contains compounds such as pinacidil, phloridzin, 14-methyl-all-*trans*-retinoic acid, 17-[(3-(1-pyrrolidinyl)propyl]imino]androst-5-en-3β-ol acetate, oleoyl serotonin, diethylcarbamazine n-oxide, 8,11,14-docosatriynoic acid, 5-amino pentanoic acid and hydroxymethyl phosphonate. Both extracts contain two similar compounds, which are 5-aminopentanoic acid and hydroxymethyl phosphonate.

Biological activities

Cytotoxic activities

The various mushroom extracts' anticancer, antimicrobial, and antioxidant activities are shown in Tables 1, 2, and 3. For the anticancer studies (Table 1), five cancer cell lines, namely; two breast cancer cell lines (MCF7 and MDA-MD-231), two colon cancer cell lines (SW948 and HT29), and a lung cancer cell lines (A549) were selected being the most common causes of cancer death in 2020, thus, there is an urgent need to find natural alternatives to common chemotherapeutic drugs used to combat these disease.[21] The percentage of inhibition of the extracts at 100 μg/mL was tested against the five cancer cell lines. The organic extracts were more potent than the aqueous extracts from both substrates. The only exceptions were the lung (A549) and colon (SW948) cancer cell lines for durian substrate mushroom extracts and breast cancer cell lines (MDA-MD-231) for the commercial (sawdust) substrate mushroom extracts. Overall, the mushroom extract from the durian substrate was the most effective except against A549 and SW948, while the aqueous extract from the durian substrate was the most effective against the A549 cancer cell lines with 29.53 ± 2.39% inhibition. On the other hand, the organic mushroom extract from the sawdust substrate was the most effective against SW948 with 60.24 ± 2.45% inhibition.

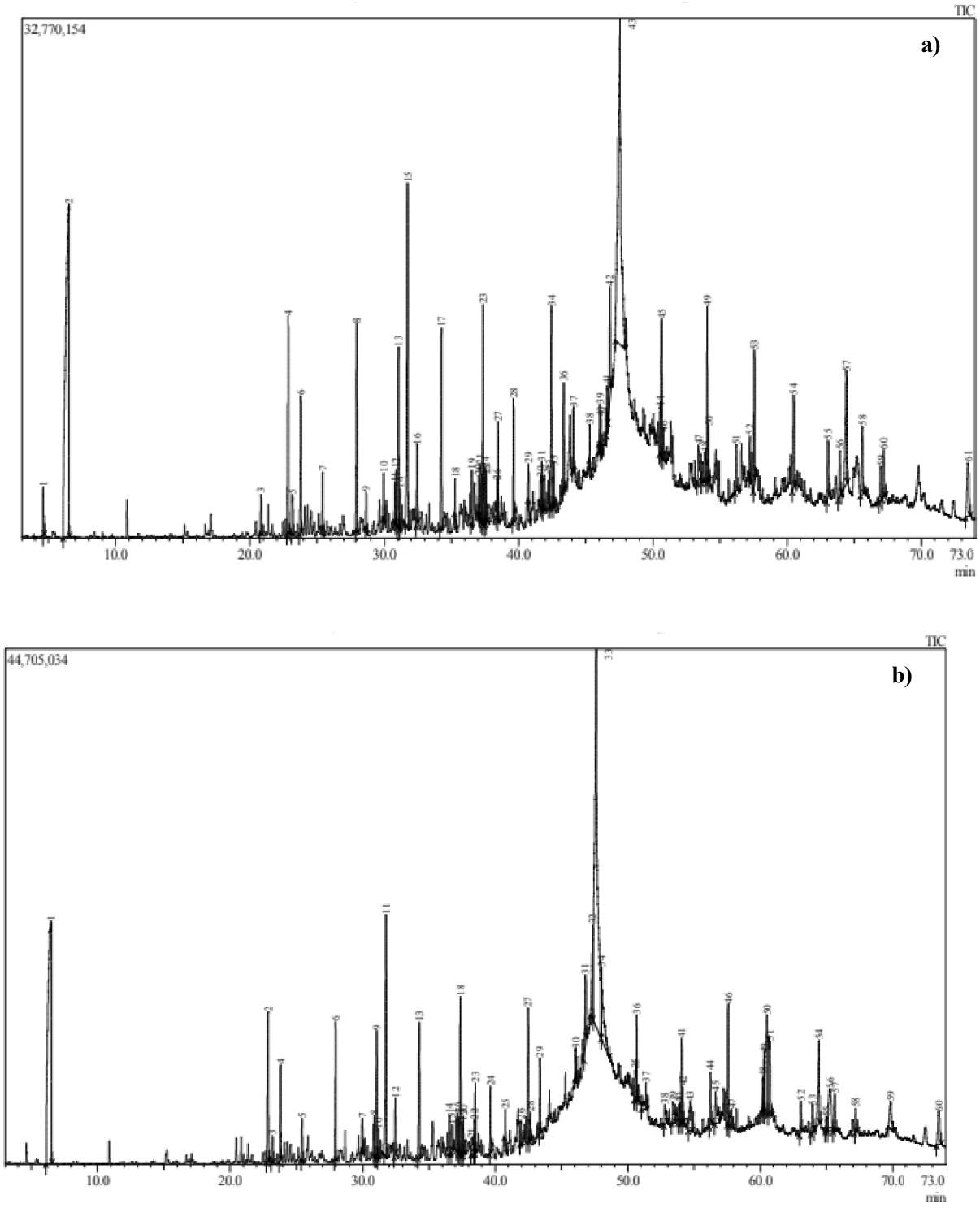


Figure 3. GCMS chromatogram for a) Dorg and b) Corg.

