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IMPROVEMENT OF ORGANIC RED PIGMENT PRODCUTION BY *MONASCUS PURPUREUS* TISTR3651 USING PATHUMTHANI-1 RICE-BASED MEDIUM IN SUBMERGED AND SOLID-STATE FERMENTATION

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ABSTRACT

Objective: This research is to study the production of natural red pigment by Monascus purpureus TISTR3615 in the submerged and solid-state fermentation system using Pathumthani-1 rice as a carbon source.

Methods: The antioxidant activity of the red pigment was evaluated in vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS radical scavenging assay, and ferric-reducing antioxidant power (FRAP) assay, including total phenolic compound.

Results and Discussion: The maximum of red pigment production was 0.55±0.02/ml (OD 680 nm) after incubation at 30°C for 24 days. The antioxidant activity based on inhibition DPPH (%), ABTS radical scavenging activity (%), and FRAP activity (mM Fe2+/g) was 97.80±1.51, 68.64±0.46, and 0.32±0.021, respectively. The total phenolic content was 164.78±2.82 µg GAE/mg.

Conclusion: It was estimated that Monascus pigments, leading to nutraceutical and pharmaceutical applications, cosmetic industry, and food industry.

Keywords: Natural pigment, Monascus purpureus, Submerged fermentation, Solid-state fermentation, Agricultural product

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INTRODUCTION

Pigments production from micro-organisms was used as a probable alternative to synthetic pigments such as synthetic food colors, some of which have a risk to consumer health and may be carcinogenic substance to humans [1]. The previous studies have been reported the natural pigments extracted from plants, animals such as annatto, grapes, beet, paprika, female insects (Coccus cacti), and micro-organisms such as *Monascus*, *Rhodotorula*, *Bacillus*, *Achromobacter*, *Phaffia*, and *Streptomyces* [2,3]. This study is a growing interest in microbial pigments due to their natural compounds providing the potential for safe uses to human health, including medicinal properties such as anti-bacterial and anti-cancer activities, nutritional effects controllable, and predictable yield [1].

Red mold rice (RMR) or *Monascus purpureus* is one of the richest producers of various pigments which reported to have a complex mixture of six chemically colored compounds of polyketide source [4]. These compounds contain a many sources of yellow-red pigment such as rubropunctatine, monascorubrine (orange), rubropunctamine, monascorubramine (red), monascine, and ankaflavine (yellow) [1,5]. In addition, *M. purpureus* are used in the production of pigment using rice, corn or cassava, oat, wheat, barley, tofu, and meat as a substrate [1,6]. Interestingly, Fabre *et al.* [7] reported that pigments from *Monascus* have potent therapeutic treatment. Lin *et al.* [8] found that red pigments indicated anti-bacterial property against *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli,* and *Pseudomonas aeriginosa*. Moreover, monacolin K is an important bioactive compound from *Monascus*, which is responsible to the potent cholesterol-lowering, anti-atherosclerotic drug lovastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor [9,10].

The cheap agricultural products and biomass residues, which are abundant in agricultural countries in the world, including Thailand, are an important sources for both energy utilization and novel product improvement. They will become the key to reducing the sustainable agricultural production system in the future. Hence, the objective of this study was focused the production of natural red pigment by *M. purpureus* TISTR3615 in the submerged and solid-state fermentation system using Pathumthani-1 rice as a carbon source. The antioxidant activity of the red pigment was evaluated *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay, including total phenolic compound.

METHODS

Micro-organism and medium cultivation

M. purpureus TISTR3615 strain was obtained from the Thailand Institute of Scientific and Technological Research. The eight culture strains were maintained on a potato dextrose agar plate at 30°C for 10 days and followed by storage at 4°C.

RMR preparation by solid-state fermentation

The Pathumthani-1 rice was obtained from Pathum Thani Rice Research Center, Pathum Thani, Thailand, used as a raw material for RMR production. Fifty grams of rice were soaked in 50 mL of distilled water. Then, the soaked rice was autoclaved for 20 min at 121°C in polyethylene bag with put-on a bottleneck and cotton. After it was cooled, five pieces of each *M. purpureus* TISTR3615 were added with dimensions of 0.6 cm^2 by Cork Borer and cultivated at 30°C for 20 days. After the end of the cultivation, the RMR was dried at 50°C for 2 days and crushed into powder.

Production of red pigment by *M. purpureus* TISTR3615 under submerged fermentation

M. purpureus TISTR3615 was cultivated into a 250 mL Erlenmeyer flask containing 100 mL of Yeast Malt Broth medium (YM Broth). These cultured samples were incubated at 30°C for 24 days. After that, the production of pigment in the cultured medium was filtrated with Whatman filter paper and measured using a UV spectrophotometer at a wavelength of 680 nm. In this experiment, YM broth was used as a blank for the detection of pigment in cultured broth medium.

