Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 7, No. 2, April 2022

Research Paper

Optimization of Antibacterial Production of Endophytic Fungi with Various Sources of C, N, and pH using The Response Surface Methodology

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Abstract
Secondary metabolites extract of McB_1 endophytic fungi from gelam (*Melaleuca cajuputi* Powell.) leaves have a high potential antibacterial activity against *Escherichia coli* ATCC8739 and *Staphylococcus aureus* ATCC6538 with flavonoids and phenol as bioactive compounds. The low production of secondary metabolites extract in the cultivation stage and the high potential antibacterial activity of bioactive compounds produced by McB_1 endophytic fungi require special treatment for optimizing the secondary metabolites product. This is possibly achieved by optimizing the composition of the cultivation media, where various sources of carbon, nitrogen, and pH produce different amounts and classes of secondary metabolites. The objectives of the research are to obtain the optimum interaction between sources of carbon, nitrogen, and pH for the production of secondary metabolite extract using Response Surface Methodology (RSM). The results showed that the highest extract (0.250 g) with the composition of sucrose as carbon source, yeast extract as nitrogen source, and pH 6. The optimization of the cultivation medium with composition 4.500 g/L sucrose, 0.480 g/L yeast extract, and pH 6.1 yielded 0.340 g secondary metabolites extract of McB_1 endophytic fungi. The chromatogram profile of the optimized secondary metabolite extract revealed the presence of flavonoids, phenols, terpenoids, and tannins.

Keywords
Endophytic Fungi, Antibacterial Compound, Response Surface Methodology (RSM)

Received: 13 November 2021, Accepted: 8 February 2022
https://doi.org/10.26554/sti.2022.7.2.149-157

1. INTRODUCTION

Endophytic fungi live in plant tissues at a certain time to form colonies and produce the same bioactive compounds as the host plants, due to the genetic transfer followed by the coevolution process (Jeffrey et al., 2008). From the result of previous research the McB_1 endophytic fungi of gelam leaves produced secondary metabolites as antibacterial against *Escherichia coli* with a Minimum Inhibitory Concentration (MIC) of 100 μg/mL, metabolite extract of 0.250 g, and fungal biomass of 2.340 g (Widjajanti et al., 2019). Efforts are required to increase the low production of secondary metabolites at the cultivation stage 0,250 g/L and the potential for the activity of bioactive compounds produced by modifying the cultivation media composition. Meanwhile, the differences in the composition of the cultivation media lead to different amounts of metabolites and their profiles. Discovering the most optimal conditions in the growth process and the formation of secondary metabolites from isolates is the basis for optimizing cultivation media (Goutam et al., 2014), which is achieved by modifying the carbon, nitrogen source, and pH (Septiana et al., 2017).

Carbon source is an important basic nutrient for fungi which is used as the main structure in providing energy for cell growth in metabolic processes. Sucrose as a carbon source also greatly affects the formation of antimicrobial compounds in endophytic fungi from *Moringa oleifera* plants (Arora and Kaur, 2019). Previous studies used glucose as carbon sources and increased the production of secondary metabolites in the form of beauvericin in *Fusarium oxysporum* (Lee et al., 2008) and *Fusarium rodolens* (Xu et al., 2009). The highest biosynthesis of flavonoid compounds was found after the use of dextrose as a carbon source in isolates of the fungus *Aspergillus tamarii* (Bose et al., 2019).

In some cases the growth of the source fungi and the nitrogen concentration greatly affect the production of secondary metabolites, it is often carried out several related studies to find the best nitrogen source in the formation of secondary metabolites. In this case, the most commonly used nitrogen sources are yeast extract, peptone, and sodium nitrate which are added to fungal nutrition (Arora et al., 2012).

The optimal growth of fungi in the pH range of 5-8 (Gupta et al., 2010). Secondary metabolite production in the fungal

group that *Fusarium incarnatum* produces the most optimum pigment at pH 5 (Himalini and Razia, 2018), while secondary metabolites were produced at the most optimum in *Geosmithia pallida* (Deka and Jha, 2018) and *Fusarium solani* at pH 6 Merlin et al. (2013). The condition of pH 7 greatly affects the formation of antimicrobial compounds in endophytic fungi *Moringa oleifera* (Arora and Kaur, 2019) while at pH 8 greatly affects the formation of naphthoquinones pigments in *Fusarium verticilioides* (Boonyapranai et al., 2008).