Antimicrobial activity

The antimicrobial studies (Table 2) were evaluated against bacteria and fungi using the well diffusion method. The mushroom extracts showed poor antimicrobial activities, with only a few extracts showing poor to moderate activities against the tested microbes. None of the extracts showed antibacterial activity against *Bacillus subtilis* and *Staphylococcus epidermidis.* Similarly, none of the extracts was effective against the two tested fungi strains (*Candida albicans* and *Aspergillus niger* except for the aqueous extract from the durian substrate mushroom with an inhibition diameter of 11.00 ± 1.00 mm against *A. niger*. The aqueous mushroom extracts from both the durian and sawdust substrates and the organic mushroom

Table 1. Cytotoxic effect of extracts and standard compounds against MCF7, MDA-MD-231, SW948, HT29, and A549.

Extracts	Percentage of inhibition (%) at 100 µg/mL				
	MCF7	MDA-MD-231	SW948	HT29	A549
Daq	19.35 ± 0.82^{A}	29.91 ± 1.80^{B}	29.02 ± 1.48^{A}	18.85 ± 2.06^{A}	29.53 ± 2.39^{B}
Dorg	60.24 ± 2.45^{D}	38.98 ± 3.90^{C}	28.75 ± 1.58^{A}	51.68 ± 5.62^{C}	17.83 ± 1.77^{A}
Caq	27.77 ± 2.02^{B}	29.28 ± 2.26^{B}	26.79 ± 2.45^{A}	26.20 ± 1.60^{B}	16.75 ± 1.65^{A}
Corg	36.86 ± 1.83^{C}	13.82 ± 1.89^{A}	41.64 ± 2.05^{B}	48.41 ± 3.64^{C}	25.51 ± 2.03^{B}
Paclitaxel IC_{50} (µg/mL)	ND	ND	0.26 ± 0.02	0.16 ± 0.01	2.23 ± 0.01
Tamoxifen IC_{50} (µg/mL)	2.28 ± 0.10	3.15 ± 0.04	ND	ND	ND

Daq = aqueous extract of mushroom from durian substrate; Dorg = organic extract of mushroom from durian substrate; Caq = aqueous extract of mushroom from sawdust substrate; Corg = organic extract of mushroom from sawdust substrate; ND = not determined. Data were analyzed using one-way ANOVA. Values presented are mean ± standard error of triplicate independent analyses. Values within the same column but with different superscripts (A, B, C, & D) are significantly different ($p < 0.05$) using the Tukey post hoc test.

Table 2. Inhibition diameters of all extracts against bacteria and fungi.

	Inhibition zone (mm)							
	Bacteria strains tested						Fungi strains tested	
Extract	S.A	K.P	E.C	P.A	S.E	B.S	A. N	C.A
Daq	11.70 ± 1.50^{A}	12.60 ± 0.80^{B}	11.00 ± 1.00^{A}	N.A	N.A	N.A	11.00 ± 1.00^{A}	N.A
Dorg	N.A	N.A	10.00 ± 1.00^{A}	N.A	N.A	N.A	N.A	N.A
Caq	11.00 ± 1.00^{A}	7.30 ± 0.80^{A}	8.00 ± 1.00^{A}	10.70 ± 0.50^{A}	N.A	N.A	N.A	N.A
Corg	N.A	14.00 ± 1.00^{B}	11.00 ± 1.00^{A}	10.70 ± 0.50^{A}	N.A	N.A	N.A	N.A
Streptomycin sulfate salt	24.33 ± 0.58^{B}	29.00 ± 1.00^{C}	28.67 ± 0.57^{B}	26.00 ± 1.73^{B}	30.67 ± 0.58	29.67 ± 0.58	N.D	N.D
Ketoconazole	N.D	N.D	N.D	N.D	N.D	N.D	25.00 ± 1.00^{B}	26.33 ± 1.53
DMSO	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A

Daq = aqueous extract of mushroom from durian substrate; Dorg = organic extract of mushroom from durian substrate; Caq = aqueous extract of mushroom from sawdust substrate and organic; Corg = organic extract of mushroom from sawdust substrate; NA = not active; ND = not determined, SA=*Staphylococcus aureus* (ATCC 25923); BS=*Bacillus subtilis* (B145); SE=*Staphylococcus epidermidis*, PA=*Pseudomonas aeruginosa* (ATCC27853); EC=*Escherichia coli*; KP=*Klebsiella pneumoniae* (ATCC 2513); CA=*Candida albicans* (C2213) and AN=*Aspergillus niger*. Data were analyzed using one-way ANOVA. Values presented are mean ± standard deviation of triplicate independent analyses. Values within the same column but with different superscripts (A, B, C, & D) are significantly different ($p < 0.05$) using the Tukey post hoc test.

extract from sawdust substrates, however, demonstrated appreciable antibacterial activities against some of the tested bacteria strains. In contrast, the organic mushroom extract from durian substrates showed no antibacterial effects against all the tested bacteria and fungi strains except against *E. coli*, with an inhibition diameter of 10.00 ± 1.00 mm. The standards, streptomycin sulfate and ketoconazole used as positive controls for the antibacterial assay and the antifungal assay, respectively, gave remarkable antimicrobial activities, as expected, being pure compounds.

Antioxidant activities

The antioxidant activities of all the extracts (Table 3) were evaluated using four different antioxidant assays: total antioxidant capacity, ABTS, FRAP, and β-carotene bleaching assays.

Table 3. Antioxidant activities of extracts, and standard compounds.

