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Novel microbial transformation of *Andrographis paniculata* by *Aspergillus oryzae* K1A

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Abstract. *Handayani R, Dinoto A, Bintang M. 2021. Novel microbial transformation of* Andrographis paniculata *by* Aspergillus oryzae *K1A. Biodiversitas 23: 110-117.* Microbial transformation is a powerful technique for altering organic substances with complex chemical structures. *Aspergillus* has been found to produce secondary metabolites from biotransformation products of various medicinal plants. *Andrographis paniculata* Nees. or sambiloto is an Asian traditional medicinal plant. This study aimed to investigate the microbial transformation of *A. paniculata* by *Aspergillus oryzae* K1A. The leaf of *A. paniculata* was fermented by *A. oryzae* K1A. Microorganisms were transferred into the flasks from the slants. The flasks were placed on rotary shakers, operating at 120 rpm at 37°C. 0.2ml of the substrate solution was added into the fermentation flasks and these flasks were maintained under the same conditions and sampling was carried out each day of 0, 1, 3, 7 and 14. The samples were analyzed by TLC, HPLC, and the LC-MS/MS method to evaluate the biotransformation products. The TLC analysis showed different spots of *A. paniculata* fermented and non-fermented on days of 7 to 14 th and the Rf value was disappeared. The peaks of *A. paniculata* fermented and non-fermented were relatively similar on days of 0 to 1 incubation and the concentrations of *A. paniculata* fermented and non-fermented were increased during incubation time in the HPLC analysis. The chromatograms LC-MS/MS study showed different peaks between *A. paniculata* fermentation and non-fermentation on 3 to 14 days of incubation, and new secondary metabolites picrasidine K ($C_{18}H_{23}N_3O_2$); bufotalinin ($C_{24}H_{30}O_6$); and β-carboline were shown in the table of *A. paniculata* fermented.

Keywords: *Andrographis paniculata*, *Aspergillus oryzae* K1A, fermentation, LC-MS/MS

Abbreviations: TLC: Thin-Layer Chromatography, HPLC: High-Performance Liquid Chromatography, LC-MS/MS: Liquid Chromatography-Mass Spectrometry/Mass Spectrometry

INTRODUCTION

Andrographis paniculata Nees. has been identified to possess several bioactivities, i.e., antibacterial, anti-inflammatory, immunomodulatory, hepatoprotective, anti-HIV, anticancer, and anti-oxidant (Premanath and Devi 2011; Sule et al. 2011; Mishra et al. 2013; Sivananthan 2013; Okhuarobo et al. 2014). Four primary active diterpenoids are found in *A. paniculata* which are andrographolide, 14-deoxy-11, 12-didehydroandrographolide, neoandrographolide, and 14-deoxyandrographolide (Song et al. 2013). Andrographolide is a diterpene lactone, and diterpenes are known to have a broad spectrum of biological activity, making them attractive for biotransformations. Investigations of diterpene biotransformation generally attempt to produce novel active compounds (De Sousa et al. 2018).

The way to discover new therapeutic targets is to improve the production of bioactive compounds through secondary metabolite engineering, which comprises isolating new compounds from natural products and analyzing their biological activity (De Oliveira Silva et al. 2013). Fungal metabolic engineering can expand the role of fungi not only in secondary metabolite biosynthesis but also in secondary metabolite biotransformation (Wakai et al. 2017). Microbial enzymatic biosynthesis of secondary metabolites is a sophisticated metabolic engineering approach to improve secondary metabolite production and generate novel molecules or derivatives (Niu and Tan 2013). Studies on secondary metabolites of *Aspergillus* species have been conducted in recent years (Sanchez et al. 2012; Amare and Keller 2014; He et al. 2018; Caesar et al. 2020). *Aspergillus oryzae* is used to manufacture secondary metabolites in traditional Chinese medicine. It has a high ability to synthesize, grows rapidly, is easy to culture, and has a powerful posttranslational modification function (Jin et al. 2021).

Fermentation of andrographolide by *Rhizopus stolonifer* produced two novel biotransformation products and eight compounds (He et al. 2010). In this case, oxidation and dehydration are the two most important enzymatic processes. Bioconversion of andrographolide by *Aspergillus ochraceus* produced five bioconversion products. Their structures were identified to be: 8β-hydroxy-8(17)-dihydroandrographolide (1), 8β-hydroxy-8(17)-dihydro-14-deoxy-11,12-didehydroandrographolide (2), 8β-hydroxy-8(17)-dihydro-14-deoxy- 11,12-didehydroandrographolide 19-oic acid (3), 14-deoxy-11,12-

didehydroandrographolide (4), and 14-deoxy-11,12-didehydroandrographolide 19-oic acid (5). Most bioconversion products showed potential cytotoxic activities against human breast cancer (MCF-7), human colon cancer (HCT-116) and leukemia (HL-60) cell lines (He et al. 2011). When the complete portions of *A. paniculata* are utilized in experiments and fermented with *A. oryzae*, several chemicals compound may form.

