ATLANTIS PRESS

Advances in Health Sciences Research, volume 26

2nd Bakti Tunas Husada-Health Science International Conference (BTH-HSIC 2019)

Fermentation and Determination of Anti-Cholesterol Monakolin K from Different *Monascus purpureus* Isolates

Anna Yuliana[1*], Khusnul[2], Syifa Ul Zannah[1], Ira Rahmiyani[1], Saeful Amin[1]
[1]Prodi Farmasi, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada
[2]Prodi Analis Kesehatan, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada
*Email : anna_yuliana@stikes-bth.ac.id

Abstract*—Objectives:** ***Monascus purpureus (M. purpureus) is the predominant mold of the red yeast rice. Red yeast rice is the product of rice fermentation by M. purpureus through solid fermentation system. The red yeast rice has long been consumed as flavoring, food coloring and traditional medicines for various diseases, one of which is to treat hypercholesterolemia, as they contain nutritious bioactive materials which are terpenoid compounds whereas mevinolin or monakolin K is the most dominant. Therefore, researches had been conducted to determine Monakolin K levels of several M. purpureus isolates, among others are isolates of LIPI F01, F147 LIPI, IPB-A, IPB-B and ITB, as the product of solid fermentation using IR 64 white rice as a substrate. The measurement of Monakolin K levels was done by extracting the sample using 80% methanol on the fermentation at 14th day, followed by examining the Monakolin K levels using HPLC (High Performance Liquid Chromatography) with UV detector at λ 238 nm using motion phase of acetonitrile: phosphoric acid 0, 2% (1: 1). The research results showed that the highest Monakolin K levels was produced by IPB-A isolates with the level was as high as 15.0517 ppm, while the lowest Monakolin K level was generated by LIPI F147 isolates, with the level was as low as 1.4264 ppm.

Keywords: **Monascus purpureus*****, fermentation, HPLC***

I. INTRODUCTION

High blood cholesterol is one of directly risky factors that causes heart disease. Heart disease is the first-rank of cause of death-disease, either in developed countries or developing countries. Heber (1999) says that the *Monascus purpureus (M. purpureus)* on red yeast rice produces compounds that inhibit the synthesis of cholesterol [1,2].

The red yeast rice is a product of rice fermentation by *M. purpureus* through solid fermentation system which appears the red color. The process of red yeast rice fermentation produces some secondary metabolites, including pigments, antibacterial agents (monascidin A) and hypercholesterolemic agent (monakolin K or also known as Lovastatin) [3,4].

The red yeast rice has long been consumed as flavoring, food coloring and traditional medicines for various diseases, which one of them is to treat hypercholesterolemia. Hypercholesterolemia is a condition in which blood cholesterol levels exceed the normal limits. So far as the science progresses today, the red yeast rice is widely used for many medical purposes, because monakolin K has the content that can lower the blood cholesterol levels. Kasim research showed that administration of the red yeast rice containing monakolin K to hypercholesterolemic patients may reduce the blood cholesterol level up to 49.28% [5,6].

Monakolin K can be used as an anti-hypercholesterolemia because the compound inhibits the liver's cholesterol synthesis by inhibiting the activity of HMG-CoA (3-hydroxy-3 methylglutaryl coenzyme A) reductase; the determinants enzyme of cholesterol biosynthesis. Monakolin K is included into the statin compounds, the compounds that have roles as competitive inhibitors of the HMG-CoA reductase which help reducing the blood cholesterol levels [7}.

In the process of red yeast rice production, *M. Purpureus* breeding is influenced by several factors, which include pH, temperature, light, medium components, as well as its mold isolate itself. *M. Purpureus* is a red mold that can be cultured on a substrate containing starch such as rice. Different varieties of rice can be used as medium to breed *M. purpureus* compounds that contain high amylose that is good for breeding the mold and its monakolin K content. Rice also contains vitamin B1, phosphorus, potassium, amino acids, salts and zinc. The essential amino acids is particularly used for monakolin K biosynthesis because of its direct precursor [8,9].

This study is aimed to determine the Monakolin K levels of several *M. Purpureus* isolates which were analyzed by using HPLC instrument (High Performance Liquid Chromatography). The difference of isolate levels was suspected to generate the difference of monakolin K levels.

II. MATERIALS AND METHODS

A. Materials

The materials used in this study were IR 64 rice, PDA (Potato Dextrose Agar), PDB (Potato Dextrose Broth), 80% methanol, acetonitrile, 0.2% phosphoric acid, and distilled water. The microbes used in this research were several *Monascus purpureus* isolates facilitated from Institut Pertanian Bogor Culture Collection (IPBCC), Indonesian Culture Collection (InaCC) Indonesian Institute of Sciences (LIPI), Bogor and collection of the Institut Teknologi Bandung (ITB).