The cultivation media can be optimized using Response Surface Methodology (RSM) to obtain the optimum factors. It also analyzes the secondary metabolite formation response influenced by several independent variables to enhance the response examined (Qiu et al., 2012). The fungus *Aspergillus niger* KE1 produced the highest lipase enzyme with an optimum nutritional composition that had been analyzed using RSM, namely 2% peptone, 0.100% olive oil, 0.500% glucose and 0.075% $MgSO_4.7H_2O$ (Ilmi , 2021). The endophytic fungus *Dichotomopilus funicola* Y3 isolated from pigeon pea (*Cajanus cajan*) produced 4.9 times higher vitexin of 78.890 mg/L with the optimum media composition determined by RSM, namely 0.060 g/L L-phenylalanine, 0.21 g/L salicylic acid, and 0.19 g/L $CuSO_4{\cdot}5H_2O$ (Gu et al., 2018).

In this study, we will try to optimize the growth medium by experimenting with variations in carbon, nitrogen, and pH sources using RSM in the hope that higher metabolite products will be obtained. Acording to Bezerra et al. (2008) Response Surface Methodology (RSM) is a combination of mathematical and statistical techniques based on the compatibility of polynomial equations with experimental data that must describe a data set with the aim of predicting statistically. Because of its high efficiency in fermentation, media composition, and process conditions, the RSM method was chosen. Furthermore, it can explain the interaction of variables, and its accuracy is high (Kiran et al., 2016; Palukurty and Somalanka, 2016; Karthikeyan et al., 2010). RSM can be applied if a response is influenced by several variables, the aim is to optimize the interaction of these variables so that the best results are obtained. The objectives of the research to obtain the optimum interaction between sources of carbon, nitrogen, and pH for the production of secondary metabolite extract using Response Surface Methodology (RSM).

2. EXPERIMENTAL SECTION

2.1 Materials

Pure culture of McB_1 endophytic fungi from the previous research that isolated from *Melaleuca cajuputi* leaves.

2.2 Methods

2.2.1 Propagation of Endophytic Fungi

Pure culture of McB_1 endophytic fungi were propagated on PDA medium in test tubes and petri dish for stock and working cultures. Subsequently, it was incubated at room temperature until the fungi grew for aproximately 5-7 days.

2.2.2 Selection of Carbon, Nitrogen Sources, and pH

Potato Dextrose Broth medium with composition of potato 200 g/L, peptone 0.500 g/L, yeast extract 0.800 g/L, $(NH_4)_2SO_4$ 3 g/L, KH_2PO_4 2 g/L, 0.500 g/L $MgSO_4$, 0.010 g/L phenylalanine, and 4% (w/v) carbon source (Merlin et al., 2013) used in cultivation. In the selection of carbon sources, the medium was modified by using carbon sources in the form of glucose, dextrose and sucrose. In the selection of nitrogen sources, the medium was modified by using nitrogen sources in the form of peptone, yeast extract, and sodium nitrate. In the selection of pH, the medium was modified by using variation pH 5, 6, 7, and 8.

2.2.3 Optimization of Cultivation Medium Composition with Response Surface Methodology (RSM)

The composition of the cultivation medium is optimized using Response Surface Methodology, and it includes three selected variables: carbon, nitrogen, and pH. The data of the selection from the previous test was used to determine the upper and lower limits of the Central Composite Design (CCD). This is also useful in the RSM method with the Design-Expert 7.0 from Stat-Ease (Qiu et al., 2012).

This stage consisted of 9 fractional factorial points 23 for a factorial design compiling from 3 variables, which is enlarged by 6 starting and 5 center points. The total of all experimental units in this stage was 19 and was optimized using the cultivation medium composition of potato 200 g/L, yeast extract 0.800 g/L, $(NH_4)_2SO_4$ 3 g/L, KH_2PO_4 2 g/L, $MgSO_4$ 0.500 g/L, phenylalanine 0.010 g/L, sources of carbon, nitrogen, and pH determined by Central Composite Design (CCD).

2.2.4 Extraction of Endophytic Fungi Secondary Metabolite

The biomass of endophytic fungi was separated from the optimization medium using filter paper. The liquid-liquid fractionation (partition) was carried out with ethyl acetate solvent in a ratio of 1:1 and the extract collected was concentrated with a rotary evaporator to obtain the ethyl acetate extract of endophytic fungi. Subsequently, the separated biomass was dried using an oven to obtain the dry weight (Bhardwaj et al., 2015).