Aspergillus oryzae has been applied to ferment several Chinese herbs to increase the active compounds. The fermentation products of each residue demonstrated a wide range of antibacterial activity (Wen et al. 2013). In addition, *A. oryzae* fermentation of *Radix astragali* produced new phenolic antioxidant (Sheih et al. (2011). As long as we knew, biotransformation products of *A. paniculata* by fermentation with *Aspergillus oryzae* have not been studied yet. Therefore, the purpose of this study was to investigate the microbial transformation of *A. paniculata* by *A. oryzae* K1A. This study provides the profiles of fermented *A. paniculata* leaf for further bioprospecting studies such as more effective antiquorum sensing agents.

MATERIAL AND METHODS

Materials and chemicals

Andrographis paniculata leaves were obtained from the Research Institute for Spices and Medicines (Balittro), Indonesia. Taxonomic identification was performed at Herbarium Bogoriense, Research Center for Biology, National Research and Innovation Agency with identification number is 1257/IPH.1.01/If.07/VII/2019. Research Center for Biology also provided *Aspergillus oryzae* K1A (LIPI). Sigma Aldrich provides pure andrographolide as a reference chemical. Thin-Layer Chromatography and LC-MS/MS were used for the qualitative analysis. TLC plate, silica gel 60 F254, acetonitrile, ethanol, chloroform, methanol, and acetic acid glacial were products of Merck (Darmstadt, Germany). Membrane filters, the pore size of 0.22 m; PTFE; P/N E252 was the product of Whatman (Little Chalfont, United Kingdom).

Leaf preparation

Andrographis paniculata leaves were harvested for 120 days and dried in the oven for 2-3 days at 40°C. The dried leaves were ground into a coarse powder using a blender and then sieved at 80 mesh size to get fine powder (Joselin and Jeeva 2014).

Growth medium

Potato medium was used for fungal cultivation and biotransformation. Potato medium was made as follows: 200g of chopped potatoes were boiled in water for 1 hour. The extract was filtered, and the filtrate was added with water to reach a volume of 1 liter, then added with 20g of glucose. The broth was autoclaved in individual Erlenmeyer flask at 120°C and 15psi for 20min and cooled before incubation (Sheih et al. 2011).

Fungal culture and biotransformation procedures

The biotransformation procedures follow (He et al. 2011). Biotransformation of *A. paniculata* leaves by *A. oryzae* was carried out in 250 mL Erlenmeyer flasks containing 50 mL of potato medium. The culture of *A. oryzae* was transferred into the flasks from the slants. The flasks were shaken at a speed of 120 rpm at 37°C on rotary shakers. *A. paniculata* leaf powder was dissolved in methanol with a concentration of 50 mg/mL. After 24 hours of incubation, 1 mL of inoculated growth medium (5% v/v) was added to another culture flasks containing 20 mL of PDB medium and incubated for 24 hours. After 24 hours of incubation, *A. paniculata* leaf powder (0.2 mg/mL) was added and incubated in an orbital shaker at 120 rpm at 37°C for 14 days. Sampling was carried out on days 0, 1, 3, 7, and 14. A culture control was a growth medium that inoculated with *A. oryzae* and maintained under identical conditions without the addition of *A. paniculata* extract. Fermentation was carried out in three repetitions. The blank is a growth medium that is not inoculated with *A. oryzae* K1A and without *A. paniculata* extract. Following incubation, each sample was added with 20 mL of ethyl acetate and incubated for 24 hours before being centrifuged to obtain pellets and supernatant. The supernatant was dried using nitrogen gas to the volume of 0.5-1 mL.

Thin-Layer Chromatography (TLC) analysis

Qualitative testing by TLC was carried out by preparing a test solution at the concentration of 10 mg/mL in ethanol from each treatment. Andrographolide (0.1 mg/mL) in methanol was standard. The mobile phase was $CHCl_3$:MeOH (9:1). The stationary phase was a silica gel 60 F_{254} TLC plate. Five µL of andrographolide (Sigma Aldrich) solution and samples were transferred to a silica gel plate. Andrographolide spots in the samples were confirmed by comparing the Rf value of the sample and the Rf value of the andrographolide standard and observed at 254 nm UV (Wang et al. 2011).

