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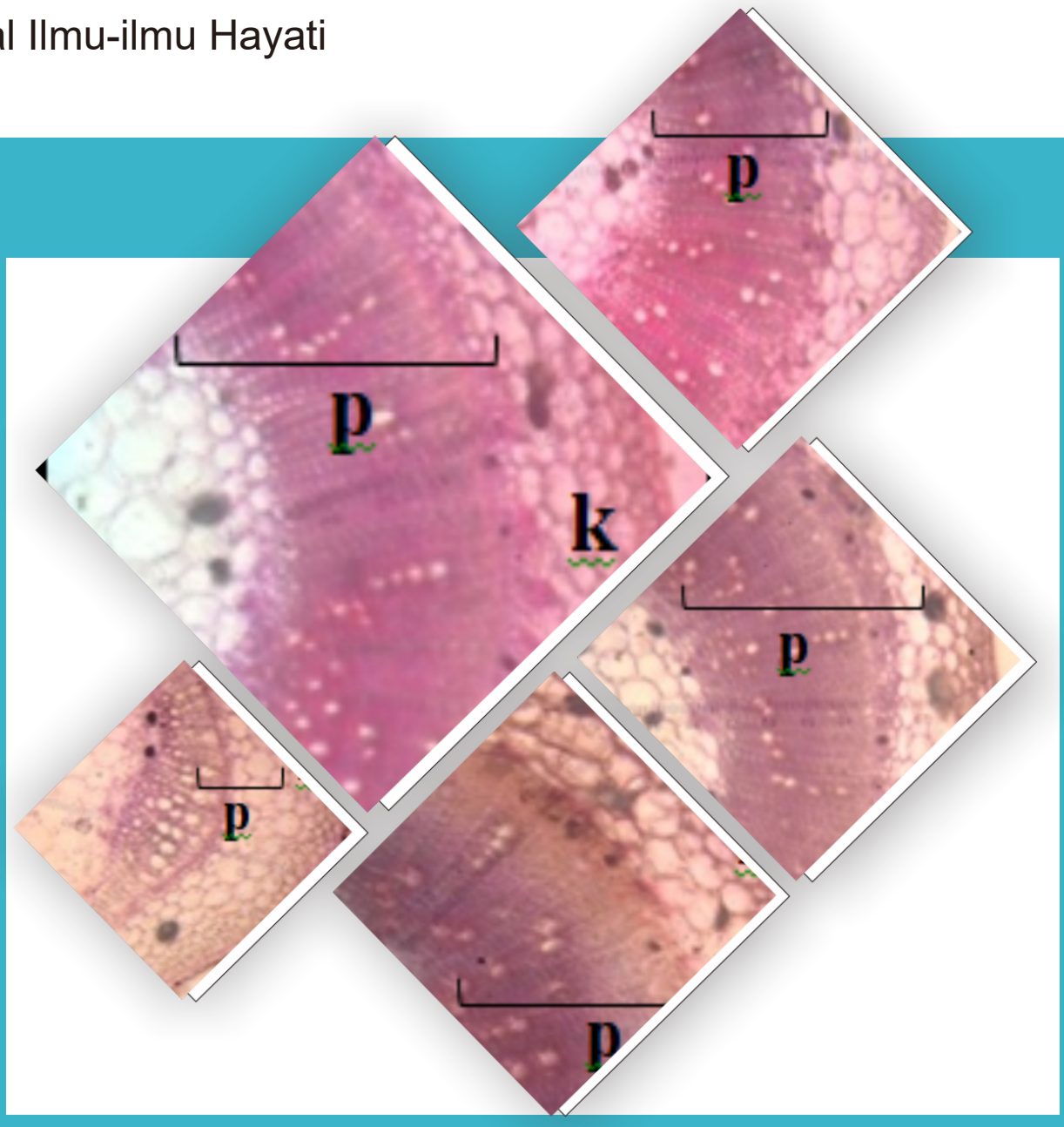

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COMPOSITION AND QUANTIFICATION OF FATTY ACIDS PRODUCED BY *Xylaria* sp. DAP KRI-5

[*Komposisi dan Kuantifikasi Asam Lemak yang Diproduksi oleh Jamur Endofit Xylaria sp. DAP KRI-5]*

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ABSTRAK

Asam lemak mempunyai nilai guna komersial sebagai suplemen makanan, produk farmasi dan sumber energi terbarukan. Sumber asam lemak sebagian berasal dari hewan, tumbuhan, dan jamur endofit. Penelitian ini bertujuan untuk menganalisis kandungan dan komposisi asam lemak dari jamur endofit Xylaria sp. DAP KRI-5. Asam lemak didapatkan dari fraksi n-heksana yang dihasilkan dari partisi ekstrak etil asetat Xylaria sp. DAP KRI-5 dengan n-heksana: metanol (1:1). Turunan FAME (Fatty acid methyl ester) dari asam lemak didentifikasi dengan menggunakan GC-MS. Kandungan asam lemak dari fraksi n-heksana Xylaria sp. DAP KRI-5 adalah 26,39% (b/b) ekstrak kering. Analisis kuantitatif dilakukan menggunakan GC-MS. Komposisi dan kuantifikasi asam lemak Xylaria sp. DAP KRI-5 adalah asam linoleat (41,177%; 112,24 mg/L); asam palmitat (25,114%; 68,45 mg/L); asam oleat (14.198%; 38,70 mg/L); asam stearat (6,575%; 17,2 mg/mL); dan asam palmitoleat 2,165%; 5,90 mg/mL). Persentase asam lemak jenuh dan tidak jenuh berturut-turut adalah 31,69 and 57,54%. Kesimpulan dari penelitian ini menunjukkan jamur endofit Xylaria sp. DAP KRI-5 berpotensi sebagai sumber asam lemak tidak jenuh. Penelitian ini merupakan laporan pertama dari asam lemak yang diproduksi oleh Xylaria sp. DAP KRI-5.