B. Methods

The research methods were accomplished through several stages, they were. The Substrates Preparation and Tools SterilizationThe rice used as the substrate was firstly washed and inserted into the Erlenmeyer then sterilized along with all the glasswares used by autoclaving at temperatures of 121˚C at 1 atm pressure for 15 minutes .

The Preparation of Breeding Medium Insulation Mediumused in this study was the PDA (Potato Dextrose Agar). A total of 39 grams of PDA medium was dissolved in 1000 ml of distilled water in Erlenmeyer, and then heated above the spiritus fire, stirred constantly until it reached a clear solution. The medium was then sterilized in an autoclave at a temperature of 121˚C and 1 atm pressure for 15 minutes.

The Breeding of *Monascus purpureus* Mold. The amount of *Monascus purpureus* isolates used in this study were numbered to 5 isolates. Each of these pure *Monascus purpureus* isolates was augmented by transferring the culture on slanted PDA agar (Potato Dextrose Agar) in a test tube with a 30 ° slope, then incubated at 28 ° C for 7 days.

Preparation of *Monascus purpureus* Inoculum. The spore suspension was made by aseptically taking *Monascus purpureus* in 7 day-aged PDA slanted agar using spatula end, and then put it into 50 ml liquid PDB (Potato Dextrose Broth) medium, then shaken it using a rotary shaker at 150 rpm speed for 3 days.

Rice Fermentation. The red yeast rice was made by inserting about 100 grams of sterilized rice in Erlenmeyer then inoculated it with 3 ml liquid *Monascus purpureus* Inoculum in 27˚-incubation at 32C for 14 days and had daily shaking (Kasim, 2006).

Monakolin K Extraction. 2 grams of Monakolin K powder was extracted using 10 ml of 80% methanol and the extracted solution was shaken using a rotary shaker at 200 rpm speed for 1 hour. The solution was then centrifuged at 1500 rpm speed for 10 minutes, and the resulted supernatant was filtered using Whatman filter paper (0.22 µm).

Monakolin K Content Analysis using HPLC (*High Performance Liquid Chromatography*)

As much as 20 ml sample was injected into the HPLC column. The HPLC used was C-18 column with a motion phase of acetonitrile: Phosphoric Acid 0.2% (1: 1), a flow rate of 1 ml / min, using an ultraviolet detector (UV) at λ 238 nm.

III. RESULT

The results of monakolin K measurement on several samples of rice fermentation by *Monascus purpureus* can be seen at a retention time appearing on the chromatogram peak. The retention time appeared in some samples was almost the same standard retention time as of Monakolin K which was in the average between 10.55 to 11.13. The chromatograms of IPB-B standard and sample produced can be seen in Figure 1 and 2.

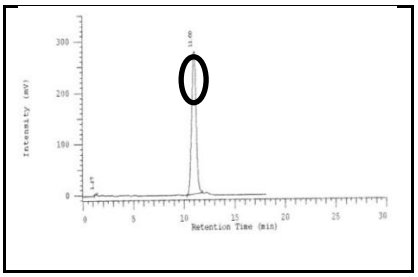

Figure 1. The chromatogram of retention time that appears on the standard.

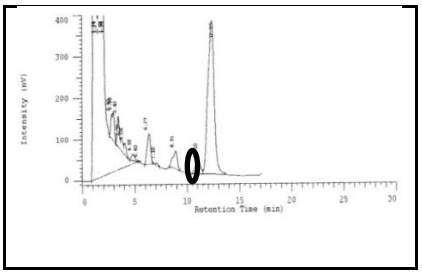

Figure 2. The chromatogram of retention time that appears on the IPB-B sample.

From the result of measurement, it was known that those five isolates tested were able to produce various levels of monakolin K, ranging from 1.4264 to 15.0517 ppm. The highest monakolin K levels were produced by IPB-A isolates with the levels of 15.0517 ppm, while the lowest was produced by LIPI F147 isolates with levels of 1.4264 ppm. The Monakolin K levels obtained were presented in Figure 3.

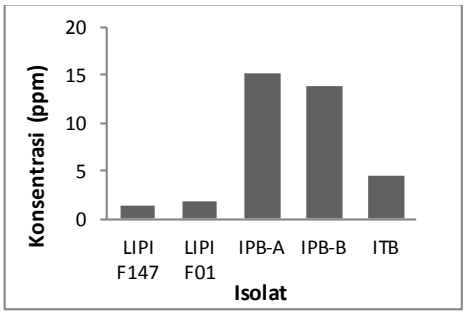

Figure 3. Monakolin K Levels of 5 *M. purpureus* Isolates

IV. DISCUSSION

The determination of monakolin k levels produced by *Monascus purpureus (M.purpureus)*, fermented through solid fermentation system using IR 64 rice as the substrate, was begun by staged breeding of each *M.purpureus* isolate on slanted PDA agar (Potato Dextrose Agar) which was incubated for 14 days.