High-Performance Liquid Chromatography (HPLC) analysis

The HPLC analysis was used to separate the compounds in fermented and non-fermented *A. paniculata* extracts using LC-20 AD equipped with a Cosmosil 5C18-MS-II column (150 nm x 4.6 mm i.d.) (Shimadzu, Japan). The method follows Song et al. (2013). The extract solution produced was injected into the HPLC column amount 10 µL. The eluent used was methanol, acetonitrile, and acetic acid at a ratio of 70:30:0.6%, and the extract from the Millipore filter was injected into the column using an absorbance of 254 nm. After 30 minutes, the chromatogram of the samples was compared to the standard of 200 ppm andrographolide to determine the andrographolide content in the samples. The chromatographic peak was identified by comparing the retention time of the standard. A single solvent injection (blank) was used as the standard solvent retention time.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS) analysis

The LC-MS/MS used is the Xevo G2-XS Qtof brand. Identification of metabolites using LC-MS/MS refers to the method used by Song et al. (2013) and Karomah et al. (2019), with modifications to achieve the optimum conditions of LC-MS/MS. The stationary phase is a non-polar column. The modification was carried out to produce a chromatogram with good separability. The mobile phase used in the modification process was 0.1% formic acid in water, 0.1% formic acid in methanol, and 0.1% formic acid in acetonitrile, seal wash period: 5,000 minutes, high-pressure limit: 18000 psi. Formic acid was added into the mobile phase to get better signal intensity, peak shape, and resolution. The gradient elution system was carried out at a 0.2 mL/min flow rate for 20 minutes with an injection volume of 2.0 µL. The MS ionization source used is ESI (+) with Q Orbitrap mass analysis. The m/z range used is from 50.00-1200. The energizes used for ionization were 6, 10, and 40 eV. A total of 60 mg of sample extract was dissolved in 5 mL of LC-MS grade methanol.

RESULTS AND DISCUSSION

Analysis of andrographolide content by TLC method

At the sampling periods of 0, 1, and 3, the TLC analysis revealed that both fermented and non-fermented samples have one spot with an Rf value similar to the Rf value of the reference andrographolide (0.48, 0.45, 0.47). The difference between fermented and non-fermented *A. paniculata* may be seen in the 7th-day incubation, where the Rf value of non-fermented *A. paniculata* was 0.42, whereas fermented *A. paniculata* was uncounted due to an uncleared location. By the 14th day, both fermented and unfermented *A. paniculata* spots were vanished, as illustrated in Figure 1 and Table 1. The Rf value is determined by the mobile and stationary phases that are used. The chemical is non-polar if the Rf value is high. Otherwise, when the Rf value is low, the compound is polar. Due to enzymatic reactions by *Aspergillus* isolates throughout the incubation interval, modifications in the *A. paniculata* fermented spot on day 14 were possible (Tianxiang et al. 2013; Hussain et al. 2016).

By comparing their spots to pure andrographolide spots, thin-layer chromatography was utilized to determine whether or not there were andrographolide components in the *A. paniculata* fermented samples. The TLC method is based on the compound's chromatogram's peak area and concentration. The area of a compound's peak is proportional to the compound's concentration. The greater the chromatogram peak area of a compound, the higher the compound content (Nugroho et al. 2012). On the Silica Gel GF254 TLC plate with chloroform: methanol as mobile phase (9:1), the standard Rf value of andrographolide is 0.55 (Temaja et al. 2012).

Table 1. Rf value of andrographolide in *Andrographis paniculata* leaf

Days of-	Rf value of andrographolide standard	Rf value of non-fermented *A. paniculata* leaf	Rf value of fermented *A. paniculata* leaf
0	0.5	0.48	0.48
1	0.5	0.45	0.45
3	0.47	0.47	0.47
7	0.42	0.42	-
14	0.45	-	-