Sample	Total antioxidant capacity (µg ascorbic acid/mg extract)	ABTS % of scavenging of extract at 10 mg/mL	FRAP (µM ferrous sulfate/mg dry extract)	β-Carotene bleaching (% of β-carotene bleaching of extract at 10 mg/mL)
Daq	10.69 ± 0.10^{B}	37.74 ± 4.75^{B}	1.18 ± 0.01^{A}	15.16 ± 7.69^{A}
Dorg	8.09 ± 0.14^{AB}	6.10 ± 0.62^{A}	0.56 ± 0.04^{A}	86.36 ± 5.20^{C}
Caq	7.93 ± 0.04^{AB}	6.51 ± 1.38^{A}	0.82 ± 0.15^{A}	76.68 ± 5.79 C
Corg	5.64 ± 0.06^{A}	46.71 ± 5.66^{C}	0.57 ± 0.07^{A}	34.34 ± 3.24B
Standard Compound	ND	Gallic acid (100 µg/mL) 94.05 ± 0.25	ND	Gallic acid (100 µg/mL) 88.58 ± 1.42

Daq = aqueous extract of mushroom from durian substrate; Dorg = organic extract of mushroom from durian substrate; Caq = aqueous extract of mushroom from sawdust substrate and organic; Corg = organic extract of mushroom from sawdust substrate; ND = not determined. Data were analyzed using one-way ANOVA. Values presented are mean ± standard error of triplicate independent analyses. Values within the same column but with different superscripts (A, B, C, & D) are significantly different ($p < 0.05$) using the Tukey post hoc test.

The aqueous mushroom extracts from the durian and commercial (sawdust) substrates were more effective in their antioxidant activities than their corresponding organic extracts except for the β-carotene bleaching assay and ABTS for the durian and sawdust substrates, respectively. Overall, the aqueous mushroom extract from the durian substrate has the highest total antioxidant capacity and FRAP with 10.69 ± 0.10 μg ascorbic acid/mg extract and 1.18 ± 0.01 μM ferrous sulfate/mg dry extract, respectively. The organic mushroom extracts from durian substrate, however, have the highest total β-carotene bleaching activity of 86.36 ± 5.20 % of β-carotene bleaching at 10 mg/mL, while the aqueous mushroom extract from sawdust substrate has the highest ABTS scavenging activity 46.71 ± 5.66 % at 10 mg/mL.

Discussions

The wide use of mushrooms as a source of food, medicine, functional purposes, and the flavoring of food has been known since ancient times.[6,22] Mushroom contains various potent bioactive-nutritional compounds and provides an adequate balanced diet for human nutrition. The mushroom benefit has led to a tremendous increase in the global cultivation, production, and application of mushrooms.[21,24] In the literature, some studies have focused on using alternative substrates for mushroom cultivation.[14,25] Mushroom cultivation remains an excellent bioconversion approach to turning lignocellulosic wastes into value-added protein-rich food thereby reducing waste accumulation and environmental pollution.[26]

On the other hand, Durian peel is an agricultural waste that is rich in lignin, cellulose, and hemicelluloses and contains high protein, low fat, and rich mineral element contents, among others.[19,20] The environment generally influences the metabolites of the various matrices. Thus, the application of metabolite profiling in the comprehensive analysis of biological matrices for potential biomarker identification is becoming more popular in recent times.[27] Therefore, in this study, we evaluated the use of durian peel as an alternative substrate for mushroom cultivation. *P. pulmonarius* mushroom was cultured in two different substrates (durian peel and rubberwood sawdust).

The mushrooms from both substrates were subjected to phytochemical analysis using GCMS and LCMS. The biological activities of both samples were also evaluated. The focus of the present studies is on the effect of the substrates on the secondary metabolites and biological functions of *P. pulmonarius* mushrooms. Devi and Krishnakumari[28] have previously reported a quantitative estimation of primary and secondary metabolites of *P. pulmonarius* hot aqueous extract. Other studies have focused on the effect of extraction techniques on the biological activity of mushroom extracts.[3,29–31] According to the literature, mushrooms and other fungal species are rich in potent antioxidants such as glutathione and ergothioneine with inhibitive potency against premature mortality. Furthermore, the mycelia of mushrooms are also excellent sources of naturally occurring antibiotics and other secondary metabolites with antibacterial and antiviral properties.[1]

The biological activity of some of the bioactive compounds such as 2-pentanone, and ■4-hydroxy-4-methyl?■ have been well reported in the literature.[32,33] The results of the anticancer studies are also in agreement with the cytotoxicity properties of four wild edible mushrooms; *Coprinus comatus* (O.F. Mull.) Pers. (Agaricaceae), *Lentinus tigrinus* (Bull.) Fr. (Polyporaceae), *Rhizopogon luteolus* Fr. and Nordholm (Rhizopogonaceae), *Tricholoma fracticum* (Britzelm.) Kreisel (Tricholomataceae) on hepatocellular carcinoma (HepG2) cells.[34]

The weak antibacterial activity of the mushroom extracts in the current study, however, contradicts the potent antibacterial property of mushrooms in the literature.[34–36] The discrepancies could be attributed to the extraction technique which inadvertently affects both the components and their antimicrobial activities.[3,37] For instance, the hexane and acetone extracts of some edible mushrooms demonstrated strong antibacterial activity while the chloroform extract showed remarkable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.[34]

Nevertheless, the results demonstrate the potential uses of durian peel as an alternative sustainable substrate for mushroom cultivation due to its high content of protein, mineral elements, lignin, cellulose, and hemicelluloses.[19] The low activity of *P. pulmonarius* extracts obtained in this study may be due to the decrease in the polysaccharides concentration, which, in turn, may be a result of the internal β-glucanase activity. At the same time, the presence of fatty acids, proteins, phenolic compounds, steroids, and terpenoids may be responsible for the high antiproliferative and antioxidant activities of the various mushroom extracts.[31,38–47] The higher antioxidant activities of the aqueous extracts also suggest the existence of remarkable polar and active constituents in the mushroom extracts as established by the LC/MS data and in agreement with the literature.[29,30] Admittedly, the nutritional composition of the various mushrooms and their secondary metabolites were not evaluated in the present study.