Kata kunci : *Asam lemak, Jamur endofit, Xylaria sp. DAP KRI-5, GC-MS*

ABSTRACT

Fatty acids have commercial uses as food supplements, pharmaceutical products, and renewable energy sources. The source of fatty acids comes from animals, plants, and endophytic fungi. This study aims to analyze the content and composition of fatty acids from the endophytic fungus *Xylaria* sp. DAP KRI-5. Fatty acids obtained from n-hexane fraction were produced from the partition of ethyl acetate extract of *Xylaria* sp. DAP KRI-5 with n-hexane: methanol (1:1). The fatty acid methyl esters (FAMEs) derived from fatty acids were identified by using GC-MS. The fatty acids content of n-hexane fraction of *Xylaria* sp. DAP KRI-5 was 26.39% (w/w) of dry extracts. Quantitative analysis was carried out using GC-MS. Composition and quantification of fatty acids of *Xylaria* sp. DAP KRI-5 were linoleic acid (41.177%; 112,24 mg/L), palmitic acid (25.114%; 68,45 mg/L), oleic acid (14.198%; 38,70 mg/L), stearic acid (6.575%; 17,2 mg/mL), and palmitoleic acid (2,165%; 5,90 mg/mL). The percentage of saturated and unsaturated fatty acids were 31.69 and 57.54%, respectively. The conclusion of this study shows that endophytic fungus *Xylaria* sp. DAP KRI-5 has the potential as a source of unsaturated fatty acids. This study is the first report of fatty acids produced by *Xylaria* sp. DAP KRI-5.

Keywords: Fatty acids, Endophytic fungus, *Xylaria* sp. DAP KRI-5, GC-MS

INTRODUCTION

Sources of fatty acids can be obtained from animals and plants, and can also derived from microbes, including endophytic fungi. Oleogenic fungi are able to accumulate lipids more than 25% of the dry weight when a carbon nutrient source is available (Murphy, 2001). This fact allows that fatty acids can be produced more economically than those sourced from plants and animals, because endophytic fungi have a short life cycle, require less energy, and are easier to increase in quantity. Fatty acid synthesis in fungi is a very important part of the formation of cell membrane lipids (Weyda *et al*., 2018). Fatty acids have potential commercial value as dietary supplements, pharmaceutical products, and renewable energy sources (Peng and Chen, 2007). Fatty acids

‡Main Contributor (*Kontributor Utama)*

make up 30-35% of the total energy intake in many industrialized countries.

Palmitic acid (16: 0) is the most common saturated fatty acid in animals, plants, and microorganisms. Palmitoleic acid (16: 1 ω-7) is widely found in animals, plants, microorganisms and is the main component of fat in whole grains. Stearic acid (18: 0) is a major fatty acid in animals and some fungi, and a minor component in most plants, namely fat in grains. Oleic acid (18: 1 ω-9) is the most common monoenoic fatty acid in plants and animals. It is also found in microorganisms. Linoleic acid (18: 2 ω-6) is the main fatty acid in plants (Rustan and Drevon 2005).

In previous research, it was found that the extract of the endophytic fungus *Xylaria* sp. DAP KRI-5 (an isolate has been deposited in the Indonesian Culture Collection (InaCC). It has the InaCC code of F230 that isolated from *Albertisia papuana* plant. This fungal extract has activity as antibacterial in vitro against *S. aureus* and *E. coli* (Fathoni *et al*., 2013). The compounds contained in the extract namely floroglusinol and cytocalasin D (Fathoni *et al*., 2013, 2019) that found in the polar solvent fraction (methanol), as well as the n-hexane fraction (nonpolar) through screening using thin-layer chromatography (TLC) shows potential as a producer of fatty acids (unpublished data). On the other hand, there is no information related to research on the fatty acid content of the endophytic fungus *Xylaria* sp. DAP KRI-5 can be used as a source of beneficial unsaturated fatty acids. This study is aimed to analyze the content and quantity of fatty acids from the endophytic fungus *Xylaria* sp. DAP KRI-5 using GC -MS, and the ratio of the fatty acid composition of this endophytic fungi is compared with other microorganisms that produce fatty acids.

MATERIALS AND METHODS

Materials

The research tools and instruments used in this study including TLC plate, chamber, petri dish, knife, tweezers, loop wire, spatula, vortex (Sibata), test tube (Pyrex), analytical balance (And hr-202i), UV cabinet (Camag), incubator, autoclave, evaporator flask (Pyrex), rotary evaporator (Heidolph WB 2000), laminar airflow, shaker (Innova^{TM} 2100), hot plate (Cimarec 2), vial, separating funnel, freeze dryer (Eyela FDU-1200), and GC-MS spectrometer (Variance).

The materials and solvent used in this study including hexane, ethyl acetate, methanol, dichloromethane, aqua dest, cerium and vanillin stains, 70% alcohol, Potato Dextrose Broth (PDB, Difco^{TM}), Potato Dextrose Agar (PDA, Difco^{TM}), Corn Meal Malt Agar (CMMA, Difco^{TM}), nitrogen gas, *Albertisia papuana* Becc leaves were obtained from the Bogor Botanical Gardens, West Java.