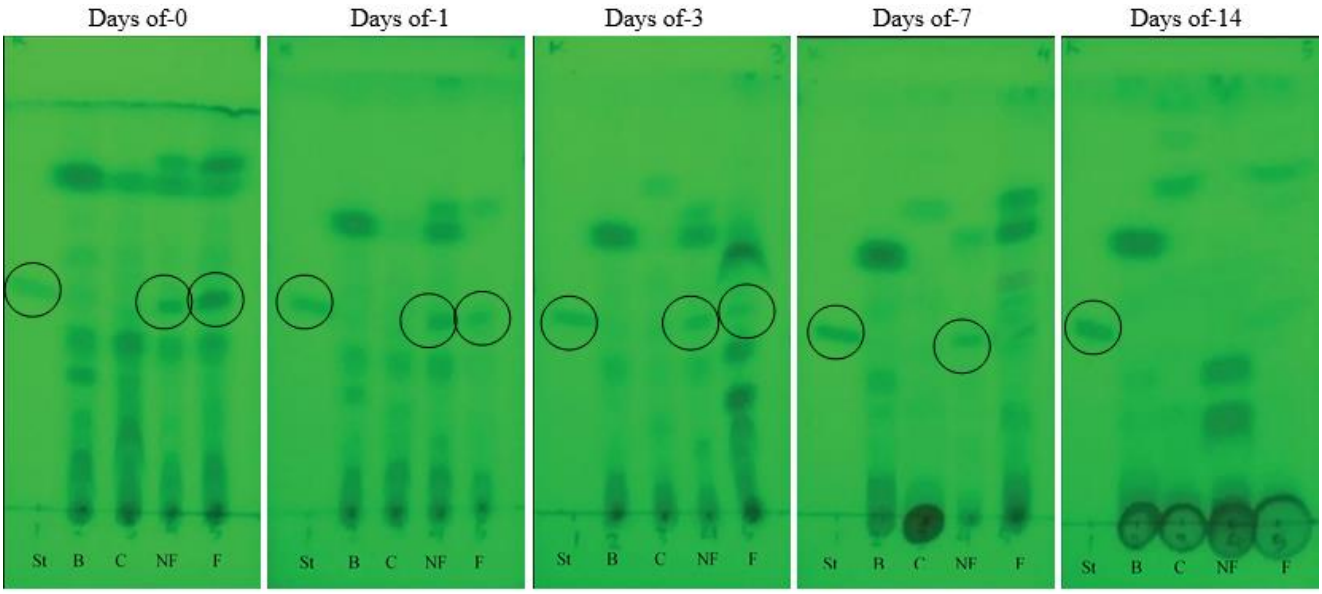

Figure 1. Chromatograms of non-fermented and *Andrographis paniculata* fermented by *Aspergillus oryzae* K1A on day 0, 1, 3, 7, and 14. St: andrographolide standard; B: blank; C: control; NF: non-fermented; F: fermented. The black circle shows the spot of andrographolide compounds, both fermented (F) and non-fermented (NF) *A. paniculata* leaves with a standard ratio of pure andrographolide (St)

Quantitative analysis of andrographolide by HPLC method

A quantitative analysis of the andrographolide concentration in fermented or non-fermented *A. paniculata* leaf extract was performed by using HPLC with pure andrographolide (Sigma Aldrich) as a standard. Chromatogram results are presented in Figure 2. The peaks of *A. paniculata* fermented and non-fermented were relatively similar on days of 0 to 1 incubation and showed a few different peaks of *A. paniculata* fermented and non-fermented on days of 3 to 14 (Figure 2). Table 2 shows the amounts of andrographolide in fermented and non-fermented *A. paniculata* leaves. Andrographolide concentrations were higher in *A. paniculata* leaf fermented by *A. oryzae* than in non-fermented leaves. A high concentration of andrographolide was detected between the 7th and 14th days of incubation in *A. paniculata* fermented; there is a correlation between incubation time and increasing andrographolide concentration. This could be due to the uncounted Rf value for *A. paniculata* fermented on the 7th and 14th days of incubation.

The amounts of andrographolide in non-fermented *A. paniculata* leaf were also elevated. The increase in andrographolide concentrations in non-fermented *A. paniculata* leaf could be attributable to the effect of the treatment prior to incubation, where Erlenmeyer containing culture fluids in a shaker incubator could allow for an oxidation-reduction process (redox). The glycolysis route and the tricarboxylic acid (TCA) cycle are two fermentation products that require microaerobic or anaerobic conditions to prevent the intended product from being oxidized by oxygen (Liu et al. 2017). Chemically, the oxidation-reduction potential is defined as a molecule's proclivity to gain electrons. The oxidizing agent (Ox) draws electrons and then becomes the reducing agent during the electron transfer process, which requires two components known as redox pairs. Oxygen enhances redox potential, whereas hydrogen decreases it. Oxygen and nitrogen are commonly used in both aerobic and anaerobic fermentation.