Conclusions

The present studies have provided experimental evidence that durian peels, in great abundance in Malaysia, are a suitable and sustainable substrate for mushroom cultivation. Overall, the mushroom grown on durian peel substrate gave higher extract yields and was generally a more effective anticancer agent than the commercial substrate. The higher antioxidant activities of aqueous extracts compared to organic extracts of *P. pulmonarius* also demonstrate the need to avoid using organic solvents. The limitations of the study include the use of only one organic solvent, and the lack of funds to study the molecular mechanism of action of *P. pulmonarius* extracts against the cancer cell lines and the effect of the substrates on the nutritional composition, secondary metabolites, and other biological activities of *P. pulmonarius* extracts. Besides that, it is important to quantify the major chemical constituents responsible for the biological activities of the mushroom extracts. It is important also in herbal standardization to ensure biochemical consistency and to optimize the safety and efficacy of herbal

products. Therefore, further studies are needed to elucidate the molecular mechanism and mode of action of *P. pulmonarius* extracts against cancer cell proliferation and to explore further the effect of the substrates on the nutritional composition, secondary metabolites, and other biological activities of *P. pulmonarius* extracts.

Materials and Methods

Chemical

The analytical or chromatographic grade solvent and acids such as Acetonitrile (75–05-8), Dimethyl sulfoxide (67-68-5), chloroform (67-66-3) and formic acid (64-18-6) were purchased from Merk (Darmstadt, Germany). Meanwhile, standard compounds and chemicals such as streptomycin sulfate (3810-74-0), ketoconazole (65277-42-1), gallic acid (149-91-7), ascorbic acid (50-81-7), Tamoxifen (10540-29-1), paclitaxel (33069-62-4), MTT (23305-68-2) and butylated hydroxytoluene (128-37-0) were purchased from Thermo Scientific Chemicals.

Mushroom culturing

Pleurotus pulmonarius spawn was purchased from Nas Agro Farm, Sepang, Selangor. The study was carried out in the chemistry research laboratory of CENAR, University Malaya. The mushroom was cultured inside six baglogs for each type of sample. Fume cardboard was used as a custom-made growth room. The temperature was controlled to 28 °C and above.

Substrate preparation and spawn inoculation

Substrate mixture; durian peels were obtained from the local durian seller (Durian SS2) in Petaling Jaya. Durian peels were dried until a constant weight was obtained and then grounded into 0.5 to 1.5 cm length pellets. The primary substrate was supplemented with 9% cocopeat and 0.9% calcium carbonate. The water content of the final mixture was adjusted to about 65–75%. The lignocellulosic substrate formula was then filled into 10×23 cm polyethylene plastic (PP) bags. The baglogs weighing approximately 0.8 kg each were sterilized in an autoclave at 121 °C for 1 h. The substrate-filled baglogs were cooled to room temperature for one whole day and then inoculated with the 15 g spawn per bag. The inoculation of the spawn was carried out in a sterile condition. The area was swabbed with alcohol and made free of dirt and dust. The mouth of the spawn was opened, and the mycelium was mixed thoroughly. Fifteen grams of the spawn was inoculated in each baglog. The mouth of each baglog was then covered with cotton and let to ferment for 60 days. The same procedure was carried out for the rubberwood sawdust substrate baglogs.

Incubation and harvest

Under dark conditions, the inoculated substrates were kept in the fume cardboard at 28 °C and 60~70% relative humidity. The surface substrates were entirely covered with mycelium, then the substrates were maintained at 28–35 °C. The inoculated substrates were kept at a relative humidity of about 90% or above. After 60 days following the appearance of the white mycelium around the surface of the baglogs, the cotton was opened. The mature mushroom was harvested 3–4 days later. The harvesting period was calculated from the inoculation of strains to the time of tube maturity in the piles. The average weight and height of each mushroom harvested from each baglog were measured and recorded.

Sample collection and extraction

The mushroom samples were inoculated, consisting of six baglogs of durian substrate and six baglogs of sawdust substrate. The extraction was adapted from Park et al.[48] with slide modification. After 60 days, fresh mushrooms were harvested and dried in the oven at 60 °C until constant weight. Then, both samples were ground into powder form using mortar and pestle and directly placed in centrifuge tubes. Ten grams of the ground sample were transferred to a 50 mL centrifuge tube and extracted with 10 mL millipore water (aqueous extract) and 10 mL chloroform (organic extract). The samples were vortexed for 2–3 min. Then, the samples were sonicated at 25 °C for 30 min. Samples were filtered and were wholly dried using miVac Quattro Concentrator to remove water or chloroform to obtain a dry extract. All the dried extracts were tested for their biological activities.

Chemical profiling of mushroom extract

The chemical profile of each extract was determined based on spectroscopic analysis using LC/MS and GC/MS to determine non-volatile and volatile metabolites, respectively. The chloroform extract was analyzed with GC/MS to determine volatile metabolites, while LC/MS was used to determine the non-volatile metabolites in the aqueous extract.

