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Optimizing solid-state fermentation for metabolite enrichment by *Aspergillus tamarii* on rice bran and wheat

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ABSTRACT

Globalization has led to an increase in the global population and drug resistance, necessitating the development of novel pharmaceuticals. Fungi represent a promising source for new drugs, producing a diverse array of secondary metabolites, some of which are integral to clinically essential drugs. This study aimed to determine the optimal conditions for enhancing metabolite production by the Indonesian fungal strain *Aspergillus tamarii* (IPBCC 880066) when cultivated on solid media, specifically rice bran and wheat. The optimal fermentation conditions, including moisture content, growth temperature, and incubation duration, were determined based on the number of spots observed on thin-layer chromatography plates. Results indicated that the optimum conditions for metabolite production were a 40% moisture content, a growth temperature of 25°C, and a 14-day incubation period. Subsequent UPLC-MS/MS analysis identified 22 metabolites produced by *A. tamarii* on rice bran and 20 metabolites on wheat. Notably, two compounds, N-(2-hydroxypropyl)-2-methylacrylamide and N^6-[(benzyloxy)carbonyl]-L-lysine, were detected in both rice bran and wheat fermented by *A. tamarii*. Some of the identified metabolites have the potential to be applied in the pharmaceutical industry. In conclusion, our study emphasizes *A. tamarii's* efficacy in diverse metabolite production under optimized conditions, providing valuable insights for maximizing fungal metabolite production in potential pharmaceutical applications.

1. INTRODUCTION

Developing new drugs is essential to address challenges arising from the increase in the world population and drug resistance. Overusing existing medications and evolving pathogens contribute to the complexities of drug resistance. Currently, only a limited number of natural products, some of which are chemically related, are employed in medicine [1]. While natural products contribute significantly, with 27% of synthetic drugs approved in clinics [2], exploring diverse natural sources is crucial for discovering new compounds to combat drug-resistant strains and enhance treatment options.

Fungi, with their extensive biodiversity, offer a promising avenue for exploration. Despite the dominance of clinically essential drugs such as penicillins, cephalosporins, and beta-lactam antibiotics produced by fungi, manufacturing bioactive compounds from fungi still needs to be explored. This underutilization is surprising given the vast biodiversity of fungi, which has the potential to yield new bioactive compounds. Recent times have identified several new metabolites from fungi, highlighting the untapped potential for discovering novel compounds [1].

Aspergillus tamarii is one of the fungi that has been known to produce several secondary metabolites. It has demonstrated the synthesis of various bioactive compounds, including antibacterial agents (indole alkaloids), anti-inflammatory compounds (dipyrrolobenzoquinone), cyclic peptides, antibiotics, butenolides, and antiphytopathogenic substances (diketopiperazine) [3,4]. Notably, studies have confirmed that *A. tamarii* Kita can synthesize kojic acid. Kojic acid and its derivatives have diverse applications in the cosmetics and pharmaceutical industries, serving as antitumor, antidiabetic, anticancer, and skin-lightening agents [5]. This fungal species has also been reported to produce statin metabolites, which are cholesterol-lowering drugs, through fermentation [3].

Solid-state fermentation provides promising results for enhancing the production of metabolites. In this fermentation process, fungi are grown on a solid medium, resembling their natural habitat [6]. Rice bran and wheat can be essential ingredients for solid-state fermentation due to their carbohydrate content and their role as energy sources for mold growth; moreover, they are very abundant as agricultural waste [7]. Rice bran and wheat were chosen for their nutrient-rich composition,

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fostering optimal growth and secondary metabolite production in *A. tamarii*. The selection was based on their alignment with the natural habitat of *A. tamarii*, commonly found in agricultural environments dominated by rice and wheat.

In addition to providing a conducive medium for fungal growth, factors such as moisture content, growth temperature, and incubation time play crucial roles in influencing fungal physiology and metabolism. Assessing these influencing factors is imperative, given the direct impact of fungal physiology and metabolism on metabolite production. Thus, the evaluation of rice bran and wheat as solid media, along with an investigation into the conditions of microbial fermentation, becomes necessary. In this context, solid-state fermentation could produce metabolite profiles that can be analyzed using both thin-layer chromatography (TLC) and liquid chromatography-mass spectrometry (LC-MS) [8,9]. TLC offers a rapid overview and initial identification, while LC-MS provides detailed information for confirmation. Integrating TLC and LC-MS enhances analytical capabilities for robust metabolite profiling, contributing to scientific rigor and a deeper understanding of complex metabolic pathways in studied biological systems.

To the best of our knowledge, comprehensive research on metabolite production from the Indonesian strain *A. tamarii* has yet to be conducted. Additionally, there has been no analysis of metabolite profiles using TLC and LC-MS methods for *A. tamarii* undergoing solid-state fermentation. The primary objective of this study is to investigate the optimal conditions for enhancing metabolite production by the Indonesian fungal strain *A. tamarii* (IPBCC 880066) cultivated on rice bran and wheat as solid media.

2. MATERIALS AND METHODS

The TLC method was used to determine the optimal conditions for the fermentation of rice bran and wheat with *A. tamarii*. Subsequently, the fermentation outcomes under these optimal conditions were analyzed using the LC-MS method.

2.1. Fungus and Materials

A. tamarii (IPBCC 880066) was obtained from the IPB Culture Collection, IPB University, Indonesia. Rice bran and wheat were purchased from an e-commerce platform in Jakarta, Indonesia. All chemical reagents used were commercially available.

2.2. Culture Preparation

A. tamarii was inoculated using the spread-plate method on a petri dish containing PDA media and incubated at 25°C for 6 days. The agar culture of *A. tamarii* was taken using a cork borer (diameter: ±10 mm), forming an agar disc, which was then transferred into a culture tube containing 20% glycerol. The agar discs containing *A. tamarii* were stored in a freezer (−20°C) as a culture stock [10].