LC-MS/MS analysis

LC-MS/MS analysis was performed on non-fermented and fermented *A. paniculata* leaf with *A. oryzae* K1A. The chromatograms of the LC-MS/MS analysis on *A. paniculata* fermented and *A. paniculata* non-fermented showed similar peaks on days 0-1 with more high peaks in *A. paniculata* fermented than *A. paniculata* non-fermented (Figure 3). The same data of the *A. paniculata* fermented and *A. paniculata* non-fermented compound showed in Table 3 for the samples on days 0 and 1.

On the 3rd day, the difference between *A. paniculata* fermented and non-fermented showed, when the *A. paniculata* fermented compounds were detected andrographolide, picrasidine K ($C_{18}H_{23}N_3O_2$), andrographolactone ($C_{20}H_{24}O_2$), saurufuran B ($C_{20}H_{28}O_3$), and bufotalinin ($C_{24}H_{30}O_6$). Meanwhile, the *A. paniculata* non-fermented compounds were candidate compound ($C_{11}H_{18}N_2O_3$); candidate compound ($C_{11}H_{18}N_2O_2$); andrographolide; andrographolactone ($C_{20}H_{24}O_2$) and saurufuran B ($C_{20}H_{28}O_3$).

On the 7th day, the differences in the chemical compounds on fermented and non-fermented *A. paniculata* (*A. paniculata*) were more significant. The non-fermented samples contained adenosine compounds, nicotinic acid, and andrographolide ($C_{20}H_{30}O_5$). Meanwhile, the fermented *A. paniculata* samples contained two new metabolites; i.e., picrasidine K ($C_{18}H_{23}N_3O_2$) *m/z* 314.1868 and β-Carboline ($C_{11}H_8N_2$) *m/z*169.0758. On the last day of treatment (days of 14), the compounds that appeared in the non-fermented *A. paniculata* samples were: candidate compound $C_{13}H_{20}O$; candidate compound $C_{11}H_{18}N_2O_2$; andrographolide and saurufuran B ($C_{20}H_{28}O_3$), and the fermented samples of *A. paniculata* showed the following compounds: candidate compound $C_{12}H_{12}N_2O_2$; candidate compound $C_{10}H_{19}NO$; andrographolide; picrasidine K and candidate compound $C_{38}H_5O_6$.

Labdane diterpenes and derivatives have a wide range of biological properties and can be used as chiral building blocks to make a variety of bioactive chemicals. Because the functionalization of unactivated C–H bonds generally requires hard reaction conditions and highly reactive oxidizing agents, which are limited in their ability to control regio- and stereoselectivity, there is a lot of interest in developing biocatalyst technology to achieve regio- and stereoselective hydroxylation of unactivated C–H bonds in complex natural products. Filamentous fungi are effective biocatalysts that can catalyze a wide range of hydroxylation processes, and using entire cell biocatalysts has advantages in terms of cofactor renewal and is considerably less expensive (Cruz de Carvalho et al. 2020).

Tabel 2. Andrographolide concentration in fermented and non-fermented *Andrographis paniculata* leaf

Incubation times (days)	Andrographolide concentrations (ppm)	
	Non-fermented *A. paniculata* leaf (NF)	Fermented *A. paniculata* leaf (F)
0	91.40	91.50
1	102.04	104.93
3	100.32	110.68
7	112.14	134.41
14	105.23	111.88