Gas chromatography-mass spectrometry (GC/MS)

The GC/MS analysis was carried out using RTX-5MS fused silica capillary column (30m ×0.25 mm i.d.; 0.25 μm film thickness). The carrier gas used was helium. The machine was run at a constant pressure of 100.0 kPa. The injection was done at 300 °C using splitless mode. The oven temperature was ramped from 40 to 160 °C (5 min hold) at 4 °C/min and 160–280 °C (15 min hold) at 5 °C/min. The temperature of the GC/MS interface was 280 °C. The analytical scanning was carried out using MS mode from 45–500 atomic mass units (AMU). The temperature of the ion source was 280 °C. Peaks' identification

was conducted with the National Institute of Standards and Technology (NIST08 and 08s). Each sample was run for an approximate total run time of about 74 min.

Liquid chromatography-mass spectrometry (LC/MS)

The extracts were analyzed using an Agilent QTOF-LCMS. The extracts were subjected to C-18 reversed-phase (RP) chromatographic separation (150 mm ×4.6 mm i.d, 3.0 μm particle size). Acidified water and acetonitrile, containing 0.1 % (v/) of formic acid, were used as eluent A and eluent B, respectively. The following gradient profile was employed: 0–18 min, 100–50 % B; 18–20 min, 50–5 % B; and 20–30 min; 5–100 B. The solvent flow rate was 0.8 mL/min. Samples (10 μL) were injected into a C18 reversed-phase column (150 mm ×4.6 mm i.d, 3.0 μm particle size). Mass spectrometric detection was performed with a quadrupole-TOF-MS operated in the positive mode. Information-dependent acquisition using a TOF-MS survey scan of 100–1100 Da (100 ms) and up to 10 dependent TOF MS scans of 100–1100 Da (100 MS) using Collision Energy (CE) of 45 V with Collision Energy Spread (CES) of ±30 V. The identification of the peaks was conducted using Metlin database and ChemSpider.

Cytotoxic activities

MTT assays were used for the cytotoxic analysis as described in the literature.[49, 50] The extracts were tested against five cancer cells; two colon cancer cell lines (SW948 and HT29); two breast cancer cell lines (MCF7 and MDA-MD-231); and a lung cancer cell lines (A549). All the cancer cells were purchased from the ATCC (American Type Culture Collection, USA). Briefly, all the cultured cells were allowed to grow to the log phase in RPMI160 medium and 5 % fetal bovine serum. Then, they were seeded in a 96-well plate at specific cell concentrations. The concentrations were 1×10^5 and 2×10^5 cells for SW948 and MCF7 cells, respectively, in 100 μL aliquots of the medium. The plates were incubated for 72 h at 37 °C in a 5 % CO_2 humidified incubator. After 72 h, 20 μL of MTT solution (5 mg/mL) was added to each well. Then, the plate was further incubated for another 3 h. The absorbance of each well was determined at 550 nm using a microplate reader. Eighty percent of the media from each well was discarded, followed by the addition of the same amount of DMSO into each well to dissolve the purple formazan crystal. The extract cytotoxicity was expressed in the percentage of cell inhibition at 100 ug/mL following the formula below. The standard drugs were, however, expressed in IC_{50} value. Tamoxifen was used as a standard compound for breast cancer cell lines, while paclitaxel was used for other cancer cell lines.

$$\text{Percentage of cell inhibition} = [(A - B)/A] \times 100\% \quad (1)$$

where A represents the average absorbance of the cell without treatment and B represents the average absorbance of the cell with treatment

Antimicrobial activities

Bacterial and fungal stock cultures were preserved on Muller Hinton agar and potato dextrose agar, respectively, and kept at 4 °C. The antimicrobial activities were studied against six bacteria comprising three Gram-negative strains [*Escherichia coli* (a clinical isolate), *Pseudomonas aeruginosa* (ATCC27853), *Klebsiella pneumoniae* (ATCC 2513)] and three Gram-positive strains [*Bacillus subtilis* (B145), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (a clinical isolate)]. The two fungal strains involved in this study, however, were *Aspergillus niger* (A121) and *Candida albicans* (C2213). All the bacteria strains were obtained from ATCC and Microbiology Laboratory, Medical Faculty, University Putra Malaysia, and all fungi strains were obtained from the Institute for Medical Research (IMR), Kuala Lumpur. The well-diffusion method was used for antibacterial and antifungal studies, according to Zamakshshari et al..[51] Each extract was screened at 10 mg/mL. Both activities were evaluated by measuring the inhibition zone diameter after incubating the plates at 37 °C for 24 h for antibacterial and 25 °C for 48 h for antifungal studies. Streptomycin sulfate (100 μg/mL) and ketoconazole were used as positive controls for the antibacterial assay and the antifungal assay. Dimethyl sulfoxide (DMSO) was used as a negative control.

