

Antibacterial Activity of Red yeast rice Extract against *Propionibacterium acnes* ATCC 11827 and Methicillin-Resistant *Staphylococcus aureus* ATCC BAA-1683

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Abstract

Propionibacterium acnes (*P. acnes*) ATCC 11827 and Methicillin-Resistant *Staphylococcus aureus* ATCC BAA-1683 are bacteria that cause skin infections, namely acne vulgaris and skin and soft tissue infection/SSTI. The increase in the number of resistant bacterial strains, such as MRSA, requires the search for alternative antibiotics, including using natural ingredients. Red yeast rice is a product of rice fermentation by *Monascus purpureus*, which is known to have antibacterial, antifungal, antitumor, antioxidant, anti-inflammatory, anti-cholesterol and immunomodulator. This study aimed to determine the antibacterial activity of several red yeast rice extracts against these bacteria that cause skin infections. The research was carried out through the stages of collecting materials and identifying the yeast isolates, extracting of red yeast rice, phytochemical screening of red yeast rice extract, confirmation of bacterial test, preparation of bacterial test suspension, testing for bacterial resistance, testing for antibacterial activity of red yeast rice extract and determining the value of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the most active extract. The results showed that the ethyl acetate and ethanol extracts had antibacterial activity against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683. Both extracts contain compounds from the polyphenols, flavonoids, quinones and saponins group. Ethyl acetate extract was the most active extract with MIC values against *P. acnes* ATCC 1182 and MRSA ATCC BAA-1683 of 50 mg/mL and 12.5 mg/mL. The MBC values of ethyl acetate extract against these bacterial tests were 100 mg/mL and 25 mg/mL. The ethyl acetate extract is more active against MRSA ATCC BAA-1683 than against *P. acnes* ATCC 11827. From the results of this study it is known that red yeast rice has activity against bacteria that cause skin infections, especially against MRSA

Keywords : *Monascus purpureus*, acne vulgaris, skin and soft tissue infection, Minimum Inhibition Concentration, Minimum Bactericidal Concentration

Introduction

Acne or acne vulgaris is a skin disease that affects many Indonesians, especially teenagers. One of the bacteria that causes acne is *Propionibacterium acnes* (*P. acnes*), which is normal flora in the pilosebaceous glands, which under certain conditions can turn into opportunistic pathogens.¹ In mild cases, acne can be treated using benzoyl peroxide or retinoids, while in moderate cases, a combination topical form of benzoyl peroxide or retinoids can be used with topical antibiotics. In severe cases, acne treatment needs to be added with oral antibiotics in the form of tetracycline, azithromycin and erythromycin.²

Other skin infections that often occur in the community are skin and soft tissue infections (SSTI), such as impetigo, abscesses and cellulitis. SSTI is caused by the bacteria *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and *Enterococcus species*. MRSA is a strain of *S. aureus* that is resistant to beta-lactam antibiotics, including methicillin, as well as other antibiotics such as oxacillin, penicillin and amoxicillin.² If the cause of SSTI is MRSA, then the antibiotic of choice can be clindamycin, doxycycline, ceftaroline or vancomycin.³

Currently, around 46% of antibiotics are used inappropriately and with incorrect indications.⁴ This condition triggers an increase in the number of bacterial strains resistant to antibiotics, so that it requires the search for alternative antibiotics, including those from natural ingredients. One of the natural ingredients that has potential as an antibiotic is red yeast rice.⁵

Red yeast rice is a product of rice fermentation by *Monascus*, especially *Monascus purpureus* (*M. purpureus*). In Asian countries, red

yeast rice is used traditionally as a colorant, flavoring and natural food preservative as well as medicine. This fermented product of *M. purpureus* is known to contain various secondary metabolites, such as flavonoids, lovastatin (monakolin K), citrinin and various pigments from the polyketide group. These compounds are known to have activity as antibacterial, antifungal, anti-tumor, antioxidant, anti-inflammatory, anti-cholesterol and immunomodulatory.⁶

Various pigments of red yeast rice are known to have activity against Gram-positive bacteria such as *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus mycoides* and *Staphylococcus aureus*, also against Gram-negative bacteria, such as *Escherichia coli*. This pigment also has antifungal activity against *Aspergillus*, *Mucor*, *Penicillium citrinum* and *Fusarium*. Until now, there is no information about the antibacterial activity of red yeast rice against *P. acnes* and MRSA.^{5,7,8}

In this study, the antibacterial activity of various extracts of red yeast rice were tested against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683. This study aimed to determine the potential of red yeast rice as a natural antibiotic to treat the infection of the two bacteria.