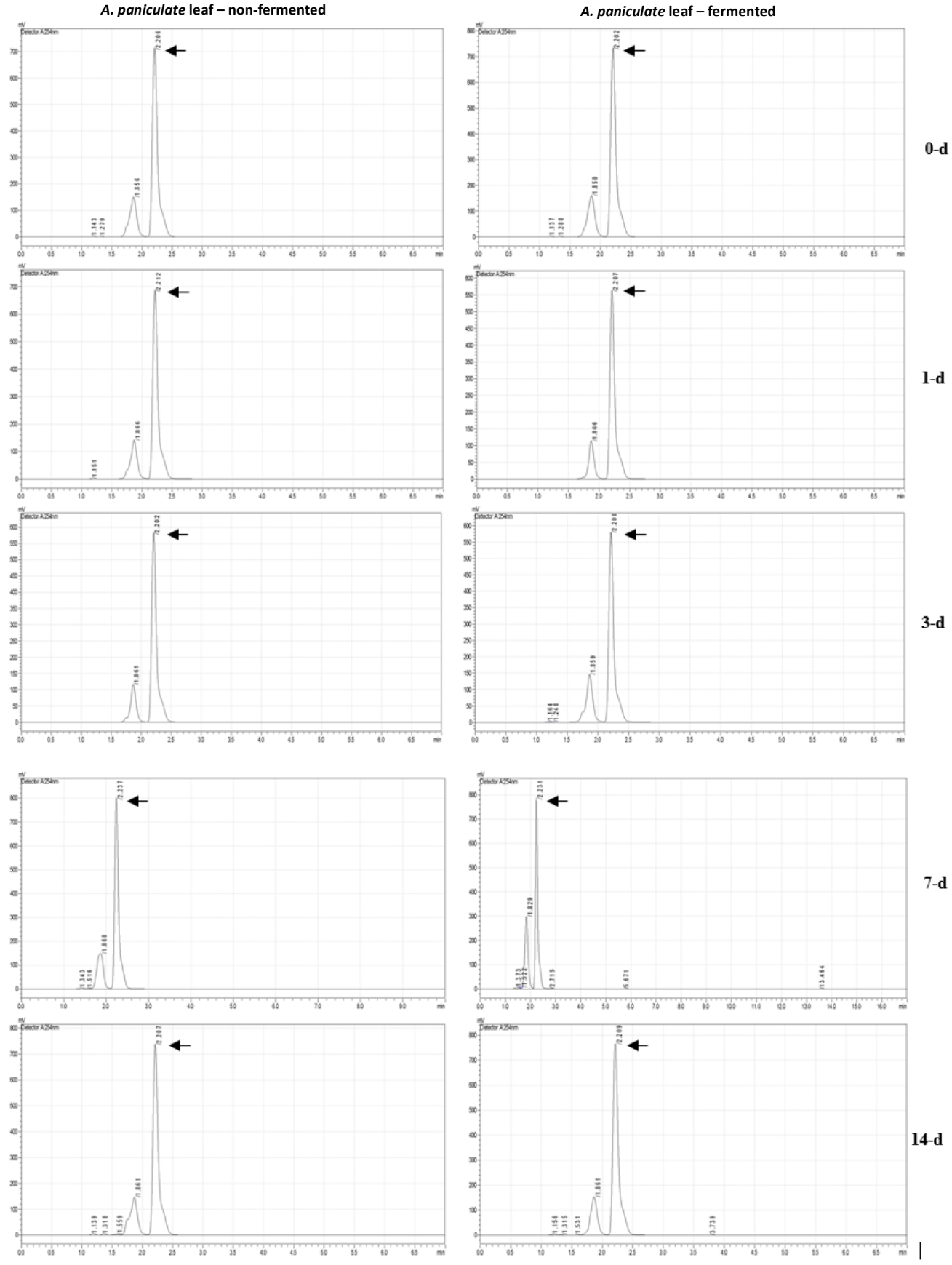

Figure 2. The chromatogram of HPLC analysis of fermented and non-fermented *Andrographis paniculata* leaf at different incubation times (days of- 0 to 14). The peak of andrographolide is shown by the black arrow icon (←)