Extraction of red pigment of *M. purpureus* TISTR3615

The 5 gm of fermented product were taken for pigment extraction as describe by Rajasekaran and Kalaivani *et al.* [10] and then filtrated through Whatman filter paper number 1. The filtrated was vaporized

by rotary evaporator at 45 °C and kept in dark bottle at 4°C until further study.

Antioxidant activity by 1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity

Antioxidant activity of all RMR samples was evaluated using the DPPH assay. First, 20 µL of RMR powder diluted appropriately in DMSO at the concentration of 0, 20, 40, 60, 80, 100, 200, and 400 µg/mL were mixed with 180 µL of DPPH in methanol (40 mg//mL) in wells of 96-well plate. Next, the plate was kept in a dark room for 30 min at room temperature. After, the absorbance of the sample was measured at 517 nm using a spectrophotometer microplate reader. Free-radical scavenging activity of RMR was determined as the percentage of reduced DPPH and was calculated following the equation (1)

$$\text{Inhibition of DPPH (\%)} = [(Ac-As)/Ac]\times 100 \quad (1)$$

When Ac is absorbance of control (20 µL of DMSO instead of sample) and as is absorbance of the sample. For this DPPH assay, ascorbic acid and DMSO were used as a positive and negative control, respectively [11].

Total phenolic content assay

The total phenolic content of each RMR powder sample was determined by reaction with Folin–ciocalteu reagent (F-C reagent). Ten microliters of RMR powder diluted in DMSO were mixed with 100 µL F-C reagent freshly diluted a ratio of F-C reagent per distilled water as 1:10 v/v. After 5 min, the solution was mixed with 100 µL of 7.5% Na_2CO_3 solution and incubated in the dark at room temperature for 60 min. After incubation, the absorbance of the solution was detected by a microplate reader at 765 nm. The TPC measurements were presented as a microgram of Gallic acid equivalents per milligram of RMR sample (µg GAE/mg) according to a Gallic acid standard curve [12].

ABTS radical scavenging assay

First, the ABTS stock solution was prepared by mixing an equal concentration of 7 mM, which contains an ABTS with 2.45 mM of potassium persulfate aqueous solutions and incubating the same for 12–16 h under dark condition. Next, the ABTS stock solution was diluted with methanol to obtain an absorbance value of 0.706±0.02 at 734 nm. After the addition of 20 µL of RMR extract sample to 180 µL of diluted ABTS solution, the absorbance was measured at 30 min after the initial mixing. Percent inhibition of absorbance was calculated using the formula,

$$\text{ABTS radical scavenging (\%)} = ((AB-AA)/AB)\times 100 \quad (2)$$

Where AB is absorbance of ABTS radical + methanol and AA is absorbance of ABTS radical + sample extract/standard. This experiment uses ascorbic acid as the standard substrate [13].

FRAP assay

This assay was perform by mixing 20 µL of RMR extract sample (10 mg/mL), and Ferrous sulfate ($FeSO_4$) (0–1.2 mM) as a standard, with 180 µL of FRAP reagent. The mixture was incubated for 6 min at 37°C, and the absorbance was measured at 595 nm. The FRAP reagent was made by mixing the following three different solutions; 10 mM of 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ), 300 mM of acetate buffer (pH 3.6), and 20 mM of FeCl3 at 1:10:1 ratio, which is mixed before use in the assay. FRAP activity was calculated as ferrous equivalents [14].

Chemical analysis using GC-MS analysis

The chemical compounds were determined by the method of Valgas *et al*. [15]. Crude extracts composition analysis was performed on an Agilent 7890 GC system instrument equipped with HP-5MS (5% diphenyl and 95% dimethylpolysiloxane) column (30 m × 0.25 mm, 0.25 µm) and interfaced to a 5975C inert XL MSD with Triple-Axis Detector. An injection volume of 2 µL was conducted (split ration of 10:1) injector temperature 250°C. The column temperature was increased from 60° to 250°C in a rate 5°C/min. The outlet temperature was 280°C. Mass spectra were taken at 70 Da. Ms transfer line temperature 250°C. The components of the extract were identified by comparison of fragmentation patterns in mass spectra with those stored on the spectrometer database and expressed in the literature. The relative percentage of individual components was calculated from the GC peak areas.

RESULTS AND DISCUSSION

Production red pigment of *M. purpureus* TISTR3615 by submerged fermentation

The fungal strain, *M. purpureus* TISTR3615, was cultivated on PDB for red pigment production. After incubation times, maximum values of all fungal growth and pigment production was increased by increasing the incubation periods to 8 h, *M. purpureus* TISTR3615 showed the red pigment (OD 0.55±0.02/ml) (Fig. 1). Results indicated that the level of glucose found on the medium promoted the mycelial growth and that was an important key for pigment production.