Methods

Tools

The tools used include analytical balance (Mettler Toledo AL204), maserator, rotary evaporator (Buchi Rotavapor R-300), autoclave (Hirayama, Japan), oven (Memmert 400-800), incubator (Sakura IF-4), microscope (Olympus CK 40), 96 wells-microplate (Iwaki, Japan), 10-100 µL volume micropipette (Biohit Proline), 100-1000 µL volume micropipette (Socorex Acura 825), blue and yellow micropipette tips, calipers, perforators and tools commonly used

in the Natural Material Pharmacy Laboratory and the Pharmaceutical Microbiology Laboratory.

Materials

Materials used include red yeast rice (PT. Global Buana Mandiri, Jakarta), n-hexane (Bratachem), ethyl acetate (Bratachem), 96% ethanol (Bratachem), sodium chloride (Merck), chloroform (Merck), ammonia (Merck), amyl alcohol (Merck), ether (Merck), hydrochloric acid (Merck), sulfuric acid (Merck), Mayer reagent (Bratachem), Dragendorff reagent (Bratachem), Liebermann Burchard reagent (Bratachem), magnesium powder (Bratachem), potassium hydroxide (Merck), Mc Farland standard solution 0.5, iron (III) chloride (Merck), gelatin (Bratachem), vanillin (Bratachem), dimethyl sulfoxide / DMSO (Merck), vaseline (Bratachem), oxacillin antibiotic disc (Oxoid), α -naphthanol (Merck), crystal violet (Merck), lugol (Bratachem), safranin (Merck), cellophane plastics, filter paper (Whatman) and distilled water.

The test bacteria and comparison fungi used were *P. acnes* ATCC 11827, MRSA ATCC BAA-1683 and *M. purpureus* ITBCCL61 from the Laboratory of Microbiology and Bioprocess Technology, Chemical Engineering Study Program, Faculty of Industrial Technology, Institut Teknologi Bandung. The bacteria and fungi medium used were Mueller Hinton Agar/MHA (Oxoid), Mueller Hinton Broth/MHB (Oxoid), Sabouroud Dextrose Agar/SDA (Oxoid), Triple Sugar Iron Agar/TSIA (Oxoid), Sulfide Indole Motility/SIM (Difco), Methyl Red-Voges Proskauer/MR-VP (Difco), Simmon's Citrate Agar/SCA (Oxoid), glucose (Oxoid), sucrose (Oxoid), lactose (Oxoid), mannose (Oxoid), maltose (Oxoid) and peptone (Oxoid).

Material Collection and Identification of Mold Isolates

Molds were isolated from red yeast rice and identified macroscopically and microscopically by comparison with *M. purpureus* ITBCCL61.⁹ As much as 1 g red yeast rice finely crushed, then suspended 5 mL of sterile distilled water. Red yeast rice suspension and *M. purpureus* ITBCCL61 culture each was inoculated onto the SDA surface in a petri dish, then incubated at room temperature for 5-7 days. Macroscopic observations were made on the shape and color of the colonies of the two molds. Microscopic observations were made of the molds grown using the moist chamber method. The forms of hyphae, conidia and ascospores of both fungi were observed and compared under a microscope.

Extraction of Red Yeast Rice

Red yeast rice was extracted by multilevel maceration method using three solvents with different polarity, namely n-hexane (non polar), ethyl acetate (semi polar) and ethanol 96% (polar).¹⁰ A total of 2 kg of red yeast rice was macerated with 2 L of n-hexane for 24 hours at room temperature. The liquid extract was filtered, then the maceration process was repeated several times until a clear filtrate was obtained. The extract residue was dried, then macerated in the same way using ethyl acetate and 96% ethanol. Each extract was thickened using a rotary evaporator at a speed of 60 rpm at 50 °C until the weight was constant. The three extracts were observed organoleptically, then the yield was determined.

Phytochemical Screening of Red Yeast Rice Extract

Phytochemical screening of red yeast rice extract was carried out using the Fransworth method (1966).¹¹ Screening was carried out on alkaloid, tannin, polyphenol, flavonoid, steroid, triterpenes, monoterpenes,

sesquiterpenes, quinone and saponin class compounds.

Confirmation of Test Bacteria

The test bacteria in the form of *P. acnes* ATCC 11827 and MRSA ATCC BAA-16 were confirmed through microscopic observation using Gram stain and through biochemical tests.¹² The biochemical tests that were carried out included the carbohydrate fermentation test, the MR-VP test, the citrate test, the motility test and the urea test.