Scaling up and Extraction of Endophytic Fungal Cultures

A fungal endophytic *Xylaria* sp. DAP KRI-5 cultivated in 2.7 L PDB medium (13x @200 ml, and 1x @100 ml) at 25^{o}C, at static conditions in a dark room, for 21 days. Biomass and growth media from the endophytic fungus *Xylaria* sp. DAP KRI-5 was extracted with the organic solvent, ethyl acetate, three times. Cultivation conditions and extraction processes used in this research are the best to obtain optimal biomass yield (unpublished data). The extract is concentrated with a rotary evaporator and after drying from the solvent, the extract is stored in a refrigerator -20^{o}C which is intended to prevent the extract from being damaged, before the extract is used for further testing. The extracts were analyzed using TLC with an eluent of dichloromethane-methanol (10: 1). Furthermore, the fatty acids were separated by a partition of two solvents (methanol: n-hexane, 1:1). The n-hexane fraction containing fatty acids was analyzed using TLC with eluents of petroleum ether/ diethyl ether/ glacial acetic acid (9: 1: 0.1). The chromatogram was monitored with UV light at a wavelength of 254 nm and 366 nm, then sprayed with 0.25% vanillin stain reagent dissolved in 10% H_2SO4/ methanol (pa).

GC MS Analysis

GC/MS analysis was performed using GC-MS (Varian-3900, GC/ MS/ MS Saturn 2000, CP-8400 autosampler) with a stationary phase of VF-17MS with a column length of 30 m and a diameter of 0.25 mm. A mobile phase used the ultra high-purity helium (He) carrier gas with a pressure of 20 Psi, injection volume: 4.0 µL, injector temperature 250 ^{o}C,

splitless, flow rate 1.3 mL/minute, column temperature programmed from 160 °C to 300 °C with two steps of increments. The initial temperature is 160 °C, then the temperature is increased to 270 °C with an increasing rate of 5 °C/minute. Then the temperature was increased to 300 °C with an increased rate of 20 °C/minute. The temperature is maintained at 300 °C for 1.5 minutes.

One milligram of the n-hexane fraction of *Xylaria* sp. DAP KRI-5 was dried with nitrogen gas and reacted with 4 mL of 10% H_2SO_4 solution in methanol at 60 °C for 2 hours. The ester product was extracted by adding 4 ml of n-hexane and partition with the n-hexane and methanol and separated between two fractions using a separating funnel. Esters produced in the n-hexane fraction were analyzed by GC-MS. The standard esterification process of palmitic acid into its ester (methyl palmitate) is carried out in the same procedure. The sample which reacted into the ester was injected (4 µl) on GC/ MS for three replications and its area was measured on a chromatogram. Some of the standard concentrations of palmitate are 0.5; 0.25; 0.125; 0.0625; 0.03125 µg each time injected at GC/ MS three replications each, the injection volume was 4 µL each, and then the area was measured.

RESULTS

Isolate of endophytic fungus *Xylaria* sp. DAP KRI-5 grown on PDA media (Figure 1.) was then cultivated on PDB media for endophytic fungal culture multiplication (scaling-up). After three weeks of incubation, extraction of the biomass and media with ethyl acetate (EtOAc) was carried out. The extract obtained from 2.7 L culture of fungal endophytic on PDB media was 2.789 g, then 1.726 g of the extract was partitioned with 2 solvent (MeOH and n-hexane) and separated with a separating funnel obtained as much as 0.456 g from the n-hexane fraction (26, 39%) and 1.271 g of the MeOH fraction (73.61%) (Table 1.) Partition using n-hexane (nonpolar) and methanol (polar) aims to separate non-polar and po-

Figure 1. Endophytic fungus of *Xylaria* sp. DAP KRI-5 (*Jamur endofit Xylaria sp. DAP KRI-5*).

Table 1. Partition Yield of EtOAC extracts of *Xylaria* sp. DAP KRI-5 (*Hasil partisi ekstrak EtOAC dari Xylaria sp. DAP KRI-5*).

Fraction (*Fraksi*)	**Weight (*Berat*) (g)**	**Percentage* (*Persentase**) (% w/w)**	**Production Capacity** (*Kapasitas produksi***) (mg/L)**
MeOH	1.271	73.61	760.82
n-Heksan	0.456	26.39	272.570

Note: * Percentage of fraction to extract weight,** Production capacity to culture media volume.
(*Ket.: * Persentase fraksi terhadap berat ekstrak, ** Kapasitas produksi terhadap volume kultur media*)

lar compounds, in order to obtain fatty acids that are non-polar in the n-hexane fraction.

Thin-layer chromatography (TLC) analysis was performed of the ethyl acetate extract of the endophytic fungus *Xylaria* sp. DAP KRI-5, methanol fraction, and n-hexane fraction on TLC silica glass plate (Silica gel GF254, Merck) (Figure 2.).

Fatty acids that have been derivatized into fatty acid methyl esters (FAMEs) were analyzed by the TLC method (Figure 3). The chromatogram profile of these FAMEs showed an increase of Rf value at the dominant spot of fatty acids (Rf: 0.6) to their esters (Rf: 0.75). This is due to a change in the functional group of carboxylic acid (RCOOH), which is polar, changes to its methyl ester (RCOOMe) which is less polar. The resulting fatty acids were analyzed qualitatively and quantitatively using GC-MS. The identification of fatty acids in the n-hexane fraction was carried out by comparing the spectrum of the unknown components contained in the n-hexane fraction with the spectrum of components in the NIST data library. The higher similarity of MS fragmentation patterns of the components, it can be concluded that these components are the same as the components in the NIST data library. The concentration of fatty acids in the fraction was carried out by comparing the area with the area of an external standard fatty acid (palmitic acid, Merck) with known concentrations.