2.3. Solid-State Fermentation

The solid substrates employed in the research were rice bran and wheat. These substrates were allocated into three 250-mL Erlenmeyer flasks: Container A contained 10 g of rice bran, Container B contained 10 g of wheat, and Container C contained 5 g of rice bran and 5 g of wheat (mixture). Subsequently, 10 mL of distilled water was added to each Erlenmeyer flask containing the solid substrate. The media were autoclaved at 121°C for 20 min [11].

Fermentation was initiated by introducing one agar disc of culture from the culture stock into an Erlenmeyer flask containing sterile media. Moisture content optimization was conducted by adding varying volumes of distilled water to the solid media, specifically 4 mL (40% moisture), 6 mL (60% moisture), and 8 mL (80% moisture). Temperature optimization involved fermenting at two different temperatures: 25°C and 30°C. The optimization of growth time was achieved by varying the fermentation duration between 7 and 14 days [12,13].

2.4. Metabolites Extraction

The fermentation results were extracted using the maceration method. Following incubation, 50 mL of MeOH was added to the Erlenmeyer, stirred, and left for 48 h. Subsequently, filtration was performed with a vacuum pump. Additionally, 50 mL of MeOH was slowly added during filtration to rinse the sample in the Erlenmeyer [14,15]. The extraction of unfermented rice bran and wheat, serving as controls, was conducted using the same method.

2.5. TLC Analysis

Under various optimization conditions, the filtrate solution from the sample was applied to a TLC plate (Merck Silica Gel 60 F_{254}, Germany) using a capillary tube. Subsequently, the TLC plate was placed into a chamber saturated with EtOAc: MeOH (7:3). Simvastatin was used as a standard for comparison [3]. The eluent, EtOAc: MeOH (7:3), was allowed to migrate to an elution distance of 6 cm. Following this, the TLC plate was removed and dried. The spots on the plate were observed under UV light at wavelengths of 254 nm and 365 nm. Additionally, the spots on the plate were observed using iodine for 15 min until the spots turned brown.

The retention factor (R_f) was utilized to express the distance traveled by the spot on the plate surface. The retention factor (R_f) was determined by dividing the spot distance by the total distance traveled by the solvent. Retention factor calculations served as the basis for determining optimal fermentation conditions. The fermentation results under optimum conditions were subsequently analyzed by LC-MS [16].

2.6. LC-MS Analysis

The fermentation filtrate obtained under optimum conditions was concentrated using a rotary evaporator at a temperature of 50°C. The resulting crude extract was then subjected to LC-MS analysis. The LC-MS instrument was the Ultra Performance Liquid Chromatography Acquity UPLC® H-Class System (Waters, US) equipped with a C18 HSS column (1.8 µm, 2.1×100 mm) maintained at a column temperature of 50°C in a room set at 25°C. The mobile phase consisted of a mixture of water with 5 mM ammonium formate as solution A and a mixture of acetonitrile with 0.05% formic acid as solution B. The flow rate was set at 0.2 mL/min with a gradient applied over 23 min, and the injection volume was 5 µL. Before injection, samples were filtered using a 0.2 µm filter.

The mass spectrometry parameters included positive electrospray ionization (ESI) mode, with a mass analysis range of 50–1200 m/z. The source temperature was maintained at 100°C, and the desolvation temperature was set at 350°C. The cone gas rate was 0 L/h, while the gas desolvation rate was 793 L/h. The collision energy was set at 4 V for low energy, and the ramp collision energy ranged from 25 to 60 V for high energy.

2.7. Data Analysis

The chromatograms obtained in .raw format were processed using the Masslynx 4.1 application to predict the molecular formula of each compound with elemental composition. The molecular formula of the compound with the highest iFit confidence (iFit conf%) was selected, and the compound name was searched on the ChemSpider web page (http://www.chemspider.com/) by selecting the compound or ID number with the most publications.

3. RESULTS AND DISCUSSION

3.1. Culture Growth and Solid-State Fermentation

A. tamarii exhibited colonies with dark green conidia. Following inoculation on a petri dish and subsequent incubation, dark green conidia appeared in the colonies as early as the 3rd day [Figure 1a]. To preserve the agar disc culture stock, it was stored in 20% glycerol [Figure 1b]. Glycerol can mitigate cell damage at low temperatures, thereby maintaining cell functionality for an extended period of time [10]. Glycerol concentration for fungal culture preservation can be 10% for 1 month of storage [10], but the glycerol concentration reported for a more extended storage period (6 months) was 50% [17]. The form of agar disc, combined with glycerol as a cryoprotectant, was adequate for fungal culture maintenance at −20°C [17]; therefore, the glycerol concentration chosen for storage between 1 and 6 months was 20%.

The growth of *A. tamarii* in solid-state fermentation commenced with the development of colonies, followed by the emergence of brownish-green conidia [Figure 1c]. In solid-state fermentation, water is needed to swell the substrate so that the microorganism can utilize the nutrient, while the moisture meets the minimum a_w of the microorganism for growth. The optimum moisture content to produce cells and enzymes can be different; the substrate type is a determinant, and the duration of fermentation combined with moisture content results differently [18].

3.2. Metabolite Profiles

The elution results of the standard and samples on TLC plates were observed under UV light at 254 nm [Figure 2a] and 365 nm [Figure 2b]. Simvastatin as the standard was marked with A, while the extracted samples were marked as follows: rice bran incubated at 25°C (B), wheat incubated at 25°C (C), a mixture of rice bran and wheat incubated at 25°C (D), and rice bran incubated at 30°C (E). As suggested by Abdelwahab *et al*. [19], statins demonstrate optimal sensitivity at a wavelength of 254 nm. Simvastatin was detected at 254 nm (a) but not 365 nm (b). Meanwhile, the R_f values for all samples are summarized in Table 1. Even though there were several spots with R_f values close to simvastatin, these spots could not be concluded to be simvastatin because they still exhibit fluorescence at a wavelength of 365 nm. Simvastatin only absorbs light at 254 nm without emitting fluorescence at 365 nm. It is possible that the compound shares polarity characteristics with simvastatin but contains different functional groups, such as the structure of a flavonoid glycoside that can emit light at a wavelength of 365 nm. To validate the UV light visualization results, elution outcomes were also observed with iodine vapor. Iodine vapor introduced to the sample resulted in brown spots with varying intensity [20].