2.2.5 Thin Layer Chromatography (TLC)

The optimized extract from McB_1 endophytic fungi was dissolved with ethyl acetate, spotted with a capillary pipette on a TLC plate (TLC, silica gel 60 F254, Merck), and eluted in a chamber that contained eluent with a ratio of n-hexane: ethyl acetate 3:2. When the eluent reached the boundary line to form a TLC chromatogram pattern, the plate was removed and the stain formed was viewed using 366 nm UV light. For visualization observations, it was sprayed with 5% H_2SO_4 reagent and heated on a hot plate at a temperature of 80°C. The stains formed were identified through color spots and the Rf value (Retardation factor) was determined using the formula below (Bele and Khale, 2011):

$$Rf = \frac{\text{Distance traveled by component}}{\text{Distance traveled by solven}}$$

2.2.6 Data Analysis

Data was analyzed with Analysis of Variance (ANOVA) at α 0.050.

3. RESULT AND DISCUSSION

3.1 The Carbon Source for McB$_1$ Endophytic Fungi

McB$_1$ endophytic fungi grew and produced secondary metabolites on all sources of sucrose, dextrose, or glucose. Meanwhile, the weight of the ethyl acetate extract and the mass of biomass produced were shown in Figure 1. After 30 days of cultivation, the highest extract yielded and biomass weight was 0.250 g and 2.450 g with a carbon source of dextrose and sucrose, respectively. Monosaccharides were found to be the most effective carbon source for fungal growth. This is also evidenced in a similar study in Bose et al. (2019) that, dextrose is a simple type of carbon that can be metabolized by fungi more easily compared to other carbon sources. The metabolism process of monosaccharide-type carbon sources is easily broken down by the fungus isolate used quickly, characterized by the highest fungal biomass weight, this causes the dextrose concentration to decrease in the cultivation media. After the dextrose carbon source is depleted, the fungus regulates the use of other carbon sources in the media to be used for the formation of secondary metabolites in the idiophase. This is explained in Stanbury and Whitaker (1987) that the limited carbon concentration will induce the formation of secondary metabolites, limited carbon is indicated due to fast metabolism for cell growth.

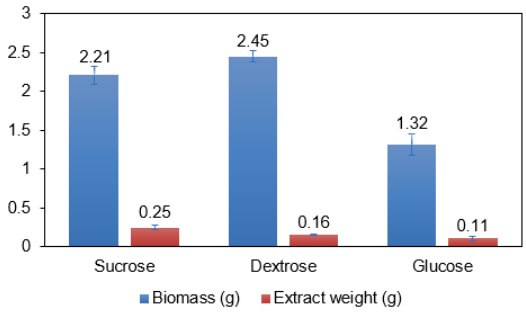

Figure 1. Fungal Biomass and Extract Weight from Carbon Source Optimization

Sucrose belongs to the disaccharide group which is broken down into glucose and fructose by enzymes before it is metabolized by fungi. According to Mao et al. (2005), this occurs through the use of substrates with a slow metabolism by fungi due to the inability to hydrolyze, characterized by non-optimal cell growth. Meanwhile, the formation of secondary metabolites is induced by slow growth due to the depletion of the main carbon source.

Figure 1 shows the biomass of McB$_1$ endophytic fungi which is not directly proportional to the secondary metabolites production. The use of sucrose produced a lower weight of biomass than dextrose, however, this is not shown in the production of secondary metabolites. Furthermore, high biomass weight did not produce higher secondary metabolite weight because cell growth has no relationship with their formation. According to Sanchez et al. (2010), biomass is formed at the cell growth stage which is dominantly synthesized at the exponential phase, while secondary metabolites are at the stationary to the lag phases.

3.2 The Nitrogen Source for McB$_1$ Endophytic Fungi

McB$_1$ endophytic fungi also grew and produced secondary metabolites on all nitrogen sources, namely peptone, yeast extract, and $NaNO_3$. The weight of the ethyl acetate extract and biomass produced are shown in Figure 2. After 30 days of cultivation, the highest extract and biomass weight were 0.390 and 1.360 g with nitrogen sources in form of yeast extract and peptone, respectively.