Composition and quantification of fatty acids of the n-hexane fraction of *Xylaria* sp. DAP KRI-5 based on the results of GC-MS analysis were linoleic acid (41.177%; 112.24 mg/L); palmitic acid (25,114%; 68,45 mg/L); oleic acid (14,198%; 38.70 mg/L); stearic acid (6,575%; 17.2 mg/mL); and palmitoleic acid 2.165%; 5.90 mg/mL). The main content of fungi, in general, is oleic acid (C18: 1) (Akpinar-Bayizit, 2014), but in this study, the main fatty acid was found, namely linoleic acid (C18: 2). This is probably related to the harvest age of the fun-

Figure 2. Chromatograms of EtOAC extracts, MeOH Fraction, and n-hexane fraction.
Note. 1: before partitioning, 2: n-hexan fraction, 3: MeOH fraction, eluent: dichloromethane / methanol = 10: 1, after sprayed with staining regent of vanillin-sulphuric acid.
(*Kromatogram ekstrak etil asetat, fraksi methanol dan fraksi heksan*. Ket*. 1 : sebelum dipartisi, 2 : fraksi n-hexan, 3 : fraksi MeOH, eluen : diklorometan/metanol=10:1, setelah disemprot penampak noda vanillin-asam sulfat*).

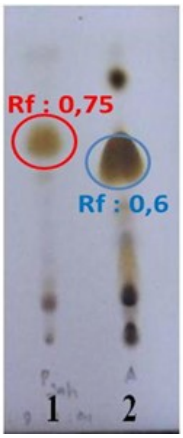


Figure 3. Chromatograms of fatty acids was produced by *Xylaria* sp. DAP KRI-5.
Note: fatty acid after esterification (1), and fatty acid before esterification (2). Eluent: petroleoum ether (PE) / diethyl ether / acetic acid (glacial) = 9/1 / 0.1.
(*Kromatogram asam lemak yang diproduksi Xylaria sp. DAP KRI-5*). *Ket.: Asam lemak setelah teresterifikasi (1), dan asam lemak sebelum teresterifikasi (2). Eluen: petroleoum eter (PE)/dietil eter/Asam asetat (glasial) = 9/1/0,1*)

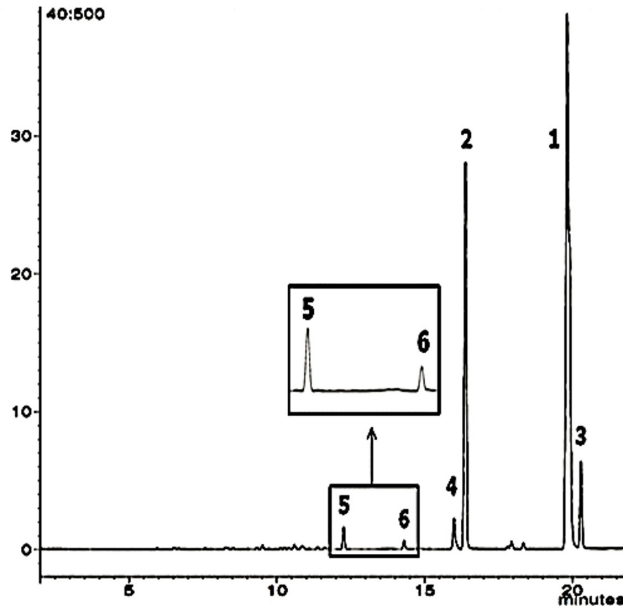
gus because linoleic acid (C18: 2 Δ9,12) is the result of further metabolism of oleic acid (C18: 1 Δ9) in the form of a desaturation process at carbon number 12 (Akpinar-Bayizit, 2014).

The difference in the fatty acid content that dominates from fungal isolates may also occur due to the duration of the lag phase and the rate of germination (Mysyakina *et al*., 2018). The results showed that the total amount of saturated and unsaturated fatty acids were 31.69 and 57.54% respectively and the other was not identified as fatty acids (10.77%). The amount of unsaturated fatty acids is more than saturated fatty acids. This is probably due to the relatively low temperature for the growth of endophytic fungi, thereby increasing the amount of unsaturated fatty acids (Suleiman *et al.,* 2018).

DISCUSSION

The results of this study indicate the three main fatty acid content of *Xylaria* sp. DAP KRI-5 are linoleic acid, palmitic acid, and oleic acid with a structure as shown in Figure 5. This fungus is a phylum; Ascomycota: class; Sordariomycetes: order; Xylariales: family; Xylariaceae: genus; *Xylaria* (https://www.gbif.org/species/5255147). Fungal species generally contain the main composition of fatty acids are namely oleic acid (C18: 1) and linoleic acid (C18: 2) as well as the dominant fatty acids contents in cell walls, mycelia, and spores. The slightly dominant fatty acids are namely palmitic acid, stearic acid, linolenic acid, and palmitoleic acid (Longo *et al.* 2013; Akpinar-Bayizit, 2014).