The TLC results served as the foundation for selecting the optimal fermentation conditions. Screening of a broad spectrum of biological properties of plants is suitable to be conducted by TLC, and the usage of other analytical methods coupled with TLC has emerged [8]. Thus, the TLC method was used for initial compound separation. The criteria for optimum fermentation conditions were based on achieving well-defined compound separation on TLC. A desirable retention factor for effective compound separation fell within the range of 0.2–0.8 [21]. Factors such as non-overlapping spots, a balanced solvent in the elution process (preventing inaccurate R_f values), and avoidance of streaking—where high sample concentration leads to line-shaped separation—were considered. Overlapping spots complicate R_f measurements and hinder the precise identification of each compound's endpoint. A properly balanced solvent system, selected based on interaction with sample molecules, is crucial for achieving accurate separation. Imbalances, such as overly polar or non-polar solvents, distort R_f values by influencing the mobility of components. Streaking, resulting from uneven sample application or irregularities in the stationary phase, introduces uncertainty in measuring the distance traveled. Maintaining clean spots is essential for precise R_f calculations, ensuring the reliability of chromatographic separation. The number of spots was also considered, as the TLC method is commonly employed for screening bioactive compounds [8,22,23].

Considering all the criteria, we concluded that the optimal fermentation condition was 40% moisture with an incubation temperature of 25°C for 14 days. Under these conditions, a favorable retention factor was observed, with no overlapping or streaking, and there was a significant separation of compounds, evident by the presence of multiple spots. The optimal conditions in this study differed from a prior one, where *A. tamarii* was grown on brewery spent grain at 40°C for 4 days,

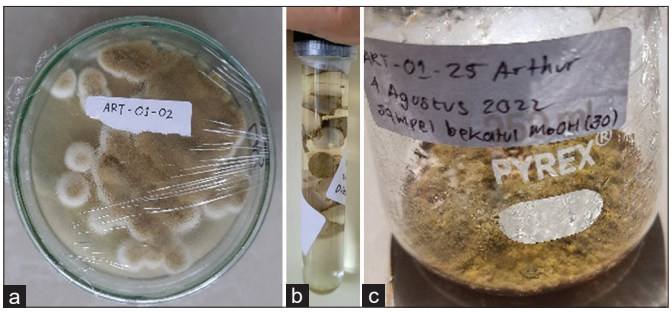

Figure 1: *Aspergillus tamarii* culture on agar plate (a), agar discs as culture stock (b), and solid-state fermentation (c).

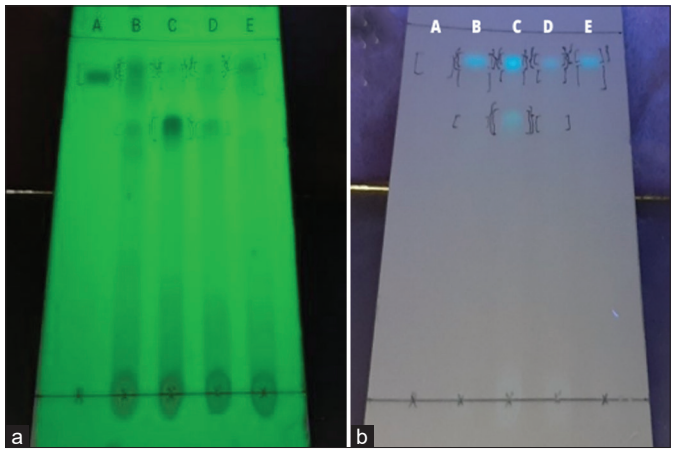

Figure 2: Thin-layer chromatography profiles of medium fermented with *Aspergillus tamarii* under UV of 254 nm (a) and 365 nm (b). Note: simvastatin (a), rice bran incubated at 25°C (b), wheat incubated at 25°C (c), mixture of rice bran and wheat incubated at 25°C (d), and rice bran incubated at 30°C (e).

Table 1: Retention factor (R*f*) on thin-layer chromatography of compounds from rice bran and wheat fermented with *Aspergillus tamarii* under certain conditions (moisture content, growth temperature, and incubation time).

Optimization condition	Media	R_f		
		$UV_{254\ nm}$	$UV_{365\ nm}$	Iodine Vapor
40% of moisture	Rice bran	0.850	0.850	0.125
	Wheat	0.783; 0.950	0.916	-
	Mixture	0.850	0.850	0.125
60% of moisture	Rice bran	0.830	0.830	-
	Wheat	0.916	0.916	0.816; 0.880; 0.930
	Mixture	0.816	0.816	-
80% of moisture	Rice bran	0.925	0.925	-
	Wheat	0.742; 0.816	0.816	0.870; 0.950
	Mixture	0.925	0.925	-
Temperature at 25°C	Rice bran	0.730; 0.860; 0.93	0.930	-
	Wheat	0.730; 0.880; 0.930	0.750; 0.940	-
	Mixture	0.725; 0.880; 0.930	0.950	-
Temperature at 30°C	Rice bran	0.880; 0.950	0.960	-
	Wheat	0.916; 0.960	0.975	-
	Mixture	0.930; 0.980	0.980	-
7 days of incubation	Rice bran	0.760; 0.960	0.975	-
	Wheat	0.740; 0.890	0.890	-
	Mixture	0.916; 0.960	0.975	-
14 days of incubation	Rice bran	0.730; 0.925	0.916	-
	Wheat	0.760; 0.930	0.933	-
	Mixture	0.760; 0.950	0.916	-
Simvastatin	Rice bran	0.75; 0.86; 0.93	-	-
	Wheat	0.783; 0.942	-	-
	Mixture	0.683	-	-
Simvastatin standard		0.816; 0.900; 0.900		

focusing on ascorbic acid production [24]. Another investigation noted that *A. tamarii* Kita UCP 1279 exhibited the highest fructosyltransferase activity after 4 days of incubation at 30°C in 3 g of wheat bran, 70% moisture, and 20% sucrose [25]. In contrast, our study screened all metabolites produced by *A. tamarii*.