Antioxidant activities

All the plant extracts were also screened for their antioxidant activities using four antioxidant assays namely; ferric reducing power (FRAP), β-carotene bleaching (BCB), total antioxidant capacity (TAOC), and ABTS 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid). The reducing power of the extracts and gallic acid was determined according to Zamakshshari et al..[52] A standard curve of gallic acid was constructed, and a standard equation was determined to find the reducing power for the extracts. The reducing power results were expressed relative to gallic acid equivalent (GAE, μg of gallic acid/mg of extract). Each extract was screened at 10 mg/mL. Similarly, the β-carotene bleaching assay was conducted according to the protocol in the literature.[53] Butylated hydroxytoluene (BHT) was used as a standard in this experiment. The absorbance was measured at 470 nm at 0 h and 2 h. The extract (10 mg/mL) and standard (100 μg/mL) were compared. TAOC assay is a non-enzymatic assay and was carried out according to a modified protocol.[54] All the extracts were screened at a 10 mg/mL concentration and analyzed at 695 nm. The total antioxidant activity was expressed as the equivalence of ascorbic acid. The free radical scavenging assay was performed using the ABTS method described in the literature[55] with slight modification. Briefly, 10 μL of the sample at 10 mg/mL concentration (diluted in DMSO) or DMSO as a blank was added to the 96-well microplate, followed by 300 μL of $ABTS^{\cdot+}$ solution. The plate was kept for 10 min at 30 °C. The absorbance was recorded at 743 nm. The ABTS was decolorized due to the scavenging activity of the antioxidant.

Statistical analysis

The results obtained from the mushroom cultivation, chemical profiling of mushroom extract, and the antioxidant and antimicrobial assays were represented as the mean ± standard deviation of triplicate independent analyses. Meanwhile, the results obtained from the cytotoxic assays were represented as the mean ± standard error of three independent experiments. The IC_{50} values for the standards (Tamoxifen and paclitaxel) were calculated with Probit Regression Analysis and associated 95% confidence limits. Data were analyzed using one-way ANOVA by Tukey post hoc test using the Statistical Package for Social Sciences (SPSS®, version 21.0, IBM Corporation, Armonk, NY, USA) to determine the significant differences among samples. The significance level was set at $p < 0.05$.

Statement of novelty

The novelty of this work lies in the evaluation of durian peel as an alternative substrate for mushroom cultivation to protect the environment from excess solid waste, thus, developing more sustainable mushroom cultivation as a climate change mitigation approach. The environmental concern, the decline in the availability, and the relatively high cost of the commercialized mushroom substrate, which is sawdust, due to declining production and a reduction of rubber plantations have been the impetus of this research. The comparison between the extracts of the two cultured mushrooms was evaluated through the analysis of their biological activities and phytochemical properties.

Author Contributions

Najihah Mohd Hashim proposed the study. All authors contributed to the study design. Material preparation, data collection and analysis were performed by Nor Hisam Zamakshsharia, Idris Adewale Ahmed, Muhammad Nazil Afiq Nasharuddin, and Nurul Syahira Zaharudin. The first draft of the manuscript was written by Nor Hisam Zamakshshari and Idris Adewale Ahmed. Najihah Mohd Hashim and Rozana Othman supervised the project. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge the technical support from University Malaya research facilities. Nas Agrofarm Sdn Bhd is also acknowledged for providing the *P. pulmonarius* seed.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

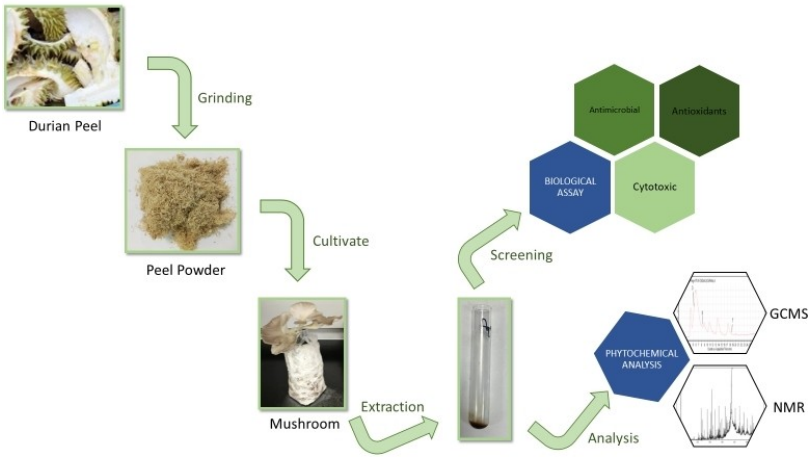
Keywords: durian peel · mushroom · cytotoxicity · antimicrobial · antioxidant