The most dominant and slightly dominant fatty acids also occur in fungi that were grown on various media and different harvest times at both the exponential phase and the stationary phase are the same of fatty acid composition (Yun *et al*., 2018) but the changes only occur in the percentage of each of the main components above. According to previous study, Calvo *et al*. (2001) stated that the fungus Ascomycycota, *Aspergillus nidulans*, showed that the ratio of conidia (asexual spores) to ascospores (sexual spores) contributes to the changes in linoleic acid content. Endogenous sporogenic factors deleted genes encoding desaturase Δ-12 (this gene changes oleic acid, C18: 1, to linoleic acid, C18: 2), resulting in a decrease in the amount of polyunsaturated fatty acids (C18: 2 and C18: 3) while oleic acid (18: 1) becomes increased. The gene encoding desaturase Δ-12 is reduced in conidia production and mycelium growth; this effect is most noticeable when cultures are grown at 26 °C in the dark (Calvo *et al.,* 2001). Based on other research, it was stated that all cell walls and mycelia obtained a similar fatty acid composition,



Gambar 4. GC-MS Analysis of fatty acid metil ester of n-hexane fraction of *Xylaria* sp. DAP KRI–5.
Note. 1: linoleic acid (C18: 2, ω-6), 2: palmitic acid (C16:0), 3: oleic acid (C18:1, ω-9), 4 and 6: stearic acid (C18:0), 5: palmitoleic acid (C16:1, ω-7).
(*Analisis GC-MS terhadap asam lemak metil ester dari fraksi n-heksana Xylaria sp. DAP KRI-5*). *Ket. 1 : asam linoleat (C18:2, ω-6), 2: asam palmitat acid (C16:0), 3: asam oleat (C18:1, ω-9), 4 dan 6: asam stearat (C18:0), 5: asam palmitoleat (C16:1 ω-7*)).

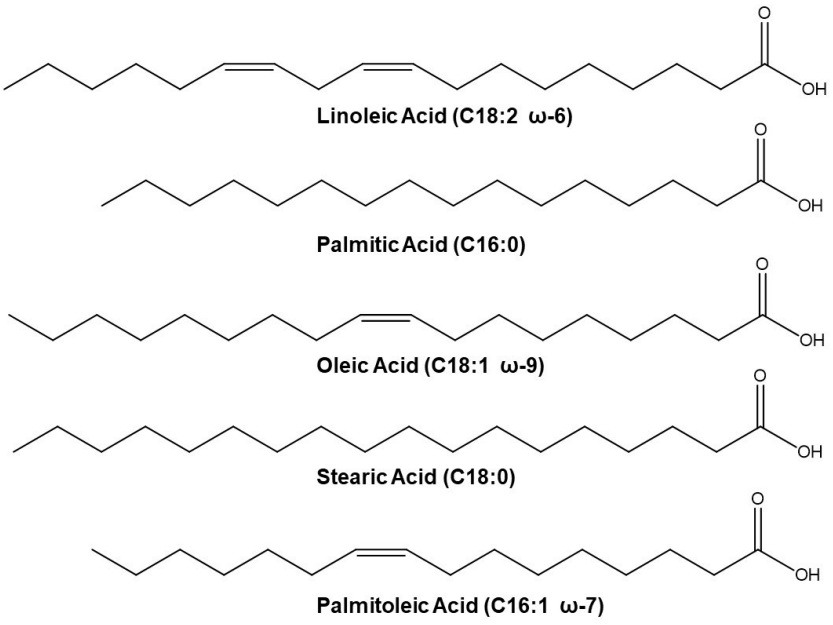


Figure 5. Chemical Structure of fatty acids produced by *Xylaria* sp. DAP KRI-5.
(*Struktur kimia asam lemak diproduksi oleh Xylaria sp. DAP KRI-5*).

Table 2. Composition and quantity of fatty acids of n-Hexane fraction of *Xylaria* sp. DAP KRI-5 (*Komposisi dan kuantitas asam lemak fraksi n-heksana dari Xylaria sp. DAP KRI-5*).

Compound (*Komponen*)	**Percentage (*Persentase*) (% b/b)**	**Production capacity (*Kapasitas produksi*) (mg/L)**
Linoleic acid (C18:2 ω-6)	41.18	112.24
Oleic acid (C18:1 ω-9)	14.20	38.70
Palmitoleic acid (C16:1 ω-7)	2.17	5.90
Total of Unsaturated Fatty acid (*Total Asam Lemak Tidak Jenuh*)	**57.55**	**156.4**
Palmitic acid (C18:0)	25.11	68.45
Stearic acid (C16:0)	6.58	17.92
Total of Saturated Fatty acid (*Total Asam Lemak Jenuh*)	**31.69**	**86.37**

Tabel 3. Production Capacity of Fatty acid in several fungi. (*Kapasitas produksi asam lemak pada beberapa jamur endofit*)

No	**Endophytic fungus (*Jamur Endofit*)**	**Growth Condition (*Kondisi Pertumbuhan*)**	**Culture age (*Usia kultur*) (hari)**	**Host Plant (Tumbuhan Inang)**	**Fatty acids Production (*Produksi Asam lemak*) (g/L)**	**Literature (*Literatur*)**
1	*Bionectria ochroleuca* XF-38	Glucose was changed by 2% cellulose microcrystals in PDB medium, 120rpm, 25°C, dark room	7 and 3	*Torreya grandis*	3.1 and 2.0	(Yang *et al.,* 2015)
2	*Colletotrichum* sp. TSC13	Glucose was changed by fructose in PDB medium, 75rpm, 25°C, dark room	7	*Taxus sumatrana* (Miq.) de Laub.	0.320	(Artanti *et al.,* 2014)
3	*Xylaria sp.* DAP KRI-5	PDB medium, static condition, 25°C, dark room	21	*Albertisia papuana*	0,243	This study

Table 4. Fatty acid composition in several fungi under different growing conditions. (*Komposisi asam lemak pada beberapa jamur pada kondisi tumbuh yang berbeda*).