The optimal condition for metabolite production seems to depend on the specific metabolites or substances analyzed. The current study found that metabolite production tends to increase between 25°C and 30°C and decrease at both minimum and maximum temperatures [26]. This finding indicated that the fungal fermentation process, with proper conditions, could maximize metabolite or enzyme production. Solid-state fermentation offers a promising bioprocess for producing metabolites from agro-waste and industrial residues. These residues, including bran, bagasse, husks, pomace, seeds, peels, and corn residue, are generated annually and often need to be more utilized or discarded [27]. Recently, significant interest has been in utilizing these abundant and cost-effective renewable substrates to produce various valuable compounds.

3.3. LC-MS Analysis Results

According to the optimal conditions resulting from the previous step, *A. tamarii* was cultivated. Subsequently, the obtained extract was separated using an LC-MS instrument to give chromatogram data. The chromatograms were processed using Masslynx 4.1 software. The molecular formula for each compound was predicted using Masslynx 4.1 software, where each chromatogram peak corresponded to a distinct compound. The chromatogram results from solid-state fermentation with *A. tamarii*, employing 40% moisture and incubated at 25°C for 14 days, are illustrated in Figures 3 and 4. In this research, the LC-MS results were presented as qualitative data, offering insights into the identity and presence of compounds rather than their specific quantitative amounts.

Figure 3 depicts the metabolites produced in the fermentation of rice bran with *A. tamarii.* There are 22 types of compounds, including three unknown compounds identified on the ChemSpider website. In contrast, wheat fermentation with *A. tamarii* resulted in 20 types of compounds, with six unknown compounds identified on the ChemSpider website [Figure 4]. Although *A. tamarii* cultivated on both solid media yields nearly similar metabolite profiles, the abundance of each detected compound varies significantly. This discrepancy may stem from differences in *A. tamarii*'*s* ability to metabolize rice bran and wheat nutrients. Another factor is the initial diversity in metabolite concentrations in rice bran and wheat, resulting in similar metabolite types during fermentation but with varying concentrations [28-30]. Meanwhile, identifying unknown compounds in this research adds curiosity and novelty to the study. Unraveling their nature could offer new insights and applications, contributing to a deeper understanding of biological processes and revealing valuable applications. The compounds with 100% abundance are detailed in Table 2.

Solid-state fermentation of rice bran by *A. tamarii* led to the production of compounds belonging to the amine, amide, ketone, carboxylic ester, sulfonic acid, hydrazide, and cyclohexane groups. Conversely, the fermentation of wheat by *A. tamarii* resulted in compounds classified as amides, amines, ketones, carboxylic esters (benzoates), and alcohol. LC-MS screening revealed the presence of N-(2-hydroxypropyl)-2-methylacrylamide and N^6-[(benzyloxy)carbonyl]-L-lysine in the results of *A. tamarii* solid-state fermentation on both rice bran and wheat. The results showed different metabolite categories produced during rice bran and wheat fermentations. The diversity of metabolites may influence the potential applications of *A. tamarii* in various industries, such as pharmaceuticals or agriculture. For example, 3,5-bis(1H-imidazol-1-ylmethyl)-4H-1,2,4-triazol-4-amine, identified in wheat fermentation, and 4-hydrazino-6-(4-morpholinyl)-N-phenyl-1,3,5-triazin-2-amine, found in rice bran fermentation, belong to the category of triazole derivative compounds. Researchers have reported that compounds with triazole residues exhibit significant pharmacological activities, including antiviral and antimicrobial properties [31].

In a study by Zarei *et al*., 453 metabolites were identified in rice bran. Based on metabolic pathways, these metabolites were categorized into 126 amino acids, 35 carbohydrates, 28 co-factors and vitamins, 11 energy-related lipids, 137 lipids, 40 nucleotides, 28 peptides, 55 secondary metabolites, and 8 xenobiotic metabolites [32]. From these categories, 209 metabolites were deemed to have potential human health benefits. Recent studies focused on only 65 rice bran metabolites from amino acids, vitamins, co-factors, and secondary metabolites, emphasizing their medicinal and human health-promoting attributes [32]. Meanwhile, wheat is rich in compounds essential