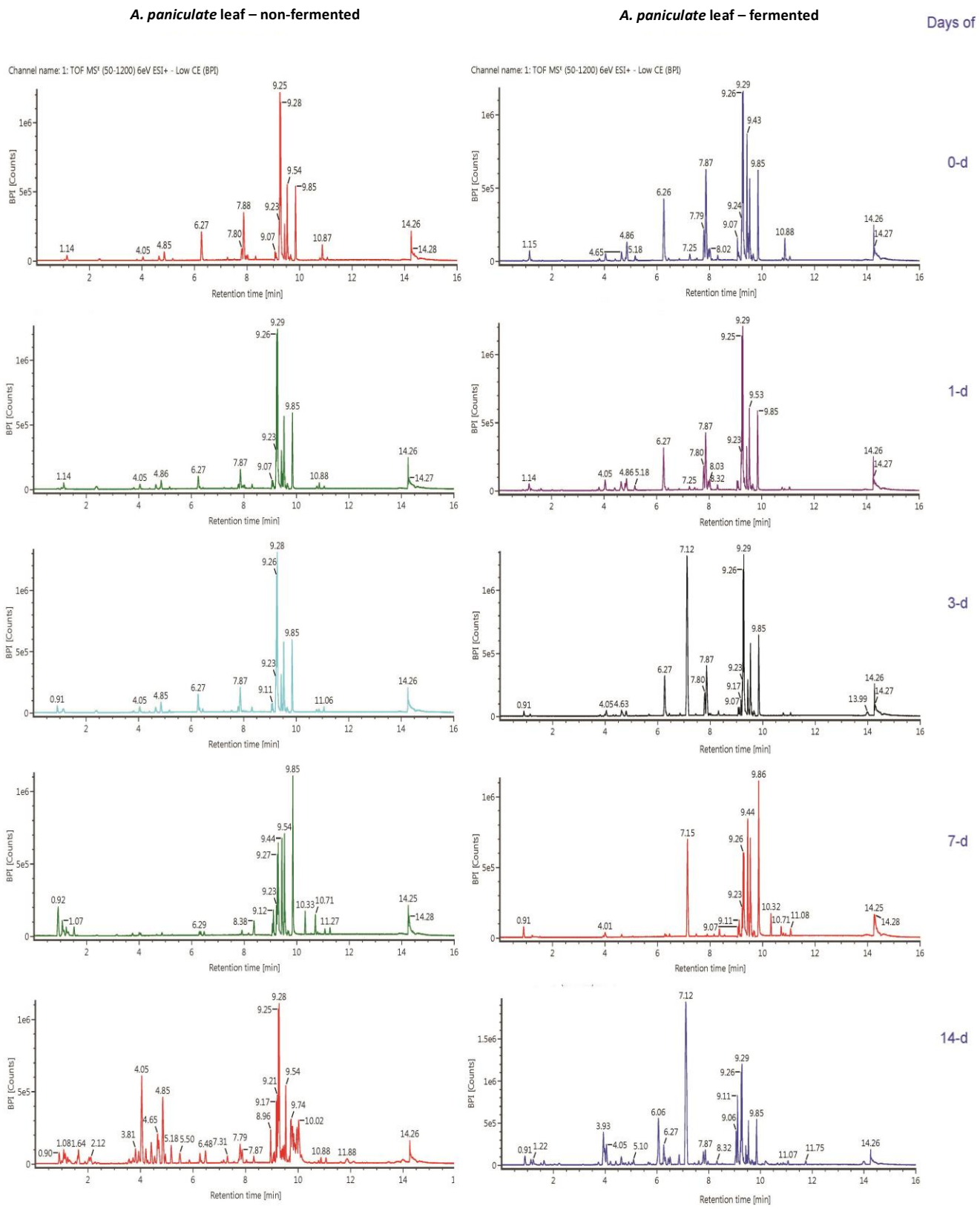

Figure 3. LC-MS/MS Chromatogram of *Andrographis paniculata* leaf fermented by *Aspergillus oryzae* KIA compared to non-fermented on 0, 1, 3, 7, and 14 days incubation

Table 3. The chemical compounds of fermented and non-fermented *Andrographis paniculata* leaf during the incubation period of 14 days

Sample days of	Non-fermented			Fermented		
	Compounds	Observedm/z	RT (min)	Compounds	Observed m/z	RT (min)
0	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1440	4.86	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1439	4.86
	Andrographolide ($C_{20}H_{30}O_5$)	351.2160	6.27	Andrographolide ($C_{20}H_{30}O_5$)	351.2160	6.27
	Saurufuran B ($C_{20}H_{28}O_3$)	317.2113	7.81	Saurufuran B ($C_{20}H_{28}O_3$)	317.2108	7.80
	Andrographolactone ($C_{20}H_{24}O_2$)	297.1853	7.88	Andrographolactone ($C_{20}H_{24}O_2$)	297.1847	7.87
	Compound candidate ($C_{54}H_{78}O_9$)	871.5742	10.87	Compound candidate ($C_{54}H_{78}O_9$)	871.5738	10.88
1	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1438	4.86	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1439	4.86
	Andrographolide ($C_{20}H_{30}O_5$)	351.2159	6.27	Andrographolide ($C_{20}H_{30}O_5$)	351.2167	6.27
	Saurufuran B ($C_{20}H_{28}O_3$)	317.2109	7.80	Saurufuran B ($C_{20}H_{28}O_3$)	317.2111	7.80
	Andrographolactone ($C_{20}H_{24}O_2$)	297.1848	7.87	Andrographolactone ($C_{20}H_{24}O_2$)	297.1851	7.87
	Compound candidate ($C_{54}H_{78}O_9$)	871.5733	10.88	Compound candidate ($C_{14}H_{13}NO_2$)	228.1019	8.03
3	Compound candidate ($C_{11}H_{18}N_2O_3$)	227.1389	4.05	-		
	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1437	4.86	-		
	Andrographolide ($C_{20}H_{30}O_5$)	351.2159	6.27	Andrographolide ($C_{20}H_{30}O_5$)	351.2164	6.27
	-		-	Picrasidine K ($C_{18}H_{23}N_3O_2$)	314.1864	7.12
	Andrographolactone ($C_{20}H_{24}O_2$	297.1847	7.87	Andrographolactone ($C_{20}H_{24}O_2$)	297.1852	7.87
	Saurufuran B ($C_{20}H_{28}O_3$)	317.2109	7.80	Saurufuran B ($C_{20}H_{28}O_3$)	317.2111	7.80
	-		-	Bufotalinin ($C_{24}H_{30}O_6$)	415.2117	9.18
7	Adenosine	268.1037	1.08	-		
	Nicotinic acid	124.0391	1.23	-		
	-		-	β-carboline	169.0758	4.01
	Andrographolide ($C_{20}H_{30}O_5$)	351.2153	6.30	Andrographolide ($C_{20}H_{30}O_5$)	351.2151	6.28
	-		-	Picrasidine K ($C_{18}H_{23}N_3O_2$)	314.1868	7.15
14	Compound candidate ($C_{14}H_{20}O$)	227.1389	4.05	Compound candidate ($C_{12}H_{12}N_2O_2$)	217.0969	3.94
	Compound candidate ($C_{11}H_{18}N_2O_2$)	211.1437	4.86	Compound candidate ($C_{10}H_{19}NO$)	170.1537	6.06
	Andrographolide ($C_{20}H_{30}O_5$)	351.2161	6.27	Andrographolide ($C_{20}H_{30}O_5$)	351.2160	6.27
	Saurufuran B ($C_{20}H_{28}O_3$)	317.211	7.80	Picrasidine K ($C_{18}H_{23}N_3O_2$)	314.1864	7.13
	-		-	Compound candidate ($C_{38}H_5O_6$)	625.3498	9.11