No	Endophytic fungus (*Jamur endofit*)	Growth Condition (*Kondisi Tumbuh*)	C16:0	C16: 1 ω-7	C18: 0	C18: 1 ω-9	C18:2 ω-6	C18:3 ω-6	Literature (*Literatur*)
1	*Bionectria ochroleuca* XF-38	Glucose was changed by 2% cellulose microcrystals in PDB medium, 120rpm, 25°C, dark room	7.38	3.0	3.46	**40.23**	23.76	0.64	(Yang *et al.,* 2015)
2	*Xylaria sp.* DAP KRI-5	PDB medium, static condition, 25°C, dark room	25.11	2.17	6.58	14.20	**41.18**	ND (*TD*)	This study
3	*Colletotrichum* sp. TSC13	Glucose was changed by fructose in PDB medium, 75rpm, 25°C, dark room	10.00	ND (*TD*)	5.00	20.00	**40.00**	5.00	(Artanti *et al.,* 2014)
3	*Cunninghamella* sp.BO30	Sucrose 20 g/L and peptone 5 g/L, pH 4, **35°C**, 9 days	**54.00**	ND (*TD*)	11.75	14.15	7.81	1.14	(Suleiman *et al.,* 2018)
4	*Cunninghamella* sp.BO30	Sucrose 20 g/L and peptone 5 g/L, pH 4, **35°C**, 9 days followed by **15°C**, 3 days	**32.00**	3.59	2.83	**43.01**	0.26	3.22	(Suleiman *et al.,* 2018)

Note: ND: No Data, (*Ket.: TD: Tidak ada Data*)

the specific ratio was dominated by linoleic acid (C18: 2), while oleic acid (C18: 1) was dominant in conidial spores (Feofilova *et al*., 2015).

Compared with the study of Yang *et al*. (2015), *Bionectria ochroleuca* XF-38 showed that the production of fatty acids or lipids was dependent on the time of cultivation. It stated that the number of fatty acids increased, but after the optimum time there was a decrease in the resulting fatty acids. Different carbon sources in the growing medium also affect the number of fatty acids produced (Yang *et al*., 2015). The difference in carbon sources in the media also affects the growth rate and phase lag (Gao *et al*., 2017). The short lag phase in microbial growth is emphasized to achieve high productivity, especially for industrial-scale production (Nazir *et al.,* 2018). The effect of agitation on culture increases dissolved oxygen (Chang *et al.*, 2013) so that optimal cell growth conditions will be achieved if the agitation speed is appropriate. Meanwhile, if the speed of agitation is increased above the optimum condition, there will be a decrease in the amount of biomass (Nazir *et al*., 2018). Harvest time also affects the fatty acid composition, such as increase in the number of monounsaturated fatty acid (MUFA) and a decrease of

polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) after the 5th day of *N.oculata* (Aussant *et al.,* 2018).

The fatty acid composition of *Xylaria* sp. DAP KRI-5 and *B.ochroleuca* XF-38 which grew at 25 ^{o}C were dominated by unsaturated fatty acids. The production of unsaturated fatty acids in the two fungal isolates were dominated by linoleic acid (C18: 2; 41.18%) in *Xylaria* sp. DAP KRI-5 and oleic acid (C18: 1; 40.23%) in *B.ochroleuca* XF-38. According to previous studies, the lower temperature of growth condition increases the amount of unsaturated fatty acids (oleic acid, C18: 1), while it also decrease the amount of saturated fatty acids (palmitic acid, C16: 0) (Suleiman *et al*., 2018). This is probably due to the slightly optimal of fungal growth condition. The changes in the composition of fatty acids from unsaturated to saturated compounds also appear to be related to the growth phase of fungi, such as in the spore phase of *P. atrovenetumn*, linoleic acid (C18: 2) decreased, while oleic acid (C18: 1) increased. On the other hand, during mycelium growth, linoleic acid (C18: 1) decreases, while there is an increase of palmitic acid (C18: 0) and stearic acid (C16: 0) (Van Etten and Gottlieb, 1965). The culture temperature factor influenced more production of unsaturated fatty acids than saturated fatty acids (Table 4.). Table 4 shows the fatty acid composition of different fungi and different culture conditions, but it showed the composition of the dominant fatty acids in general, namely oleic acid, linoleic acid, and stearic acid. The use of a low temperature of 15 to 25^{o}C compared to a high temperature of 35^{o}C indicates a difference in the composition of saturated and unsaturated fatty acids. Saturated fatty acids (stearic acid, C16: 0) increases in the use of relatively high-temperature conditions, while unsaturated (oleic acid, C18: 1 and linoleate, C18: 2) decrease (Suleiman *et al.*, 2018). At low temperatures, fungal cells will also reduce membrane fluidity, and usually, cells compensate by increasing the synthesis of polyunsaturated fatty acids (PUFA) (Aussant *et al*., 2018).