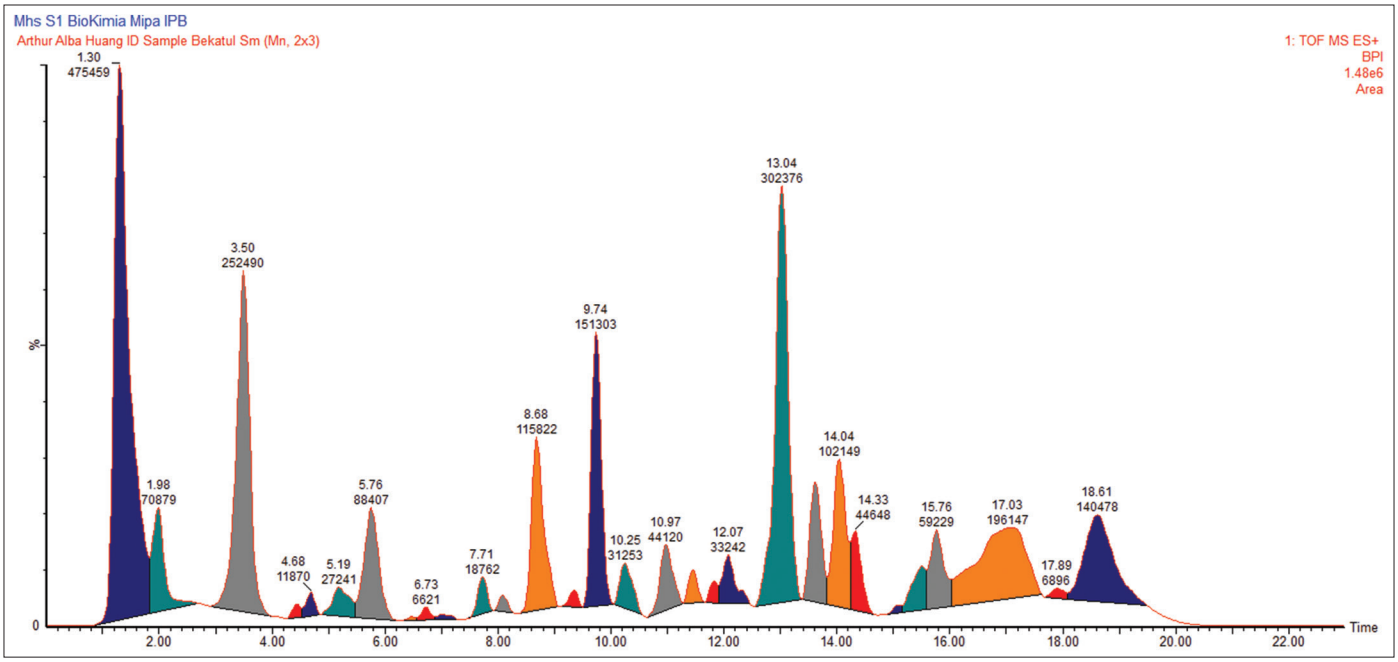

Figure 3: Chromatogram depicting the results of solid-state fermentation of rice bran with *Aspergillus tamarii* under 40% moisture, incubated at 25°C for 14 days.

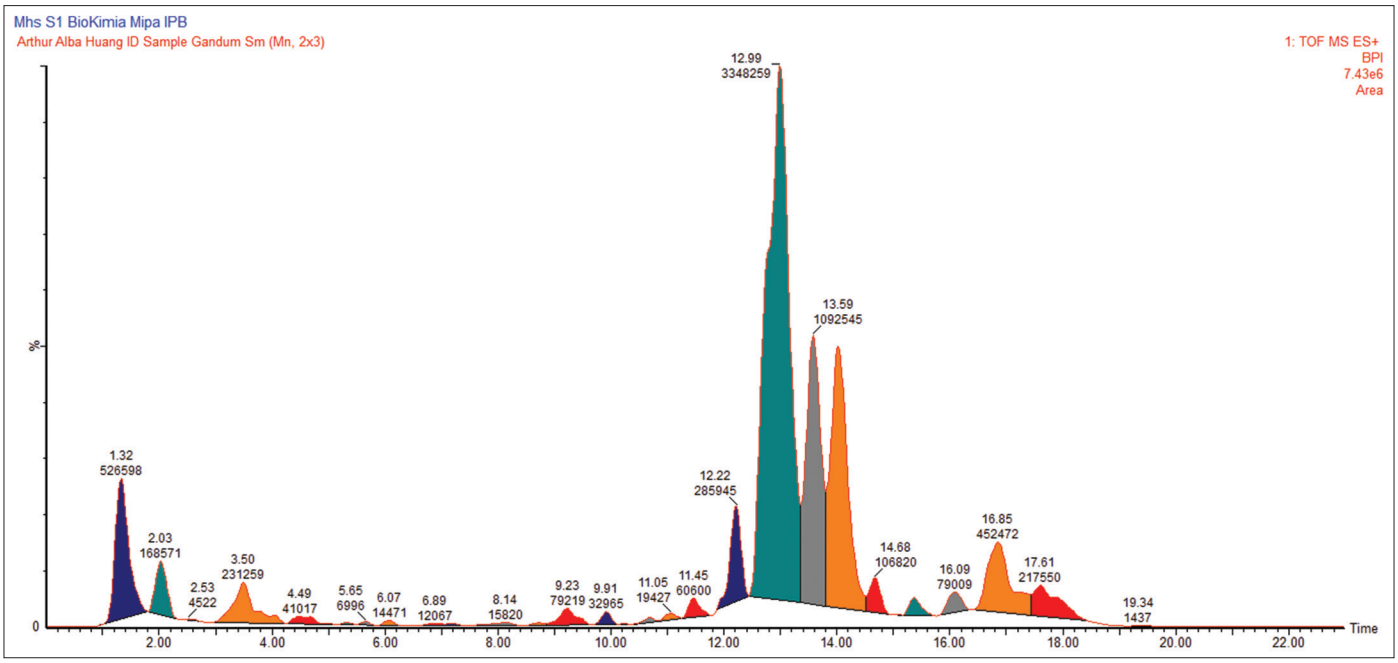

Figure 4: Chromatogram depicting the results of solid-state fermentation of wheat with *Aspergillus tamarii* under 40% moisture, incubated at 25°C for 14 days.

for human nutrition, encompassing nutrients, antinutrients, lipids, phytochemicals, and fiber. Tais *et al*. identified 248 unique compounds in whole wheat grains, successfully grouping 37 compounds [33]. The categorized wheat metabolites include hydroquinone, hydroxycinnamic acid amide, benzoxazionide, flavonoids, lignans, and various phenolic compounds.

