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Growth Inhibitory Effects of Red and Yellow Passion Fruits against MRSA and ESBL-producing Bacteria

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Abstract

Background: *Red passion fruit (Passiflora edulis Sims) and yellow passion fruit (Passiflora edulis f. flavicarpa) are native Indonesian fruits with numerous health benefits. This study used de Man, Rogosa, and Sharpe (MRS) medium for fermentation. Probiotics are beneficial microorganisms obtained from fermented food or drink (Hamid et al., 2020). Probiotics and lactic acid bacteria, which are known to have various benefits, particularly as antibacterial, are among the active components identified in passion fruit pulp.* ***Objective:*** *This study examinedthe antibacterial activity of red and yellow passion fruits.* ***Methods:*** *Freshly collected passion fruit pulps were fermented in MRS medium in a shaker incubator for 48 hours at 24°C. The filtrates from the fermented broth were tested against clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase (ESBL)-producing bacteria. The analyses applied the streak plate method and the total plate count method.* ***Results:*** *The Minimum Inhibitory Concentrations (MICs) of red passion fruit ferment filtrate against MRSA 10 and MRSA 11 were 50% and 60%, respectively, and of yellow passion fruit ferment filtrate against MRSA 10 and MRSA 11 were both 30%. Meanwhile, the MICs of red passion fruit ferment filtrates were 35% against ESBL 41 and ESBL 43 and 25% against ESBL 45 and ESBL 47, whereas the MICs of yellow passion fruit (Passiflora edulis f. favicarpa) ferment filtrates were 25% against ESBL 41 and ESBL 43, and 12.5% against ESBL 45 and ESBL 47. Red passion fruit (Passiflora edulis Sims) and yellow passion fruit (Passiflora edulis f. flavicarpa) ferment filtrates became growth inhibitors against the clinical isolates of MRSA and ESBL-producing bacteria with an optimal fermentation time of 24 hours and an optimal concentration of 75%.* ***Conclusion:*** *The results of this study found that the fermented filtrates of red and yellow passion fruits in MRS media could be developed as an antibacterial against MRSA and ESBL-producing bacteria.*

Keywords: *Passiflora, Methicillin-resistant Staphylococcus aureus, and Extended-spectrum beta-lactamase.*

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INTRODUCTION

Indonesia is home to a plethora of plant species, many of which are fruit trees, including the *Passiflora edulis* plant, which produces passion fruit or granadilla. The *Passifloraceae* family consists of over 500 species in the genus *Passiflora*. Yellow and red passion fruits are common throughout Indonesia, particularly in West Java and West Sumatra. Unripe red passion fruit is red with white spots; the rind is rather thick and hard. Red passion fruit can bear quite a lot; the fruit is round to slightly oval in shape, and the juice has a sweet and sour taste with a guava-like aroma (Karsinah and Manshur, 2010). Meanwhile, unripe yellow passion fruit is light green, and the ripe one is golden yellow with white spots; the rind is tough and thick. Besides containing protein, fat, carbohydrates, fibre, vitamins, and minerals, passion fruit is also rich in flavonoids (Karsinah and Manshur, 2010; Shiamala et al., 2013). According to Cushnie and Lamb (2005), flavonoids can reduce the synthesis of nucleic acids, the function of cell membranes, and energy expenditure.

It is fascinating to study how the phenolic chemicals in yellow passion fruit, which have antibacterial and antioxidant properties, can be used as a source of medicine (Shiamala et al., 2014). A prior study by Joachim et al. (2016) also found that the triterpene components in the methanol extract of passion fruit can prevent the growth of several bacteria, including *E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas euginosa*, and *Providencia stuartii*. In addition, *Passiflora edulis* seed oil rich in unsaturated fatty acids has been shown to have antibacterial activity against *Escherichia coli, Salmonella enteritidis, Staphylococcus aureus,* and *Bacillus cereus* (Marlene et al., 2019). Furthermore, Marwah et al. (2019) revealed that yellow passion fruit ferment filtrate could inhibit the growth of *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), and extended-spectrum beta-lactamase (ESBL)-producing bacteria.

This study aims to determine the MIC and antibacterial activity of yellow passion fruit ferment filtrates and red passion fruit ferment filtrates against MRSA and ESBL-producing bacteria.

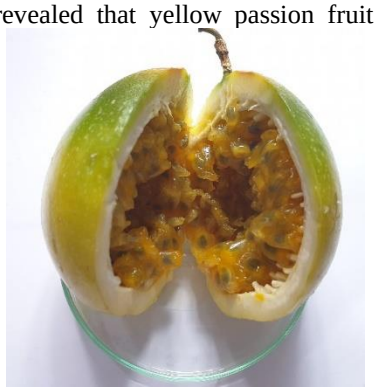

Figure 1 : The yellow passion fruit

MATERIALS AND METHODS

Materials

This study used distilled water, de Man, Rogosa, and Sharpe (MRS) media, MRSA 4, MRSA 10, MRSA 11, MRSA 23, ESBL 41, ESBL 43, ESBL 45, and ESBL 47 from Jombang Regional General Hospital (RSUD Jombang), red passion fruit ferment filtrate, yellow passion fruit ferment filtrate, Nutrient Agar (Merck), and NaCl p.a (Merck).