Production red pigment of *M. purpureus* TISTR3615 by solid-state fermentation

The agricultural products are wildly used as an alternative to apply in biological processes for high value-added products, including rice, soybean, and corn meal which were selected to reduce their waste management [15]. *M. purpureus* TISTR3615 strains can produce red pigment. Results showed that the fungal strains displayed more red color when compared with other fungal strain on solid-state fermentation which used Pathum Thani-1 rice as solid-substrate after incubation at 30°C for 10 days (Fig. 2). This result agreed with previous data of Chairote *et al*. [16] who used the Thai glutinous rice as solid-state fermentation for red pigment production. Moreover, the red pigment production of *M. purpureus* TISTR3615 depended on carbon sauces, nitrogen sources, trace element concentration, vitamin, and environmental cultivation to increasing the mycelial growth and pigment production, including oxygen and surface for attachment of fungal hyphae [17].

Antioxidant activity of *M. purpureus* TISTR3615 under solid-state fermentation using Pathumthani-1 rice as a carbon source

The antioxidant activity of red mold pigment from *M. purpureus* TISTR3615 using Pathumthani rice1 as solid substrate fermentation was analyzed by different *in vitro* assay and represented in Table 1. The inhibition of DPPH-scavenging activities, ABTS activity, and FRAP value of red mold pigment of *M. purpureus* TISTR3615 was 97.80±1.51%, 68.64±0.46%, and 0.32±0.021 $mMFe^{2+}/g$, respectively. The antioxidant activity levels of red mold pigment in this study are comparable to those extracts of *Monascus*–fermented with rice and brown rice [18], *M. purpureus*–fermented rice, corn, and sorghum [19], *Monascus*-durian seed [20] *M. purpureus*-purple rice [21].

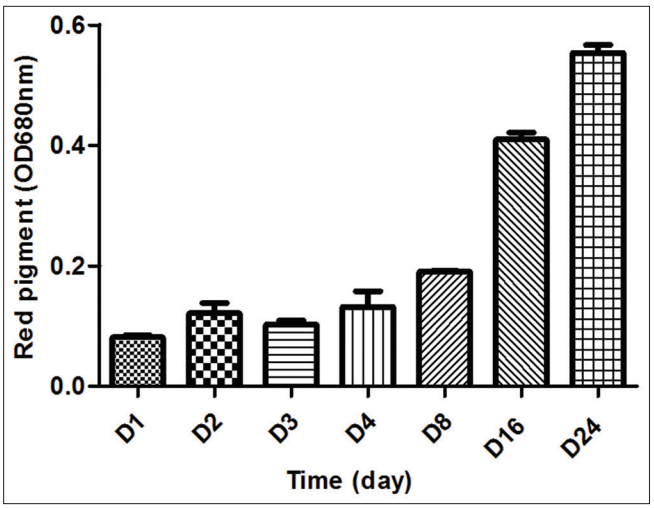

Fig. 1: The production of red pigment of *Monascus purpureus* TISTR3615 under submerged fermentation was observed for 24 days after fermentation

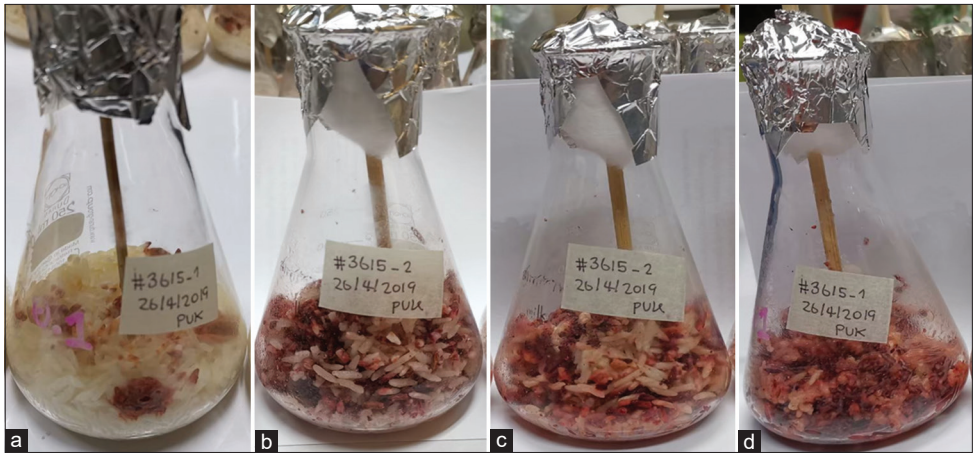

Fig. 2: The production of red pigment of *Monascus purpureus* TISTR3515 under solid-state fermentation; (a) 3 days, (b) 5 days, (c) 7 days and (d) 10 days