Certain *Aspergillus* species, such as *Aspergillus niger*, are known for their abundance of primary and secondary metabolites. According to Yu *et al*., *A. niger* produced 166 secondary metabolites identified up to 2020 [34]. These compounds fall into categories such as pyrones (gamma-naptilpyrone, alpha-pyrone, and gamma-pyrone), alkaloids (pyranonigrin derivatives, pyridone, fumonisin, nigerloxin, and ergosteriamide), cyclopentapeptides (diketopiperazine and malformin), polyketides (citric acid, itaconic acid, 2-phenylethanol, *p*-hydroxyphenylacetic acid, gallic acid, benzoic acid derivatives, and asperielone), and sterols (14-dehydroergosterol, nigerasterol A and B, and ergosteriamide).

The versatile application of LC-MS analysis extends to detecting metabolites, environmental contaminants, and food contaminants through non-targeted approaches. In this study, a non-targeted approach offers an advantage in capturing a broader spectrum of compounds, encompassing known and unknown entities. This approach will

Table 2: Predicted compounds in LC-MS/MS from solid-state fermentation of rice bran and wheat with *Aspergillus tamarii* at 40% moisture, incubated at 25°C for 14 days.

Retention time (min)	Measured mass	Calculated mass	Formula	Predicted compound	Rice bran	Wheat
1.30	144.1028	144.1025	$C_7H_{14}NO_2$	N-(2-Hydroxypropyl)-2-methylacrylamide	✓	✓
1.98	162.0565	162.0555	$C_9H_8NO_2$	1H-Indole-3-carboxylic acid	✓	
2.03	143.0346	143.0344	$C_6H_7O_4$	5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one (asam kojik)		✓
2.53	264.1452	264.1434	$C_8H_{14}N_{11}$	Unknown		✓
3.50	281.1505	281.1501	$C_{14}H_{21}N_{2O4}$	N^6-[(Benzyloxy) carbonyl]-L-lysine	✓	
4.45	265.1554	265.1525	$C_{10}H_{17}N_8O$	4-{5-[(4-Methyl-1-piperazinyl) methyl]-1H-1,2,3-triazol-1-yl}-1,2,5-oxadiazol-3-amine	✓	
4.49	263.1393	263.1387	$C_9H_{20}N_6OCl$	N^2-Methoxy-N^2, N^4, N^4, N^6, N^6-pentamethyl-1,3,5-triazine-2,4,6-triamine hydrochloride (1:1)		
4.68	304.1658	304.1634	$C_{12}H_{18}N_9O$	N-[6-(4-Methyl-1-piperazinyl)-4-pyrimidinyl]-2-(1H-tetrazol-1-yl) acetamide	✓	
4.99	243.1336	243.1291	$C_3H_{11}N_{14}$	Unknown		✓
5.19	346.2119	346.2104	$C_{15}H_{24}N_9O$	3-(5-Methyl-1H-tetrazol-1-yl)-N- {2-[4-(2-pyrimidinyl)-1-piperazinyl] ethyl} propanamide	✓	
5.35	211.1451	211.1420	$C_7H_{15}N_8$	N-[1-(4-Methyl-4H-1,2,4-triazol-3-yl) ethyl] imidodicarbonimidic diamide		✓
5.65	211.1449	211.1420	$C_7H_{15}N_8$	N-[1-(4-Methyl-4H-1,2,4-triazol-3-yl) ethyl] imidodicarbonimidic diamide		
5.76	252.1607	252.1573	$C_{10}H_{18}N_7O$	[4,6-Bis (dimethylamino)-1,3,5-triazin-2-yl](methoxymethyl) cyanamide	✓	
6.07	245.1310	245.1263	$C_{10}H_{13}N_8$	3,5-Bis (1H-imidazol-1-ylmethyl)-4H-1,2,4-triazol-4-amine		✓
6.48	241.1450	241.1413	$C_9H_{17}N_6O_2$	N-[3-(Dimethylamino) propyl]-5-nitro-4,6-pyrimidine diamine	✓	
6.73	280.1919	280.1886	$C_{12}H_{22}N_7O$	2-Hydrazino-4-(4-morpholinyl)-6-(1-piperidinyl)-1,3,5-triazine	✓	
6.89	388.1879	388.1918	$C_{10}H_{22}N_{13}O_4$	Unknown		
6.99	249.1132	249.1127	$C_{14}H_{17}O_4$	Diethyl benzylidenemalonate	✓	
7.19	344.2254	344.2298	$C_{15}H_{30}N_5O_4$	2-{(4S)-3-[(Ethylcarbamoyl) amino]-4-methoxy-1-pyrrolidinyl}-N-(isopropylcarbamoyl) propanamide		✓
7.71	308.2227	308.2259	$C_{15}H_{34}NO_3S$	3-(Dodecylamino)-1-propanesulfonic acid	✓	
8.09	288.1620	288.1573	$C_{13}H_{18}N_7O$	4-Hydrazino-6-(4-morpholinyl)-N-phenyl-1,3,5-triazin-2-amine	✓	
8.14	560.3566	560.3534	$C_{24}H_{42}N_{13}O_3$	Unknown		✓
8.68	331.0814	331.0818	$C_{17}H_{15}O_7$	5,7-Dihydroxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4H-chromen-4-one (tricin)	✓	
8.73	548.3607	548.3633	$C_{21}H_{46}N_{11}O_6$	Unknown		✓
9.23	379.2959	379.2961	$C_{22}H_{39}N_2O_3$	1-Cycloheptyl-5-{[4-(3-methoxypropyl)-1-piperidinyl] carbonyl}-2-piperidinone		✓
9.35	261.1502	261.1464	$C_{12}H_{17}N_6O$	N'-([1,2,4]Triazolo[4,3-b] pyridazin-6-yl) cyclohexanecarbohydrazide	✓	
9.74	614.3769	614.3752	$C_{28}H_{56}NO_{13}$	35-Amino-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacont-1-yl methacrylate	✓	
9.91	665.2889	665.2849	$C_{36}H_{33}N_{12}O_2$	Unknown		✓
10.22	403.2024	403.1981	$C_{20}H_{27}N_4O_5$	2-(Diethylamino) ethyl 4-{[(1,3-dimethyl-2,4,6-trioxotetrahydro-5 (2H)-pyrimidinylidene) methyl] amino} benzoate		
10.25	403.2008	403.2022	$C_{25}H_{27}N_2O_3$	N'-[(2-Isopropyl-5-methylphenoxy) acetyl]-4-biphenylcarbohydrazide	✓	
10.97	642.4053	642.4024	$C_{23}H_{48}N_{17}O_5$	Unknown	✓	
11.05	288.1588	288.1573	$C_{13}H_{18}N_7O$	4-Hydrazino-6-(4-morpholinyl)-N-phenyl-1,3,5-triazin-2-amine		
11.45	318.3018	318.2981	$C_{14}H_{36}N_7O$	Unknown	✓	
11.45	318.3009	318.3008	$C_{18}H_{40}NO_3$	2-Amino-1,3,4-octadecanetriol		✓
11.83	439.3325	439.3298	$C_{24}H_{39}N_8$	4,4'-Bis[(3,5,6-trimethyl-2-pyrazinyl) methyl]-1,1'-bipiperazine	✓	
12.07	337.1555	337.1525	$C_{16}H_{17}N_8O$	1-Ethyl-7-[2-methyl-6- (4H-1,2,4-triazol-3-yl)-3-pyridinyl]-3,5-dihydropyrazino[2,3-b] pyrazin-2 (1H)-one	✓	
12.99	520.3415	520.3432	$C_{18}H_{42}N_{13}O_5$	Unknown		✓
13.04	520.3392	520.3346	$C_{23}H_{46}N_5O_8$	2-Methyl-2-propanyl 4-(20-azido-3,6,9,12,15,18-hexaoxaicos-1-yl)-1-piperazinecarboxylate	✓	
13.59	496.3455	496.3459	$C_{20}H_{46}N_7O_7$	Unknown		✓
13.61	496.3412	496.3459	$C_{20}H_{46}N_7O_7$	Unknown	✓	