The highest metabolite production was obtained after the use of yeast extract, which did not produce maximum fungal biomass, however, there was high production of biomass from peptone. Merlin et al. (2013) stated that the highest secondary metabolites production by the endophytic fungi of *Fusarium solani* was observed after the use of yeast extract. According to Septiana and Simanjuntak (2017), yeast extract is the best nitrogen source in producing antioxidant compounds by isolates of endophytic fungi from turmeric roots. Described by Wang et al. (1979), the amino acids found in yeast extract and peptone contain glutamic acid, glutamine, cysteine, methionine, arginine, asparagine, proline, and phenylalanine. Besides containing amino acids, yeast extract also contains several minerals such as sodium, chloride, calcium, magnesium, potassium phosphate, and sulfate as elements used in the growth process by fungi.

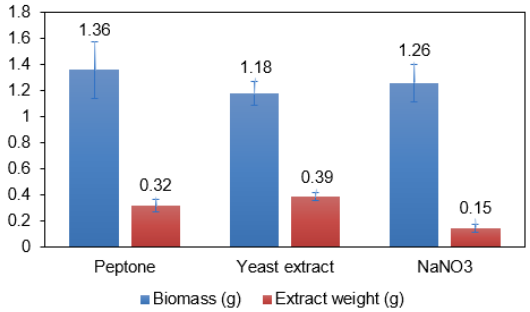

Figure 2. Optimization of Nitrogen Source Yields Fungal Biomass and Extract Weight

3.3 pH Range for McB$_1$ Endophytic Fungi

The McB$_1$ endophytic fungi were able to grow and produce secondary metabolites at all tested pH values, which include

6, 7, and 8. The extract weight of the ethyl acetate and fungi biomass produced with variations in pH are shown in Figure 3. After 30 days of cultivation, the highest extract was 0.560 g at pH 6, while the biomass weight was 2.270 g at pH 7. Since the most optimum secondary metabolite production was obtained at pH 6, therefore, the fungi produced maximally in acidic pH. It has been previously examined by Gazi and Kanda (2004) that cell growth and the formation of secondary metabolites in fungi tend to be produced at optimal pH with a range of 5-7. Research conducted by Merlin et al. (2013) also found that the isolates from the endophytic fungi of *Fusarium solani* formed the highest secondary metabolites at pH 6.

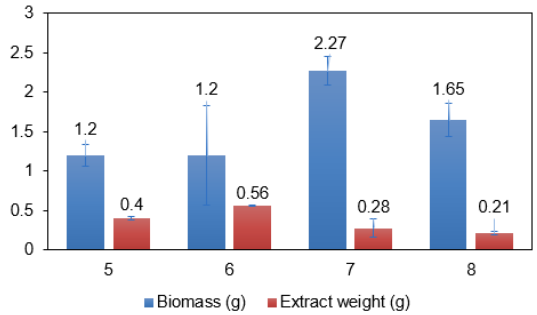

Figure 3. Fungal Biomass and Extract Weight as a Result of pH Optimization

Acording to Rousk et al. (2009) pH affects the growth of fungi, starting from the growth of mycelium or the growth of fruiting bodies. The pH will affect the permeability of the fungal membrane, therefore the fungus will be unable to absorb important nutrients when a certain pH is not suitable. Acording to Arora and Chandra (2010) the inhibition of the nutrient uptake process will affect the metabolic rate of the fungus. pH also related to the permeability characteristics of the fungal membrane so that it affects the uptake and loss of ions in media nutrient for their growth. The effect of pH on secondary metabolite production varies greatly depending on the fungus species. The production of quinidine increased considerably in *Fusarium solani* at an initial pH of 6.2, but not in *Diaporthe sp.* at an initial pH of 6.8 (Rahmawati et al., 2021).

3.4 Results of Cultivation Medium Optimization for McB_1 Endophytic Fungi

In McB_1 endophytic fungi, three factors were used to affect the weight of secondary metabolite extract with sucrose as carbon source with a value ranging from 0.360 g/L to 0.680 g/L and yeast extract as nitrogen source with a value between 0.160 g/L to 0.830 g/L, while the pH ranges from 5.1 to 6.8. Meanwhile, the range and level of the factors used can be reviewed in Table 1. The extract weight of the ethyl acetate and biomass of endophytic fungi optimized using sucrose, yeast extract, and pH are presented in Table 2.