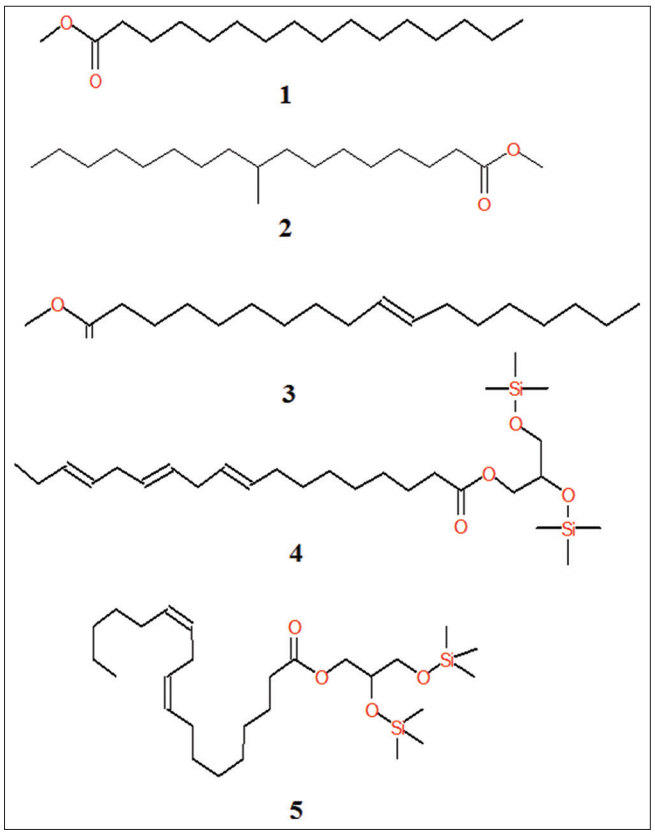

Fig. 3: The chemical structure of red pigment of *Monascus purpureus* TISTR3515 under solid-state fermentation using Pathum thani rice1 as carbon source. (1) Hexadecanoic acid, methyl ester, (2) hexadecanoic acid, methyl ester, (3) 10-Octadecenoic acid, methyl ester, (4) 9,12,15-Octadecatrienoic acid,2,3-bis [(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)-, (5) 1-Monolinoleoylglycerol trimethylsilyl ether

Other studies also reported that Monascus fermented rice, soybean, and adlay possess DPPH radical scavenging activity [22].

GC-MS analysis of crude extracts of red pigment

The crude extracts of red pigment of *M. purpureus* TISTR3615 under solid-state fermentation were determined by GC-MS. The active principles with their retention time (RT), molecular formula, molecular weight, concentration (peak area %), and the chemical structure were analyzed. Table 2 shows that the components present in the ethyl acetate extract as identified by GC-MS and Fig. 3 shows the chemical structure of red pigments. These compounds, 9,12,15-Octadecatrienoic acid,2,3-bis [(trimethylsilyl) oxy]propyl ester, (Z,Z,Z), were the first report that produced by *M. purpureus* and played important role for biologically activity such as anti-oxidant, anti-diabetic, and anti-inflammatory [23], whereas 1-Monolinoleoylglycerol trimethylsilyl ether are being used for antimicrobial, anti-oxidant, anti-inflammatory, anti-arthritic, antiasthma, and diuretic [24].

Table 1: Antioxidant activity of red pigment of *Monascus purpureus* TISTR3615 under solid-state fermentation using Pathumthani rice1 as a carbon source

Sample	Antioxidant activity			
	DPPH[b]	ABTS[c]	FRAP[d]	TPC[e]
SSF[a]	97.80±1.51	68.64±0.46	0.32±0.021	164.78±2.82
Vit. C[f]	93.54±2.16	96.70±0.01	1.14±0.00	-

[a]Solid-state fermentation, [b]% Inhibition [c]% Scavenging activity, [d]mM Fe II/g of sample, [e]µg of GAE/g of sample, [f]Vitamin C

Table 2: The chemical analysis of red pigment from *Monascus purpureus* TISTR3615

RT[a]	Molecular weight[b]	Compounds	Formula
13.437	270	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
16.097	298	Heptadecanoic acid, 9-methyl-, methyl ester	$C_{19}H_{38}O_2$
15.764	296	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
22.143	496	9,12,15-Octadecatrienoic acid, 2,3 bis [(trimethylsilyl) oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$
26.355	498	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$

[a]Retention time, [b]Molecular weight

CONCLUSION

From the above study, the results revealed that the crude extracts of red pigment of *M. purpureus* TISTR3615 under solid-state fermentation (Pathumthani1 rice) were potential of antioxidant activity and it

possible apply to use as a natural coloring agent and natural for food additive as well as cosmetic application in the future.

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DECLARATION OF INTEREST

This manuscript has not been published and is not under consideration for publication elsewhere. We have no conflicts of interest to disclose.

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