✓ *appeared on the related chromatogram*, LC-MS/MS: Liquid chromatography with tandem mass spectrometry.

facilitate the demonstration of the results of growth optimization for the produced metabolites. However, LC-MS results are primarily qualitative, with compound responses influenced by compound nature, eluent composition, and the specific instrument used for analysis. Instrument-related aspects, including the program or ionization model, play a crucial role in the response and separation of compounds in LC-MS, emphasizing the importance of selecting suitable programs for practical analysis [35].

4. CONCLUSION

This study aimed to optimize metabolite production in the Indonesian fungal strain *A. tamarii* on solid media, specifically rice bran and wheat. The results identified optimal conditions for solid-state fermentation as 40% moisture with an incubation temperature of 25°C for 14 days. Under these conditions, the fermentation demonstrated the maximum production of metabolites. LC-MS analysis of *A. tamarii*'s rice bran fermentation yielded 22 detected compounds, including 3 unknowns, while wheat fermentation resulted in 20 compounds, including 6 unknowns. Two common compounds, N-(2-hydroxypropyl)-2-methylacrylamide and N6-[(benzyloxy)carbonyl]-L-lysine, were identified in both rice bran and wheat. The metabolites produced during rice bran fermentation comprised a diverse range, including amines, amides, ketones, carboxylic esters, sulfonic acids, hydrazides, and cyclohexanes. In contrast, wheat fermentation predominantly produces metabolites in the form of amides, amines, ketones, carboxylic esters (benzoates), and alcohols. These findings contribute comprehensive insights into the metabolite profile produced by *A. tamarii* through solid-state fermentation. The data is anticipated to serve as a valuable guide for further understanding the potential metabolites derived from the fermentation process in *A. tamarii*, thereby enriching the field of fungal metabolomics.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements and guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included in this research article.

10. PUBLISHER'S NOTE

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REFERENCES

1. Hoeksma J, Misset T, Wever C, Kemmink J, Kruitjzer J, Versluis K, *et al*. A new perspective on fungal metabolites: Identification of bioactive compounds from fungi using zebrafish embryogenesis as read-out. Sci Rep 2019;9:17546.
2. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2019;79:629-61.
3. Anilkumar NK, Krishna AR, Chockalingan J, Balakrishnan L, Janarthanan NT, Ramachandran P, *et al*. Elucidation of antifungal, antioxidant and anticholesterol activity of efficiency of fungal statin isolated from *Aspergillus tamarii.* Biosci Biotechnol Res Commun 2020;13:1597-604.
4. Huu DT, Phuong HT, Tran PT, Souvannalath B, Trung HL, Ho DV, *et al*. Secondary metabolites from the grasshopper-derived entomopathogenic fungus *Aspergillus tamarii* NL3 and their biological activities. Nat Prod Commun 2022;17:1-5.
5. Saeedi M, Eslamifar M, Khezri K. Kojic acid applications in cosmetic and pharmaceutical preparations. Biomed Pharmacother 2019;110:582-93.
6. Thomas L, Larroche C, Pandey A. Current developments in solid-state fermentation. Biochem Eng J 2013;81:146-61.
7. Valentino MJ, Ganado LS, Undan JR. Single cell protein potential of endophytic fungi associated with bamboo using rice bran as substrate. Adv Appl Sci Res 2016;7:68-72.
8. Kowalska T, Sajewicz M. Thin-layer chromatography (TLC) in the screening of botanicals-its versatile potential and selected applications. Molecules 2022;27:6607.
9. Switzerland: Evans Analytical Group Laboratories, Liquid Chromatography Mass Spectrometry (LC-MS). Available from: https://www.eag.com/techniques/mass-spec/liquid-chromatography-mass-spectometry-lc-ms [Last accessed on 2023 Dec 04].
10. Homolka L. Methods of cryopreservation in fungi. In: Gupta VK, Tuohy MG, Ayyachamy M, Turner KM, O'Donovan A, editors. Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology. New York: Springer; 2013.
11. Bhaskar T, Varjani S, Pandey A, Rene ER. Waste Biorefinery. 1st ed. Amsterdam: Elsevier; 2021.
12. Kamath PV, Dwarakanath BS, Chaudhary A, Janakiraman S. Optimization of culture conditions for maximal lovastatin production by *Aspergillus terreus* (KM017963) under solid state fermentation. HAYATI J Biosci 2015;22:174-80.
13. Manzoni M, Bergomi S, Rollini M, Cavazzoni V. Production of statins by filamentous fungi. Biotechnol Lett 1999;21:253-7.
14. Srikandace Y. Aktivitas Antioksidan Kapang Endofitik *Aspergillus* spp dariBiotaLautdariBeberapaMediaFermentasi;2016.p.802-5.Available from: https://ifory.id/proceedings/2016/4chq7e9cp/snips_2016_yoice__srikandace_4affe3d877b561bc53aabec275ad83fb.pdf [Last accessed on 2023 Dec 04].
15. Sukmawati IK, Purnamaasri D, Suwendar S. Activity of ethanol extract and fractions of basil leaves (*Ocimum sanctum* L.) against fungi *Candida albicans*, *Microsporum gypseum*, and *Aspergillus flavus*. J Farmasi Galenika 2017;3:30-5.
16. Sari SP, Ikayanti R, Widayanti E. Thin Layer Chromatography (TLC): Chromatogram pattern approach to confirm rhodamine B in cheek blush. J Syifa Sci Clin Res 2022;4:494-500.
17. Saxena A, Gupta S. Experimental comparison: Methods for the preservation of fungal cultures. Curr Res Environ Appl Mycol 2019;9:208-12.
18. Ridder ER, Nokes SE, Knutson BL. Optimization of solid-state