Preparation of Test Bacteria Suspension

P. acnes ATCC 11827 and MRSA ATCC BAA-1683 were inoculated onto the MHA surface, then incubated at 37 °C for 18-24 hours. Both bacterial cultures were suspended in a 0.9% (w/v) sodium chloride solution. The turbidity of the suspension was adjusted to the turbidity of the McFarland standard solution of 0.5 which was equivalent to 1.5×10^8 CFU/mL.

Bacteria Resistance Testing

The MRSA ATCC BAA-16 resistance test to oxacillin was carried out using the agar diffusion method with the paper disc technique.¹⁴ The bacterial suspension was inoculated on the surface of the MHA in a petri dish, then a 1 µg oxacillin antibiotic disc was placed on the agar surface. The plates were incubated at 37 °C for 18-24 hours, then the diameter of the inhibition was measured using a caliper.

Testing Antibacterial Activity of Red Yeast Rice Extract

Red yeast rice extract was tested for its antibacterial activity against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683 using the agar diffusion method with the perforation technique.¹³ The three extracts were diluted using 2% DMSO (v/v), resulting in several variations in extract concentration. A total

of 20 µL of each tested bacterial suspension was put into a sterile petri dish, then 20 mL of liquid MHA was added. The mixture was shaken slowly until homogeneous, then allowed to solidify.

The medium was perforated using a perforator with a diameter of 8 mm, then filled with 50 µL of various extract concentrations. As a comparison, a petri dish containing MHA (negative control), MHA inoculated with the test bacteria (positive control) was prepared, and MHA inoculated with 2% DMSO (v/v) (solvent control). All petri dishes were incubated at 37 °C for 18-24 hours. The antibacterial activity of the extract in the form of the inhibition area around the hole was measured using a caliper. Measurement results were compared between extracts to determine the most active extract.

Determination of Minimum Growth Inhibitory Concentration (MIC) and Minimum Killing Concentration (KBM) of Red Yeast Rice Extract

The most active red yeast rice extract is determined by the Minimum Growth Inhibitory Concentration (MIC) and the Minimum Killing Concentration (KBM) using the microdilution method.¹⁵ The extract was dissolved in 2% DMSO (v/v) as a stock extract solution. The concentration of the tested bacteria was adjusted, so that each well contained 3.75×10^5 CFU/mL.

Column 1 to column 12 of microplates each inserted 100 µL MHB. Column 3 and 4 were added by 100 µL of Red yeast rice extract, then homogenized. The stratified dilution was carried out by pipetting 100 µL from column 4 to column 5, then the mixture was homogenized. The next dilution was carried out in the same manner until the column 12, then the 100 µL from the last dilution was discarded. To the column 2, 4 until column



Figure 1. Macroscopic observation results of (A) mold isolated from red yeast rice (B) Culture of *M. purpureus* ITBCCL61

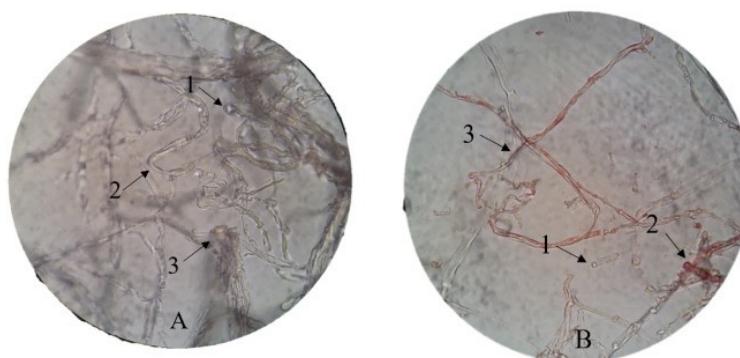


Figure 2. Microscopic observation results of (A) molds isolated from red yeast rice (B) culture of *Monascus purpureus* ITBCCL61 (1) conidia, (2) ascospores, (3) hyphae

12, 10 μ L of the tested bacterial suspension were added. Column 1 functions as negative control, column 2 functions as positive control, while column 3 functions as extract control. The microplates were covered using cellophane plastic, then incubated at 37 °C for 18-24 hours. MIC value was determined from the column with the smallest extract concentration that appeared clear (no bacterial growth).

Determination of the KBM value of the extract is carried out by scraping the solution from the last few columns that appeared clear on 5 mL MHA in a petri dish. Petri dishes were incubated at 37 °C for 18-24 hours. The MBC value was determined from the extract concentration which did not show the growth of bacterial colonies.