[1] R. W. Mwangi, J. M. Macharia, I. N. Wagara, R. L. Bence, *Biomed. Pharmacother.* **2022**, *147*, 112621.
[2] L. Wang, M. A. Brennan, W. Guan, J. Liu, H. Zhao, C. S. Brennan, *Food Chem.* **2021**, *351*, 129320.
[3] B. Jacinto-Azevedo, N. Valderrama, K. Henríquez, M. Aranda, P. Aqueveque, *Food Chem.* **2021**, *356*, 129651.
[4] S. Boonsong, W. Klaypradit, P. Wilaipun, *Agric. Nat. Resour.* **2016**, *50*, 89–97.
[5] Y. Sudjaroen, K. Thongkao, *Int. J. Green Pharm.* **2017**, *11*, S174–S181.
[6] G. N. Maity, P. Maity, S. Khatua, K. Acharya, S. Dalai, S. Mondal, *Int. J. Biol. Macromol.* **2021**, *168*, 743–749.
[7] B. Du, F. Zhu, B. Xu, *J. Funct. Foods.* **2018**, *47*, 334–342.
[8] X. Li, Y. Guo, Y. Zhuang, Y. Qin, L. Sun, *Int. J. Food Sci. Technol.* **2018**, *53*, 1855–1864.
[9] F. Ahmad Zakil, R. Mohd Isa, M. S. Mohd Sueb, R. Isha, *Mater. Today: Proc.* **2022**, *57*, 1329–1337.
[10] V. Sopit, *Biotechnol.* **2007**, *6*, 578–582.
[11] Y. Basiron, *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 289–295.
[12] S. Rezaeian, H. Pourianfar, *Waste Biomass Valorization* **2017**, *8*, 2631–2642.
[13] Y. Zou, F. Du, H. Zhang, Q. Hu, *Waste Biomass Valorization* **2019**, *10*, 2879–2885.
[14] M. Yamauchi, M. Sakamoto, M. Yamada, H. Hara, S. Mat Taib, S. Rezania, M. D. Mohd Fadhil, F. H. Mohd Hanafi, *J. King Saud Univ. Sci.* **2019**, *31*, 490–494.
[15] B. H. Hameed, H. Hakimi, *Biochem. Eng. J.* **2008**, *39*, 338–343.
[16] S.-T. Ong, S.-Y. Tan, E.-C. Khoo, S.-L. Lee, S.-T. Ha, *Desalin. Water Treat.* **2012**, *45*, 161–169.
[17] E. C. Arollado, K. M. G. Ponsaran, M. M. Loquias, *Acta Med. Philipp.* **2018**, *52*, 453–457.
[18] Z. Xiao, M. Niu, Y. Niu, *Molecules* **2022**, *27*, 1264.
[19] Y.-F. Zhan, X.-T. Hou, L.-L. Fan, Z.-C. Du, S. E. Ch'ng, S. M. Ng, K. Thepkaysone, E.-W. Hao, J.-G. Deng, *Chin. Herb. Med.* **2021**, *13*, 461–471.
[20] Matura, Woatthichai, N. *Afr. J. Biotechnol.* **2013**, 12, 5244–5251.■■Please check the authors names.■■
[21] WHO, 2022, World Health Organization, Cancer, fact Sheet retrieved on 22.5.2023 at 7.53 pm, https://www.who.int/news-room/fact-sheets/detail/cancer. Accessed on 22nd May 2023.
[22] I. H. Cho, Y.-S. Kim, H.-K. Choi, *J. Pharm. Biomed. Anal.* **2007**, *43*, 900–904.
[23] I. Abcha, P. Criado, S. Salmieri, H. Najjaa, H. Isoda, M. Neffati, M. Lacroix, *Eur. Food Res. Technol.* **2019**, *245*, 2641–2654.
[24] S. Saari, W. R. Wan Ishak, *Int. Food Res. J.* **2016**, *23*, 262–268.
[25] F. Xu, Z. Li, Y. Liu, C. Rong, S. Wang, *Saudi J. Biol. Sci.* **2016**, *23*, 607–613.
[26] C. Öztürk, F. Atila, *Sci. Hortic.* **2021**, *288*, 110403.
[27] Y. G. Chua, B. C. Bloodworth, L. P. Leong, S. F. Li, *Rapid Commun. Mass Spectrom.* **2014**, *28*, 1387–1400.
[28] M. R. Devi, S. Krishnakumari, *J. Pharmacogn. Phytochem.* **2015**, *4*, 198.
[29] E. R. Carbonero, A. C. Ruthes, C. S. Freitas, P. Utrilla, J. Gálvez, E. V. d Silva, G. L. Sassaki, P. A. J. Gorin, M. Iaomini, *Carbohydr. Polym.* **2012**, *90*, 814–819.
[30] T. C. Finimundy, L. Barros, R. C. Calhelha, M. J. Alves, M. A. Prieto, R. M. V. Abreu, A. J. P. Dillon, J. A. P. Henriques, M. Roesch-Ely, I. C. F. R. Ferreira, *Food Chem.* **2018**, *245*, 150–158.
[31] T. C. Finimundy, G. Gambato, R. Fontana, M. Camassola, M. Salvador, S. Moura, J. Hess, J. A. P. Henriques, A. J. P. Dillon, M. Roesch-Ely, *Nutr. Res.* **2013**, *33*, 76–84.