The 19 treatment points of the design gave a response in form of the McB_1 endophytic fungi extract weight, as presented in Table 2. The highest extract weight was obtained in the center point at the 17^{th} standard with the metabolite extract weight of 0.390 g.

Table 1. The Composition of Medium Optimization for The McB_1 Endophytic Fungi and The Range of Factors Tested

Optimization Factor	Range and Level				
	-1.680	-1	0	1	1.680
Sucrose (g/L)	0.630	2	4	6	7.360
Yeast Extract (g/L)	0.160	0.300	0.500	0.700	0.830
pH	5.1	5.5	6	6.5	6.8

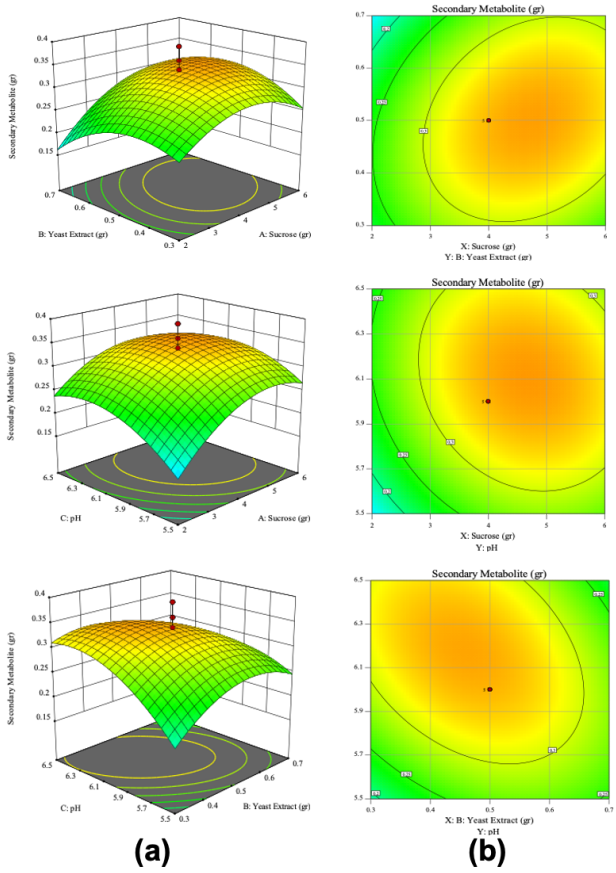

Figure 4. 3D-Surface Graphics for Surface and Contour Responses The Surface of McB_1 Endophytic Fungi Extract. (a) 3D-Surface Graphics for Response Surface (b) Contour Graph for Response Surface

3.5 Response Model of McB_1 Endophytic Fungi

The data model was selected based on the response from the weight of the secondary metabolite extract by reading the results of the sequential analysis of the sum of squares in line with the smallest p-value ($p>0.050$). According to the analysis of the summary statistics and the sequential sum of the square, it was concluded that the appropriate model was selected by the pro-

Table 2. Weight of McB_1 Endophytic Fungi Extract from Cultivation Medium Optimization with 3 Factors using RSM

Standard	Sucrose (X_1, g/L)	Yeast Extract (X_2, g/L)	pH (X_3)	Extract Weight (Y, g)
1	2	0.300	5.5	0.130
2	6	0.300	5.5	0.180
3	2	0.700	5.5	0.090
4	6	0.700	5.5	0.230
5	2	0.300	6.5	0.290
6	6	0.300	6.5	0.270
7	2	0.700	6.5	0.100
8	6	0.700	6.5	0.190
9	0.630	0.500	6	0.110
10	7.360	0.500	6	0.270
11	4	0.160	6	0.160
12	4	0.830	6	0.250
13	4	0.500	5.1	0.180
14	4	0.500	6.8	0.250
15	4	0.500	6	0.280
16	4	0.500	6	0.360
17	4	0.500	6	0.390
18	4	0.500	6	0.340
19	4	0.500	6	0.31

gram. This was conducted to determine the optimum response of the secondary metabolite of the McB_1 endophytic fungi and explain the relationship between the three factors. Therefore, the quadratic model in the selection analysis represented the most appropriate model used in this research.