Note: RT: Retention time

Aspergillus oryzae is an obligate aerobic fungus, in which oxygen serves as a terminal electron acceptor for the electron transport chain in the respiration process. This fungus produces useful secondary metabolites (Jin et al. 2021). *Aspergillus oryzae* produces many enzymes that degrade various high molecular mass biomolecules such as carbohydrates, polypeptides, nucleic acids, and strong promoters of biosynthetic genes (Marui et al. 2010). *Aspergillus oryzae* is equipped with powerful enzyme systems that can break down proteins into amino acids and synthesize amino acids during metabolism (JieHua et al. 2014).

The results of the biotransformation of *A. paniculata* leaf using *A. oryzae* K1A showed the presence of several new compounds in the leaf samples of *A. paniculata* which were the result of microbial enzymatic processes. *Aspergillus oryzae* K1A is a microbe that is widely known to be capable of hydrolyzing amylase, lipase, and protease enzymes, thereby converting protein into essential amino acids. According to Cai et al. (2012) L-Tryptophan, is an essential amino acid, as a key precursor in the biosynthesis of ß-carboline. Structural analysis of andrographolide derivatives showed that various enzymatic reactions occur during biotransformation such as enzymatic hydrolyzation, hydroxylation, oxidation, glycosylation, epoxidation, and elimination (Wang et al. 2011). *A. paniculata* leaf contains proteins 1,144 mg/g (Valdiani et al. 2017) and alkaloid (Harmine), an indole alkaloid in the Carboline group (Kurzawa et al. 2015) which is easily bound with other compounds and synthesized in a newly formed derivative molecular structure (Laine et al. 2014).

The biotransformation products of *A. paniculata* by *A. oryzae* K1A were picrasidine K ($C_{18}H_{23}N_3O_2$), and bufotalinin ($C_{24}H_{30}O_6$). This process can be explained through the *Wittig reduction reaction.* Where cyclization mechanism in the enzymatic protease process by *A. oryzae* converts protein of *A. paniculata* into amino acids and with carboline D-ring; 1-formyl-9H-b-carboline and 3-formyl-9H-b-carboline. The Aldo-X bifunctional building blocks (AXB3s) may allow the diversity-oriented synthesis of β-carboline derivatives and open up new opportunities to introduce significant diversity at the C1 and C3 positions of the b-carboline core (Devi et al. 2018).

In conclusion, microbial transformation of *A. paniculata* (*A. paniculata*) leaves by *A. oryzae* K1A through an enzymatic reaction produced two secondary metabolites Picrasidine K ($C_{18}H_{23}N_3O_2$) and Bufotalinin ($C_{24}H_{30}O_6$). The bioactivities assay of the biotransformation product to inhibit the growth of bacteria or antiquorum sensing is needed.

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