Results and Discussion

Material Collection and Identification of Mold Isolates

Macroscopic observations of mold isolates and *M. purpureus* ITBCCL61 showed colonies with white hyphae, which a change in color to orange and then to red, with increasing incubation time (Figure 1). The same colony shape and color is also shown by *M. purpureus* isolated by Manan, et al. (2017). The growth medium also turned red, due to the presence of *M. purpureus* pigments which was diffused into the medium.⁹

The microscopic observations of the two mold cultures show the same characteristics of hyphae, conidia, and ascospores (Figure 2). Both have somatic hyphae, which were composed of branched mycelium, clear to

Table 1. Results of Phytochemical Screening of Red Yeast Rice Extract

Compound Group	n-hexane Extract	Ethyl Acetate Extract	Ethanol Extract
Alkaloids	-	-	-
Tannins	-	-	-
Polyphenols	-	+	+
Flavonoids	+	+	+
Steroids and Triterpenes	-	-	-
Monoterpenes and Sesquiterpenes	-	-	-
Quinone	+	+	+
Saponins	-	+	+

Information: (+) = positive
(-) = negative

brown and insulated (septate). Conidia are globular and clear in color, while ascopora are round or slightly oval in shape. The same observation results are also shown by *M. purpureus* that was isolated by Manan, et al., 2017.⁹ Based on these observations, it is found that the mold isolate was *M. purpureus*.

Red Yeast Rice Extraction

The results of organoleptic observations of red yeast rice extract showed that the three extracts were in the form of a thick liquid, distinctive smell, with n-hexane extract with a slightly orange color, ethyl acetate extract with a red color, and ethanol extract in red. Of the 2 kg of red yeast rice, 53.36 g of n-hexane extract (2.67% w/w), 84.22 g of ethyl acetate extract (4.21% w/w), and 192.09 g (9.60% w/w) of ethanol extract were obtained. The extract with the highest yield was ethanol extract, followed by ethyl acetate and n-hexane extracts. The same results were obtained by Ismail, et al. (2016) which produced a yield of 9.44% w/w of the ethanol extract of red yeast rice.¹⁶

Phytochemical Screening of Red Yeast Rice Extract

The results of phytochemical screening of

red yeast rice extract show that the n-hexane extract contained compounds from the flavonoid and quinone groups, while the ethyl acetate and ethanol extracts contained compounds from the polyphenol, flavonoid, quinone and saponin groups (Table 1). A research by Kongbuntad & Asenphet (2016) showed that the water extract of red yeast rice contained terpenoids, triterpenoids, flavonoids, phenols, plobatin, coumarin and saponin research, while the research of Ismail, et al (2016) showed that the ethanol extract of red yeast rice contained flavonoids, alkaloids and saponins. The difference in results is due to differences in the red yeast rice that are used as research objects.^{16,17}

Based on the results of phytochemical screening, it is suspected that the antibacterial activity of red yeast rice extract comes from polyphenol compounds, flavonoids, quinones and saponins. Polyphenol and flavonoid compounds are known to cause extracellular and intracellular proteins to form complexes with bacterial cell walls. The formation of this complex causes damage to the microsomes, lysosomes and bacterial cell walls. Flavonoids can also interfere with the intercalation

Table 2. Results of Biochemical Test of *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683

Biochemical Test	<i>P. acnes</i> ATCC 11827	MRSA ATCC BAA-1683
Glucose	-	+
Sucrose	+	+
Lactose	+	-
Manosa	-	+
Maltose	-	+
MR	-	-
VP	+	+
Citric	-	-
Motility	+	-
Urea	-	-

(formation of hydrogen bonds) between nucleotide bases, thereby inhibiting DNA and RNA synthesis. In addition, flavonoids can attack the dehydrogenase enzyme which is needed in energy metabolism, thereby inhibiting respiration and growth of bacterial cells.¹⁸

Quinones can form irreversible complex compounds with nucleophilic amino acid residues in transmembrane proteins, also with polypeptides in the cell wall and with various enzymes on the surface of the cell membrane, thereby disrupting the stability of bacterial cells.¹⁹ Saponins also reduce surface tension, thereby increasing cell wall permeability and resulting in the release of intracellular compounds.¹⁸

Confirmation of Bacteria Test

Microscopic observations of the results of Gram stain show that *P. acnes* ATCC 11827 is a Gram-positive rod-shaped bacterium, while MRSA ATCC BAA-1683 is a round Gram-positive bacterium with a grape-like arrangement. The results of biochemical tests

showed that *P. acnes* ATCC 11827 was a motile bacterium that ferments sucrose and lactose, with a positive VP result. MRSA ATCC BAA-1683 is a bacterium that ferments glucose, lactose, mannose and maltose, with a positive VP result (Table 3). The results of microscopic observations and biochemical tests are in accordance with the research of Shinkafi & Ndanusa (2013) for *P. acnes* and research by Domon, et al. (2016) for MRSA.^{20,21}