fermentation parameters for the production of xylanase by *Trichoderma longibrachiatum* on wheat bran. Trans ASAE 1998;41:1453-9.
19. Abdelwahab NS, El-Zeiny BA, Tohamy SI. TLC-densitometric method for determination of some cholesterol lowering drugs in different combinations. J Chromatogr Sep Tech 2012;3:126.
20. Rosamah E. Kromatografi Lapis Tipis: Metode Sederhana dalam Analisis Kimia Tumbuhan Berkayu. Samarinda: Mulawarman University Press; 2019.
21. Syaefudin S, Dwicesaria MA, Hasanah U, Nurcholis W. Biochemical Research Techniques: A Beginner's Guide to Isolating Bioactive Compounds. Bogor: IPB Press; 2022.
22. Nichols L. Thin-Layer Chromatography; 2023. Available from: https://chem.libretexts.org/ancillary_materials/demos_techniques_and_experiments/general_lab_techniques/thin_layer_chromatography [Last accessed on 2023 Dec 04].
23. Wulandari L, Retnaningtyas Y, Mustafidah D. Development and validation of a thin-layer chromatography densitometry method for simultaneous determination of theophylline and ephedrine hydrochloride levels in tablet formulations. J Kimia Terapan Indone 2013;15:15-21.
24. Banjo TT, Kareem SO, Adeogun AI. Ascorbic acid production by *Aspergillus flavus* and *Aspergillus tamarii*; Kinetic and thermodynamic study. Curr Microbiol 2020;77:1476-82.
25. Batista JM, Brandao-Costa RM, da Cunha MN, Rodrigues HO, Porto AL. Purification and biochemical characterization of an extracellular fructosyltransferase-rich extract produced by *Aspergillus tamarii* kita UCP1279. Biocatal Agric Biotechnol 2020;26:101647.
26. Bose P, Gowrie SU, Chathurdevi G. Optimization of culture conditions for growth and production of bioactive metabolites by endophytic fungus-*Aspergillus tamarii*. Int J Pharm Biol Sci 2019;9:469-78.
27. Kumar V, Ahluwalia V, Saran S, Kumar J, Patel AK, Singhania RR. Recent developments on solid-state fermentation for production of microbial secondary metabolites: Challenges and solutions. Bioresour Technol 2021;323:124566.
28. Liu N, Song M, Wang N, Wang Y, Wang R, An X, *et al*. The effects of solid-state fermentation on the content, composition and *in vitro* antioxidant activity of flavonoids from dandelion. PLoS One 2020;15:e0239076.
29. Xiao Y, Wu X, Yao X, Chen Y, Ho CT, He C, *et al*. CMetabolite profiling, antioxidant and α-glucosidase inhibitory activities of buckwheat processed by solid-state fermentation with *Eurotium cristatum* YL-1. Food Res Int 2021;143:110262.
30. Ma Y, Ling TJ, Su XQ, Jiang B, Nian B, Chen LJ, *et al*. Integrated proteomics and metabolomics analysis of tea leaves fermented by *Aspergillus niger*, *Aspergillus tamarii* and *Aspergillus fumigatus*. Food Chem 2021;334:127560.
31. Marinescu M. Benzimidazole-triazole hybrids as antimicrobial and antiviral agents: A systematic review. Antibiotics (Basel) 2023;12:1220.
32. Zarei I, Brown DG, Nealon NJ, Ryan EP. Rice bran metabolome contains amino acids, vitamins & cofactors, and phytochemicals with medicinal and nutritional properties. Rice (N Y) 2017;10:24.
33. Tais L, Schulz H, Böttcher C. Comprehensive profiling of semi-polar phytochemicals in whole wheat grains (*Triticum aestivum*) using liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. Metabolomics 2021;17:18.
34. Yu R, Liu J, Wang Y, Wang H, Zhang H. *Aspergillus niger* as a secondary metabolite factory. Front Chem 2021;9:701022.
35. Liigand J, Wang T, Kellogg J, Smedsgaard J, Cech N, Kruve A. Quantification for non-targeted LC/MS screening without standard substances. Sci Rep 2020;10:5808.

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