And then the *Monascus purpureus* inoculum or suspension was created. It was made to increase the grow rate. Afterward the fermentation was begun. The mold was produced through solid fermentation system, wherein the growth of red dotted colonies started to appear on a portion of rice on the fourth day, in which the red dotted colonies of these 5 isolates increasingly and evenly spread out through the entire rice. The pigmentation continuously increased along with the length of fermentation time.

The samples of rice that had been overgrown by each *Monascus purpureus* isolate was then extracted by 80% methanol solvent, then homogeneously was shaken by a rotary shaker and the resulted supernatant was centrifuged to be injected in HPLC for having Monakolin K levels analysed further.

The monakolin K content in red yeast rice can be influenced by the intensified color production during fermentation. The fermented rice of IPB-A and IPB -B isolates was dark red, whereas the LIPI F147 and LIPI F01 isolates were oranged yellow and IPB isolates was red. Above all, the other factors that also affect the monakolin K synthesis was the temperature, humidity and pH. The cultivation temperature was one of the factors that affect the growth, synthesis and secretion of monakolin K.

V. CONCLUSION

The results of monakolin K determination that had been carried out as to five *Monascus purpureus (M. purpureus)* isolates, using IR 64 rice as the substrate which was analyzed by HPLC (High Performance Liquid Chromatography), showed various levels among those isolates. The highest monakolin K levels was produced by IPB-A isolates with the levels of 15.0517 ppm, while the lowest was produced by LIPI F147 isolates with levels of 1.4264 ppm.

ACKNOWLEDGMENT

This research was supported by Directorate General of Higher Education, Ministry of National Education, Indonesia for financial supporting of this research.

REFERENCES

[1] Kasim, E dkk. Kandungan Pigmen dan Lovastatin pada Angkak Beras Merah Kultivar Bah Butong dan BP 1804 IF 9 yang Difermentasi dengan *Monascus purpureus* Jmba. *Bioverditas* 7(1): 7-9.J. Clerk Maxwell, A Treatise on Electricity and Magnetism, 2006, 3rd ed., vol. 2. Oxford: Clarendon, 1892, pp.68–73.

[2] Yuliana, A., Singgih, M., Julianti, E. dan Blanc, P. J. Derivates of azaphilone monascus pigments, Biocatalysis and Agricultural Biotechnology, 2017. 9, 183-194.K. Elissa, "Title of paper if known," unpublished.

[3] Triana, E., Yulinery, T., dan Nurhidayat, N. Analisis Citrinin, Lovastatin dan Pigmen pada Angkak Hasil Fermentasi Beras IR 42 dengan *Monascus purpureus* Hasil Mutagenesis Etidium Bromida. 2014. 4(1): 459-466

[4] Marlia Singgih, Benny Permana, Selvira, Anna Yuliana¨ Studi In Silico Metabolit Sekunder Kapang Monascus sp. Sebagai Kandidat Obat Antikolesterol dan Antikanker. ALCHEMY Jurnal Penelitian Kimia, 2019. Vol. 15(1), 104-123.

[5] Dewi, M.S. Kajian Aktivitas Antioksidan dan Kadar Antikolesterol pada Angkak dengan Variasi Varietas Beras unggulan (IR 64) dan Beras Lokalc (Rojo Lele) dan Merah Putih [Skripsi]. 2010. Surakarta : Prodi Teknologi Hasil Pertanian Universitas Sebelas Maret.

[6] Asadayanti, D.D dkk. Peningkatan Kadar Lovastatin Angkak oleh *Monascus purpureus*. *Berita Biologi*, 2010. 10(3): 313-321

[7] Danuri, H. Optimizing Angkak Pigments and Lovastatin Production By *Monascus purpureus*. *Jurnal of Biosciences*, 2008. 15(2): 61-66

[8] Yuliana, A, Singgih M, Julianti E. Solubility and Toxicity Level of *Monascus pigments*. Trends in Technical & Scientific Research. 2018. Volume 2(4), 001-003

[9] Marlia Singgih, Elin Julianti, Anna Yuliana. Pembentukan zat warna Monascus purpureus Hasil Fermentasi Padat Dengan Limbah Ampas Kelapa Sebagai Substrat dan Uji Aktivitasnya Terhadap Escherichia coli dan Candida albicans. Jurnal Farmasi Galenika. 2018. Volume 5 No. 2. 41-55