The results of this research indicate that the n-hexane fraction of the endophytic fungus *Xylaria* sp. DAP KRI-5 is a potential source of essential and non-essential fatty acids. Essential fatty acid such as linoleic acid (LA) is the shortest chain ω-6 fatty acids and the most common PUFAs in commercial vegetable oils including seeds of cotton, corn, soybeans, and sunflowers with amounts over 50% (Kenar *et al*., 2017). Omega-6 fatty acids such as LA (18: 2, ω− 6) from the endophytic fungus *Xylaria* sp. DAP KRI-5 is a precursor to the fatty acid gamma linolenic acid (GLA, 18: 3 ω − 6); dihomo gamma-linolenic acid (DGLA, 20: 3 ω-6); and arachidonic acid (AA, 20: 4 ω− 6). The LA is an essential fatty acid that is needed by human because LA cannot be produced by their body (Hadley *et al*., 2016).

Fatty acids have the potential as a source of environmentally friendly fuels (biofuels), and also useful for medicine. Fatty acids such as oleic, linoleic, linolenic, stearic, and palmitic acids have antidiabetic activity by inhibiting α-glucosidase enzyme with an IC_{50} value of 2.15-95.27 µg/mL (Wheni and Tachibana, 2017). The inhibition of α-glucosidase was also found in fatty acids which were high content of linoleic acid, such as fatty acids was produced by *Colletotrichum* sp. TSC13 that cultivated on various types of growing media (Artanti *et al.,* 2014).

Esters of fatty acids derived from vegetable oils are known to be potent antioxidants with IC_{50} of 1.86-9.42 µg/mL and antifungal against *Paracoccidioides* spp., *Candida glabrata*, *C. krusei*, *C. parapsilosis* with the MIC value range of 15.6 -500 µg/mL (Pinto *et al*., 2017). Oleic acid derivative in the form of its esters is reported as anticancer action against human breast cancer cells (MCF-7) and human colon cancer cells (HT-29) with IC_{50} value in the range of 48-294 µg/mL (Dayley Jr *et al*., 2011). The main components of the macrofungi *Laetiporus sulphureus* were C18: 2ω-6, C18: 1ω-9 and C16: 0 with a ratio of unsaturated fatty acids/ saturated fatty acids (UFA/ SFA) of more than 3.4 and had antibacterial activity. and antifungal in vitro with MIC/ MBC/ MFC values in the range of 0.05-17 mg/mL (Sinanoglou *et al*., 2015).

CONCLUSION

Composition and quantification of fatty acids of the n-hexane fraction of *Xylaria* sp. DAP KRI-5 using GC-MS are as follows: linoleic acid (C18: 2 ω-6; 41.18%; 112.24 mg/L), palmitic acid (C: 16; 25.11%; 68.45 mg/L), oleic acid (C18: 1 ω-9; 14.20%; 38.70 mg/L), stearic acid (C18; 6.58%; 17.2 mg/mL), and palmitoleic acid (C16: 1 ω-7; 2.17%; 5.90 mg/mL). The total amount of unsaturated and saturated fatty acids was 57.54 and 31.69%, respectively. The n-hexane fraction of *Xylaria* sp. DAP KRI-5 has the potential as a source of essential fatty acids, namely omega-6 fatty acids (linoleic acid).

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Pedoman Penulisan Naskah Berita Biologi

Berita Biologi adalah jurnal yang menerbitkan artikel kemajuan penelitian di bidang biologi dan ilmu-ilmu terkait di Indonesia. Berita Biologi memuat karya tulis ilmiah asli berupa makalah hasil penelitian, komunikasi pendek dan tinjauan kembali yang belum pernah diterbitkan atau tidak sedang dikirim ke media lain. Masalah yang diliput harus menampilkan aspek atau informasi baru.

Tipe naskah

1. **Makalah lengkap hasil penelitian (*original paper*)**
   Naskah merupakan hasil penelitian sendiri yang mengangkat topik yang *up to date*. Tidak lebih dari 15 halaman termasuk tabel dan gambar. Pencantuman lampiran seperlunya, namun redaksi berhak mengurangi atau meniadakan lampiran.
2. **Komunikasi pendek (*short communication*)**
   Komuniasi pendek merupakan makalah hasil penelitian yang ingin dipublikasikan secara cepat karena hasil termuan yang menarik, spesifik dan atau baru, agar dapat segera diketahui oleh umum. Hasil dan pembahasan dapat digabung.
3. **Tinjauan kembali (*review*)**
   Tinjauan kembali merupakan rangkuman tinjauan ilmiah yang sistematis-kritis secara ringkas namun mendalam terhadap topik penelitian tertentu. Hal yang ditinjau meliputi segala sesuatu yang relevan terhadap topik tinjauan yang memberikan gambaran '*state of the art*', meliputi temuan awal, kemajuan hingga issue terkini, termasuk perdebatan dan kesenjangan yang ada dalam topik yang dibahas. Tinjauan ulang ini harus merangkum minimal 30 artikel.