Resistance of Bacteria Test

The results of the resistance test showed that oxacillin produced an inhibitory diameter of 8.03 mm against MRSA ATCC BAA-1683. Based on the provisions of CSLI (2018), the diameter of the oxacillin inhibition of ≤ 10 mm indicates that bacteria were resistant to oxacillin, while the diameter of the inhibition ≥ 13 mm indicates that the bacteria were sensitive to oxacillin. These results indicated that MRSA ATCC BAA-1683 was resistant to oxacillin.¹⁴

Antibacterial Activity of Red Yeast Rice Extract

The extract concentrations used for the

Table 3. Test Result of Antibacterial Activity of Red Yeast Rice Extract against *P. acnes* ATCC 11827

Extract (mg/mL)	Inhibition Diameter (mm)		
	n-hexane	Ethyl Acetate	Ethanol
100	0	10.35	9.53
200	0	11.64	10.30
400	0	13.11	13.47
600	0	14.57	14.86

Information: perforator diameter = 8mm

Table 4. Test Result of Antibacterial Activity of Red Yeast Rice Extract against MRSA ATCC BAA-1683

Extract (mg/mL)	Inhibition Diameter (mm)		
	n-hexane	Ethyl Acetate	Ethanol
100	0	10.48	9.75
200	0	11.88	11.90
400	0	14.64	14.96
600	0	15.77	16.72

Information: perforator diameter = 8mm

antibacterial activity test were 100, 200, 400 and 600 mg/mL in DMSO 2% v/v. The results of the antibacterial activity test show that ethyl acetate and ethanol extracts have activity against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683, but n-hexane extract does not have activity against these two bacteria (Tables 3 and 4).

Ethyl acetate extract was the most active extract against the two tested bacteria. The increase in the inhibitory diameter of the extract is directly proportional to the concentration of the extract. This was also observed in the inhibition diameter of the red pigment in red yeast rice against *Bacillus subtilis*, *Bacillus megaterium*, and *Bacillus mycoides*. The inhibitory diameter of the ethyl acetate extract was smaller than the 10 mg/mL inhibitory diameter of the orange yellow pigment from red yeast rice against *E. coli* by 21.3 mm.^{5,8}

Determination of Minimum Growth Inhibitory Concentration (MIC) and Minimum Killing Concentration (KBM) of Red Yeast Rice Extract

MIC was only determined for the most active extract, namely ethyl acetate extract. The concentration of the extract stock solution used was 400 mg/mL. From the results of the determination, it was found that the MIC value of ethyl acetate extract against *P. acnes* ATCC 11827 was 50 mg/mL, while for MRSA ATCC BAA-1683 was 12.5 mg/mL (Table 5). The MIC value was greater than the MIC value of the orange yellow pigment from red yeast rice of 2.5 mg/mL. This was because in this study extracts were still used, not pigment isolates as in previous studies.⁸

The MBC value of ethyl acetate extract against *P. acnes* ATCC 11827 was 100 mg/mL, while for MRSA ATCC BAA-1683 was 25 mg / mL

Table 5. Results of Determination of the MIC Value of Red Yeast Rice Ethyl Acetate Extract against *P. acnes* ATCC 11827 dan MRSA ATCC BAA-1683

Extract (mg/mL)	<i>P. acnes</i> ATCC 11827	MRSA ATCC BAA-1683
200	-	-
100	-	-
50	-	-
25	+	-
12.5	+	-
6.25	+	+
3.125	+	+
1.562	+	+
0.781	+	+

Information: (-) = no bacterial growth; (+) = there was bacterial growth

(Table 6). From the results of MIC and MBC determination, it was found that ethyl acetate extract is more active against MRSA ATCC BAA-1683 than against *P. acnes* ATCC 1827.

Conclusion

Based on the results of this study, it can be concluded that the ethyl acetate extract and ethanol extract of red yeast rice have antibacterial activity against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683. Both extracts contain polyphenol compounds, flavonoids, quinones and saponins. Ethyl acetate extract is the most active extract with MIC values against *P. acnes* ATCC 1182 and MRSA ATCC BAA-1683 of 5 mg/mL and 12.5 mg/mL, respectively. The MBC values of ethyl acetate extract against *P. acnes* ATCC 11827 and MRSA ATCC BAA-1683 are 100 mg/mL and 25 mg/mL, respectively. The ethyl acetate extract of red yeast rice is more active against MRSA ATCC BAA-1683 than *P. acnes* ATCC 11827. Based on the results of this study, it is found that red yeast rice has the potential to be used as an alternative antibacterial for bacteria that causes skin infections, especially against MRSA.

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