[32] P. A. Nikitina, E. I. Basanova, E. B. Nikolaenkova, I. A. Os'kina, O. A. Serova, N. I. Bormotov, L. N. Shishkina, V. P. Perevalov, A. Ya Tikhonov, *Bioorg. Med. Chem. Lett.* **2023**, *79*, 129080.
[33] R. Jadhav, S. Pawar, C. Khilare, A. Nikumbh, *Mater. Today: Proc.* **2023**, *73*, 487–493.
[34] G. Sadi, B. Emsen, A. Kaya, A. Kocabaş, S. Çınar, D. I. Kartal, *Pharmacogn Mag.* **2015**, *11*(Suppl 1), S6–S18. ■■Dear author, please check this journal. ■■
[35] G. Gebreyohannes, A. Nyerere, C. Bii, D. Berhe Sbhatu, *Evid.-Based Complement. Altern. Med.* **2019**, 6212673. ■■ Dear Author, if the journal has volumes, please add the journal number. ■■
[36] M. Fogarasi, Z. M. Diaconeasa, C. R. Pop, S. Fogarasi, C. A. Semeniuc, A. C. Fărcaş, D. Ţibulcă, C.-D. Sălăgean, M. Tofană, S. A. Socaci, *Agron.* **2020**, *10*, 1972.
[37] I. A. Ahmed, M. A. Mikail, M. bin Ibrahim, N. bin Hazali, M. S. B. A. Rasad, R. A. Ghani, R. A. Wahab, S. J. Arief, M. N. A. Yahya, *Food Chem.* **2015**, *172*, 778–787.
[38] I. Adewale Ahmed, M. Abimbola Mikail, N. Hisam Zamakshshari, M. Rais Mustafa, N. Mohd Hashim, R. Othman, *Saudi J. Biol. Sci.* **2022**, 103363. ■■ Dear Author, if the journal has volumes, please add the journal number. ■■
[39] I. A. Ahmed, M. A. Mikail, M. bin Ibrahim, N. bin Hazali, M. S. B. A. Rasad, R. A. Ghani, R. A. Wahab, S. J. Arief, M. N. A. Yahya, *Food Chem.* **2015**, *172*, 778–787.
[40] I. A. Ahmed, M. A. Mikail, M. R. Mustafa, M. Ibrahim, R. Othman, *Saudi J. Biol. Sci.* **2019**, *26*, 1519–1524.
[41] I. A. Ahmed, M. A. Mikail, N. Zamakshshari, A. H. Abdullah, *Biogerontology* **2020**, *21*, 293–310.
[42] L. Barros, R. C. Calhelha, J. A. Vaz, I. C. F. R. Ferreira, P. Baptista, L. M. Estevinho, *Eur. Food Res. Technol.* **2007**, *225*, 151–156.
[43] S. A. Heleno, M. A. Prieto, L. Barros, A. Rodrigues, M. F. Barreiro, I. C. F. R. Ferreira, *Food Bioprod. Process.* **2016**, *100*, 25–35.
[44] M. P. Singh, S. N. Rai, S. K. Dubey, A. T. Pandey, N. Tabassum, V. K. Chaturvedi, N. Singh, *Crit. Rev. Biotechnol.* **2022**, *42*, 913–930.
[45] S. N. Rai, D. Mishra, P. Singh, E. Vamanu, M. P. Singh, *Biomed. Pharmacother.* **2021**, *137*, 111377.
[46] V. K. Chaturvedi, N. Yadav, N. K. Rai, R. A. Bohara, S. N. Rai, L. Aleya, M. P. Singh, *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 13761–13775.
[47] S. M. M. Hossen, M. S. Hossain, S. Akbar, U. Tahmida, J. Mawa, N. Emon, *Heliyon* **2021**, *7*, e07997.
[48] Y. J. Park, E. S. Jung, D. Singh, D. E. Lee, S. Kim, Y. W. Lee, J.-G. Kim, C. H. Lee, *Food Res. Int.* **2017**, *102*, 544–552.
[49] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55–63.
[50] M. M. Al-Oqail, *Saudi Pharm. J.* **2021**, *29*, 244–251.
[51] N. H. Zamakshshari, I. A. Ahmed, N. A. M. Didik, M. N. A. Nasharuddin, N. M. Hashim, R. Abdullah, *Biomass Conv. Bioref.* **2022**, 1–9. ■■ Dear Author, if the journal has volumes, please add the journal number. ■■
[52] N. Zamakshshari, I. A. Ahmed, M. N. A. Nasharuddin, N. Mohd Hashim, M. R. Mustafa, R. Othman, M. I. Noordin, *J. Appl. Res. Med. Arom. Plants* **2021**, *24*, 100320.
[53] U. P. Nyayiru Kannaian, J. B. Edwin, V. Rajagopal, S. Nannu Shankar, B. Srinivasan, *Heliyon* **2020**, *6*, e03411.
[54] L. Sun, J. Zhang, X. Lu, L. Zhang, Y. Zhang, *Food Chem. Toxicol.* **2011**, *49*, 2689–2696.
[55] S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, J. M. Mérillon, *J. Agric. Food Chem.* **2009**, *57*, 1768–1774.

Manuscript received: January 23, 2023
Accepted manuscript online: May 26, 2023
Version of record online: ■■, ■■■

RESEARCH ARTICLE



Assist. Prof. N. Hisam Zamakshshari, Assoc. Prof. I. Adewale Ahmed*, M. Sc. M. Nazil Afiq Nasharuddin, M. Sc. N. Syahira Zaharudin, Assoc. Prof. N. Mohd Hashim, Assoc. Prof. R. Othman*

1 – 11

Comparative Study on Biological Activities and Chemical Profiling of Mushroom (*Pleurotus pulmonarius* (Fr.) Quel.) Cultured on Lignocellulosic Agro Wastes

SPACE RESERVED FOR IMAGE AND LINK

Share your work on social media! *Chemistry & Biodiversity* has added Twitter as a means to promote your article. Twitter is an online microblogging service that enables its users to send and read short messages and media, known as tweets. Please check the pre-written tweet in the galley proofs for accuracy. If you, your team, or institution have a Twitter account, please include its handle @username. Please use hashtags only for the most important keywords, such as #catalysis, #nanoparticles, or #proteindesign. The ToC picture and a link to your article will be added automatically, so the **tweet text must not exceed 250 characters**. This tweet will be posted on the journal's Twitter account (follow us @ChemBiodiv) upon publication of your article in its final form. We recommend you to re-tweet it to alert more researchers about your publication, or to point it out to your institution's social media team.

ORCID (Open Researcher and Contributor ID)

Please check that the ORCID identifiers listed below are correct. We encourage all authors to provide an ORCID identifier for each coauthor. ORCID is a registry that provides researchers with a unique digital identifier. Some funding agencies recommend or even require the inclusion of ORCID IDs in all published articles, and authors should consult their funding agency guidelines for details. Registration is easy and free; for further information, see http://orcid.org/.

Assist. Prof. Nor Hisam Zamakshshari
Assoc. Prof. Idris Adewale Ahmed http://orcid.org/0000-0002-2215-3900
M. Sc. Muhammad Nazil Afiq Nasharuddin
M. Sc. Nurul Syahira Zaharudin
Assoc. Prof. Najihah Mohd Hashim
Assoc. Prof. Rozana Othman