Struktur naskah

1. **Bahasa**
   Bahasa yang digunakan adalah Bahasa Indonesia atau Inggris yang baik dan benar.
2. **Judul**
   Judul diberikan dalam bahasa Indonesia dan inggris. Judul ditulis dalam huruf tegak kecuali untuk nama ilmiah yang menggunakan bahasa latin, Judul harus singkat, jelas dan mencerminkan isi naskah dengan diikuti oleh nama serta alamat surat menyurat penulis dan alamat email. Nama penulis untuk korespondensi diberi tanda amplop cetak atas (*superscript*). Jika penulis lebih dari satu orang bagi pejabat fungsional penelitian, pengembangan agar menentukan status sebagai kontributor utama melalui penandaan simbol dan keterangan sebagai kontributor utama dicatatan kaki di halaman pertama artikel.
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4. **Pendahuluan**
   Pendahuluan berisi latar belakang, permasalahan dan tujuan penelitian. Perlu disebutkan juga studi terdahulu yang pernah dilakukan terkait dengan penelitian yang dilakukan.
5. **Bahan dan cara kerja**
   Bahan dan cara kerja berisi informasi mengenai metode yang digunakan dalam penelitian. Pada bagian ini boleh dibuat sub-judul yang sesuai dengan tahapan penelitian. Metoda harus dipaparkan dengan jelas sesuai dengan standar topik penelitian dan dapat diulang oleh peneliti lain. Apabila metoda yang digunakan adalah metoda yang sudah baku cukup ditulis sitasinya dan apabila ada modifikasi maka harus dituliskan dengan jelas bagian mana dan hal apa yang dimodifikasi.
6. **Hasil**
   Hasil memuat data ataupun informasi utama yang diperoleh berdasarkan metoda yang digunakan. Apabila ingin mengacu pada suatu tabel/ grafik/diagram atau gambar, maka hasil yang terdapat pada bagian tersebut dapat diuraikan dengan jelas dengan tidak menggunakan kalimat 'Lihat Tabel 1'. Apabila menggunakan nilai rata- rata maka harus menyertakan pula standar deviasinya.
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   Pembahasan bukan merupakan pengulangan dari hasil. Pembahasan mengungkap alasan didapatkannya hasil dan arti atau makna dari hasil yang didapat tersebut. Bila memungkinkan, hasil penelitian ini dapat dibandingkan dengan studi terdahulu.
8. **Kesimpulan**
   Kesimpulan berisi infomasi yang menyimpulkan hasil penelitian, sesuai dengan tujuan penelitian, implikasi dari hasil penelitian dan penelitian berikutnya yang bisa dilakukan.
9. **Ucapan terima kasih**
   Bagian ini berisi ucapan terima kasih kepada suatu instansi jika penelitian ini didanai atau didukungan oleh instansi tersebut, ataupun kepada pihak yang membantu langsung penelitian atau penulisan artikel ini.
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Format naskah

1. Naskah diketik dengan menggunakan program Microsoft Word, huruf New Times Roman ukuran 12, spasi ganda kecuali Abstrak spasi tunggal. Batas kiri-kanan atas-bawah masing-masing 2,5 cm. Maksimum isi naskah 15 halaman termasuk ilustrasi dan tabel.
2. Penulisan bilangan pecahan dengan koma mengikuti bahasa yang ditulis menggunakan dua angka desimal di belakang koma. Apabila menggunakan Bahasa Indonesia, angka desimal ditulis dengan menggunakan koma (,) dan ditulis dengan menggunakan titik (.) bila menggunakan bahasa Inggris. Contoh: Panjang buku adalah 2,5 cm. Lenght of the book is 2.5 cm. Penulisan angka 1-9 ditulis dalam kata kecuali bila bilangan satuan ukur, sedangkan angka 10 dan seterusnya ditulis dengan angka. Contoh lima orang siswa, panjang buku 5 cm.
3. Penulisan satuan mengikuti aturan *international system of units*.
4. Nama takson dan kategori taksonomi ditulis dengan merujuk kepada aturan standar yang diakui. Untuk tumbuhan menggunakan *International Code of Botanical Nomenclature* (ICBN), untuk hewan menggunakan International *Code of Zoological Nomenclature* (ICZN), untuk jamur *International Code of Nomenclature for Algae*, *Fungi and Plant* (ICFAFP), *International Code of Nomenclature of Bacteria* (ICNB), dan untuk organisme yang lain merujuk pada kesepakatan Internasional. Penulisan nama takson lengkap dengan nama author hanya dilakukan pada bagian deskripsi takson, misalnya pada naskah taksonomi. Penulisan nama takson untuk bidang lainnya tidak perlu menggunakan nama author.
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6. Untuk range angka menggunakan en dash (–), contohnya pp.1565–1569, jumlah anakan berkisar 7–8 ekor. Untuk penggabungan kata menggunakan hyphen (-), contohnya: masing-masing.
7. Ilustrasi dapat berupa foto (hitam putih atau berwarna) atau gambar tangan (*line drawing*).
8. Tabel
   Tabel diberi judul yang singkat dan jelas, spasi tunggal dalam bahasa Indonesia dan Inggris, sehingga Tabel dapat berdiri sendiri. Tabel diberi nomor urut sesuai dengan keterangan dalam teks. Keterangan Tabel diletakkan di bawah Tabel. Tabel tidak dibuat tertutup dengan garis vertikal, hanya menggunakan garis horisontal yang memisahkan judul dan batas bawah.
9. Gambar
   Gambar bisa berupa foto, grafik, diagram dan peta. Judul gambar ditulis secara singkat dan jelas, spasi tunggal. Keterangan yang menyertai gambar harus dapat berdiri sendiri, ditulis dalam bahasa Indonesia dan Inggris. Gambar dikirim dalam bentuk .jpeg dengan resolusi minimal 300 dpi, untuk *line drawing* minimal 600dpi.
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Gambar ilustrasi yang terdapat di sampul jurnal Berita Biologi berasal dari salah satu naskah yang dipublikasi pada edisi tersebut. Oleh karena itu, setiap naskah yang ada ilustrasinya diharapkan dapat mengirimkan ilustrasi atau foto dengan kualitas gambar yang baik dengan disertai keterangan singkat ilustrasi atau foto dan nama pembuat ilustrasi atau pembuat foto.

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