3.6 Results of Analysis of Variance (ANOVA) and Interaction Between Factors on The Response of McB_1 Endophytic Fungi

Based on the data processing using Design Expert 11.0.0 and Analysis of Variance (ANOVA), the yeast extract, pH, and the 2FI model (interaction between 2 factors) have insignificant values. As shown in the table, each p-value has 0.551, 0.086, 0.170, 0.394 and 0.067, respectively, where p>0.050. This indicated that the use of nitrogen sources such as yeast extract, variations in pH, and the relationship between the two factors did not affect the production of secondary metabolites. According toBaş and Boyacı (2007), a significant data model provides accurate data to explain the relationship between the dependent and independent variables in the response surface analysis method.

The use of a carbon source in form of sucrose had a significant value of 0.015 where p <0.050, which affected the production of secondary metabolite extracts. Similarly, the use of sucrose affected on the formation of secondary metabolites of McB_1 endophytic fungi. This is because sucrose is a disaccharide that is difficult to synthesize and is not favored by fungi. Therefore, other carbon sources are used until the condition of the first preferred source is exhausted, which causes metabolic imbalance and physiological stress. Martin and Demain (1980) stated that in a medium, a carbon source such as sucrose is used

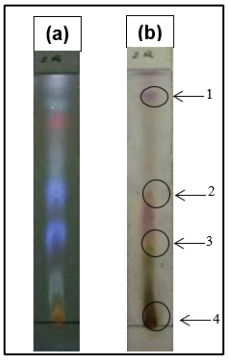

Figure 5. Results of TLC on Secondary Metabolite Extract of McB_1 Endophytic Fungi (a) TLC of McB_1 Endophytic Fungi Extracts at 366 nm UV Light (b) Chromatogram of McB_1 Endophytic Fungi Extract After Spraying H_2S0_4 5%

longer by fungi and after the preferred source, while the latter is used for the biosynthesis of secondary metabolites.

The use of sucrose, yeast extract, and pH is shown in form of contour plots and response surface graphs in Figure 4. Based on the analysis, there is an increase in the concentration of sucrose from 0.680 g to 4 g and yeast extract from 0.160 to 0.500 g. Furthermore, the use of sucrose more than 4 g and yeast extracts more than 0.500 g can reduce the production of secondary metabolites. The optimum concentration to increase the production of metabolites were 4 g of sucrose and 0.500 g of yeast extract. This decrease in production was explained by Tudzynski (2014) which stated that some nitrogen and excess

Table 3. Composition Formula for Medium Optimization of McB_1 Endophytic Fungi

Isolate	Sucrose	Yeast Extract	pH	Secondary Metabolites	Desirability
Level	4.500 g/L	0.480 g/L	6.1	0.340 g	0.853

carbon sources used in the cultivation medium cause inhibition of secondary metabolite formation by the substrate. Since these conditions significantly affect the specific growth process, the cell growth rate becomes constant and there is no physiological imbalance that induces the formation of secondary metabolites leading to inhibition by the substrate.

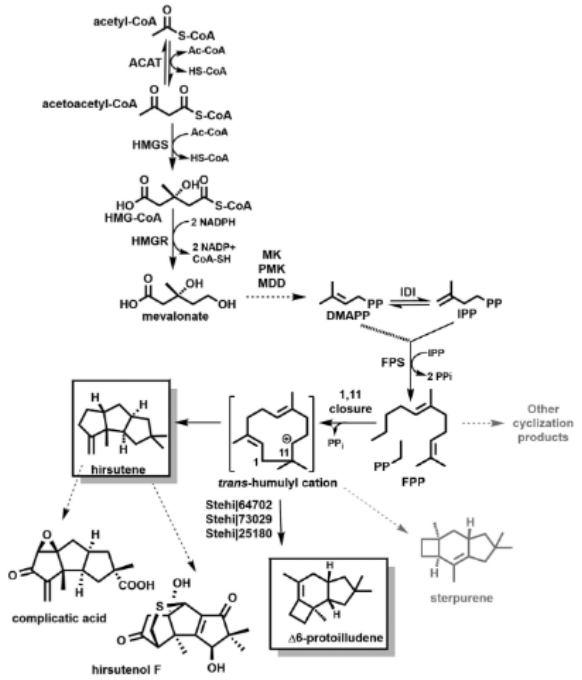

Figure 6. The Mechanism for The Biosynthesis of Terpenoids through The Mevalonic Acid Pathway (Flynn and Schmidt-Dannert, 2018)

Based on the ANOVA yeast extract, pH and the 2FI model (interaction between 2 factors) have nonsignificant values. Each p-value shown in the table is 0.551, 0.086, 0.170, 0.394 and 0.067 where p>0.050. This indicates that the use factor nitrogen source in the form of yeast extract, variations in pH and the relationship between the two factors did not affect the production of secondary metabolites of McB_1 fungal isolate. Based on Figure 4, as the pH value increases from 5.5 to 6.5, the production of secondary metabolites also increases, and the optimum value is obtained at pH 6. According to Arora and Chandra (2010), the appropriate pH is among the factors that determine growth, product formation, and enzymes used in metabolic processes. The optimum pH under within the acidity range was able to affect the work of the enzymes produced. This showed that fungi can grow optimally and produce secondary metabolites at a pH within the acidic range.

3.7 The Formulation for Medium Optimization of McB_1 Endophytic Fungi

Figure 7 provides optimal point solutions for each factor suggested by Design Expert 11.0.0. Meanwhile, the production of secondary metabolites was increased with influencing factors, namely sucrose, yeast extract, and pH range using the Design Expert 11.0.0 program with the specified limits to produce the optimum value. The criteria for optimizing the response to the production of secondary metabolites are adjusted to the limits in Table 3.

The mathematical model for the secondary metabolite production of McB_1 endophytic fungi obtained in the optimization was $Y = -8.297 + 0.185X_1 + 3.022X_2 + 2.443X_3 + 0.062X_1X_2 - 0.015X_1X_3 - 0.350X_2X_3 - 0.013X_1^2 - 1.213X_2^2 - 0.179X_3^2$, where the variables with the most optimum response were sucrose (X_1) of 4.500 g/L, yeast extract (X_2) of 0.480 g/L and pH (X_3) of 6.1. Moreover, the estimated response obtained was 0.340 g with a desirability of 0.853, which is approximately 1. According to Raissi and Farsani (2009), desirability is a value showing the program's ability to fulfill the desires based on the criteria set on the final product. Therefore, the most optimal formula accepted is that with the maximum desirability value in a range of 0 to 1. The desirability value closer to 1 indicates that the program's ability to produce the desired product is getting more perfect.

3.8 TLC Secondary Metabolite Extract from Optimization of McB_1 Endophytic Fungi

Thin-Layer Chromatography was used to examine the optimized secondary metabolite extract (TLC). The fungi produced secondary metabolites from several groups of compounds as the medium composition of the cultivation process changed, including phenols, flavonoids, terpenoids, and tannins (Figure 5).

The bioactive compounds were identified by examining the color spots formed on the TLC plate. Based on Figure 5, the secondary metabolite extract of the McB_1 endophytic fungi in TLC obtained yellow phenol compounds and flavonoids with an orange color. According to Bungihan and Matias (2013), yellow-colored compounds are classified as phenolic, while flavonoids have a brownish-yellow to red color. Furthermore, the two other spots observed were blackish-brown spot which was identified as tannins, while purple spots were identified as terpenoid. Bioactive compounds from the tannin group form green, brown to black spots, while terpenoid formed pink to purple or violet after being sprayed with 5% H_2SO_4 and heated.

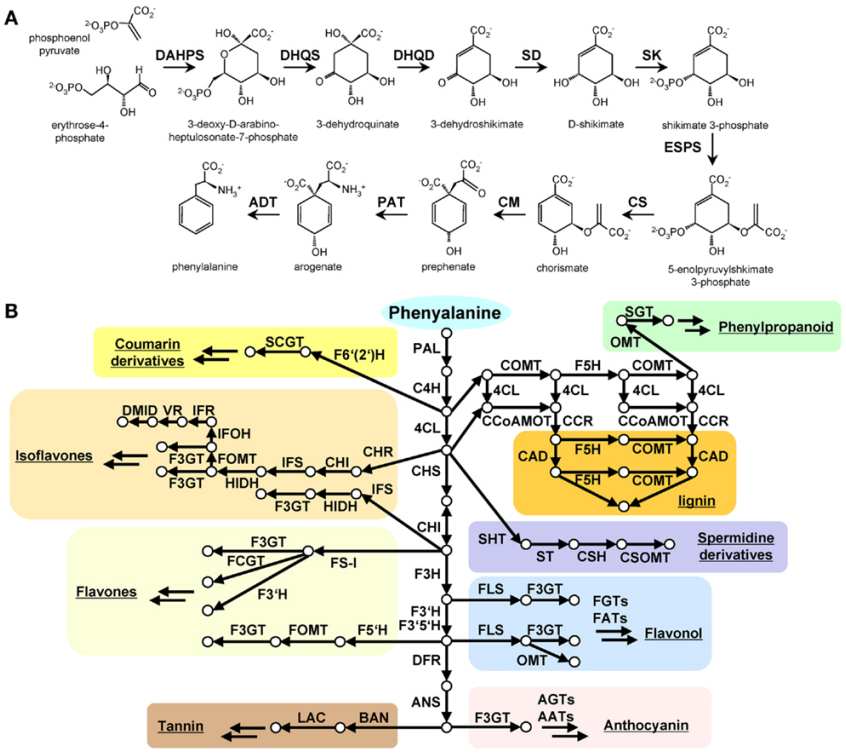

Figure 7. The Mechanism of Tannin Biosynthesis through The Shikimic Acid Pathway (Tohge et al., 2013)

Based on the results of TLC (Figure 5), two new groups of the compound were added, namely terpenoids and tannins were added to the secondary metabolite compounds of the McB_1 endophytic fungi. In Widjajanti et al. (2019), the group of compounds produced by McB_1 endophytic fungi was phenolic and flavonoids, due to changes in the composition of the cultivation medium used. In a previous study, cultivation used the PDB medium (200 g of potatoes and 20 g of glucose in 1 L of distilled water). In the optimization, a medium with a more complete composition was used (200 g potato, 4 g sucrose, 0.800 g yeast extract, 3 g $(NH_4)_2SO_4$, 0.500 g $MgSO_4$, 0.010 g phenylalanine in 1 L of aquadest). Abo-Elmagd (2014) also stated that the nutrient source can induce or eliminate a metabolite compound produced by fungi.

The terpenoid group is formed due to differences in the composition of the carbon source. The formation of acetic acid in cellular processes is influenced by carbon metabolism. Hence, it was used as a substrate in primary metabolism to produce acetyl CoA as the main ingredient in the formation of terpenoid compounds during the biosynthesis of the mevalonic acid pathway (Figure 6). According to Agusta (2006) and Flynn and Schmidt-Dannert (2018), acetic acid was condensed to produce acetyl coenzyme A, after activation, which is the reaction step of terpenoid biosynthetic. The reaction of acetyl coenzyme A produced Isopentenyl Pyrophosphate (IPP), which is isomerized into Dimethyl Allyl Pyrophosphate (DMAPP) by the isomerase enzyme. Moreover, IPP is an active isoprene unit that combined head-to-tail with DMAPP, which is the first step of isoprene polymerization to produce terpenoids.

Based on the chromatogram profile, blackish-brown spots formed were assumed to be tannin compounds, due to the use of L-phenylalanine in the composition of the medium optimization. Meanwhile, the use of L-phenylalanine as a precursor for the formation of tannins is presented in Figure 7. According to Tohge et al. (2013), the shikimic acid pathway is used in the synthesis of hydrolyzable tannin groups and compounds based on the amino acid phenylalanine. Since the amino acid phenylalanine is used in the formation of gallic acid, therefore, the tannins are formed through esterified gallic acid derivatives. From fungus McB_1 isolate tannin support as an antibacterial compound.

4. CONCLUSION

The optimization of the cultivation medium with composition 4.500 g/L sucrose, 0.480 g/L yeast extract, and pH 6.1 yielded 0.340 g secondary metabolites extract of McB_1 endophytic fungi. The optimized secondary metabolite extract's chromatogram profile revealed the presence of flavonoids, phenols, terpenoids, and tannins. The chromatogram profile of the optimized secondary metabolite extract showed the presence of flavonoids, phenols, terpenoids, and tannins that as an antibacterial.

5. ACKNOWLEDGEMENT

The DIPA of the Public Service Agency of Universitas Sriwijaya 2021 funded the research and publication of this article. SP DIPA-023.17.2.677515/2021, on November 23, 2020. In accordance with the Rector's Decree Number: 0010/UN9/SK.LP2M.PT/2021, on April 28, 2021.

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