Tools

The tools used in this study were analytical balance (Sartorius-Werke GMBH type 2472), autoclave (Huxley HV-340 Speedy), ultrasonic vibrators (Julabo), vortex (Thermo), micrometer syringes (Hamilton), pH paper (Macherey-Nagel), pH meter (S1 Analytics), öse wire, 15 cm diameter petri dish, and 0.2 µm membrane filters (Minisart).

Plant source and determination

In August 2020, yellow and red passion fruits were collected from Krembung, Sidoarjo, East Java, Indonesia. A botanist from the Faculty of Science and Technology, Universitas Airlangga, verified the identification of the yellow passion fruit (Figure 1), and a botanist from Herbarium Malangensis, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang verified the identification of red passion fruit (Figure 2).

Figure 2 : The red passion fruit

Preparation of fermentation media

To make MRS broth, 52 grams of MRS broth powder was dissolved in 1 liter of distilled water and mixed well. After that, it was sterilized in an autoclave at 121°C for 15 minutes.

Preparation of growth media

Growth media were prepared by dissolving 18 g of nutrient agar (NA) powder (Oxoid) in 1 L of distilled water, then divided into 10 mL and autoclaved for 15 minutes at 121°C for sterilization.

Preparation of samples

Red and yellow passion fruits were cleaned and dried. Then, 10 g of the fruit pulps were weighed and added to 200 mL of MRS broth media. The samples were then fermented with a rotary shaker for 48 hours at 150 rpm and filtered with a 0.2 m filter membrane after

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48 hours of fermentation. Lastly, the passion fruit ferment filtrates were put into the vial.

Preparation of bacterial test

All bacteria were collected from RSUD Jombang; each of which was then inoculated and allowed to grow in nutrient agar medium for 24 hours at 37°Celsius. To produce a bacterial suspension for the OD measurement, 10 mL of sterile saline (0.9 percent NaCl) was added to the culture and then vortexed until all colonies were removed from the surface of the agar medium. A spectrophotometer was used to perform OD measurements at a wavelength of 580 nm.

Methods

Streak Plate Method

For the analysis using the Streak Plate method, 5 mL of yellow and red passion fruit filtrates with concentrations of 6.25%, 12.5%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, and 100% according to dilution in distilled water (v/v) were added to 5 mL of diluted nutrient agar and then vortexed until homogeneous. The samples were then divided into four equal portions in a sterile petri, each for a different type of bacteria. After the nutrient agar was solid, the next step was to streak the bacteria 1 cm wide with three replications for each type of bacteria. The temperature was maintained at 37°Celsius for 24 hours during the incubation period. After incubation, the progress of bacterial growth was checked. This experiment was repeated with 48-hour incubation.

Total Plate Count Method

Aside from the Streak Plate method, the Total Plate Count method was also applied in this study. For passion fruit ferment filtrate with a concentration of 100%, 9 mL of 0.9% sterile saline was taken and labelled 10^{-1} until 10^{-10}. As much as 1 mL of 100% ferment filtrate was then added to a 10^{-1} saline solution and vortexed until homogenous. The next step was to transfer it from 10^{-1} to 10^{-2} and vortex it until homogeneous. These steps were repeated until 10^{-10}. The result was then added to 10 mL diluted nutrient agar, vortexed until homogeneous, poured into a sterile petri dish and allowed to solidify. The same procedure was applied for ferment filtrate with a concentration of 75%. The temperature was maintained at 37°Celsius for 24 hours during the incubation period. After incubation, the progress of bacterial growth was checked.

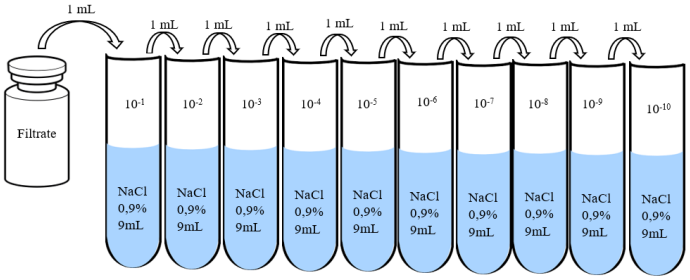

Figure 3: Schematic diagram of Total Plate Count

RESULTS AND DISCUSSION

Granadilla, which is another name for passion fruit (*Passiflora edulis*) or *markisa* in Indonesian, is a member of the *Passifloraceae* family which consists of over 500 species. This tropical fruit is rich of protein, fat, carbohydrates, fibre, vitamins, minerals, and flavonoids (Karsinah and Manshur, 2010; Ramaiya et al., 2013). Numerous investigations have proven that flavonoid molecules are antimicrobial, whose antibacterial activity can be divided into three categories: 1) inhibitor of nucleic acid production, 2) inhibitor of cell membrane function, and inhibitor of energy-producing metabolic processes (Cushnie and Lamb, 2005).

Botanical, chemical, and pharmacological reports on the genus *Passiflora* have been reviewed in previous studies. In addition, several in vitro and in vivo pharmacological studies have discovered the promising bioactivities in purple passion fruit (*P. edulis* Sims f. *edulis*), such as antioxidant, antimicrobial, anti-inflammatory, antihypertensive, hepatoprotective and lung-protective activity, anti-diabetic, sedative, and antidepressant activity, as well as anxiolytic activity. Most of the pharmacological effects of *P. edulis* Sims f. *edulis* are due to its bioactive compounds, i.e., polyphenols, triterpenes, and polysaccharides (He et al., 2020).

A previous study by Nurrosyidah et al. (2020) revealed that red passion fruit can inhibit the growth of MRSA and ESBL-producing bacteria.

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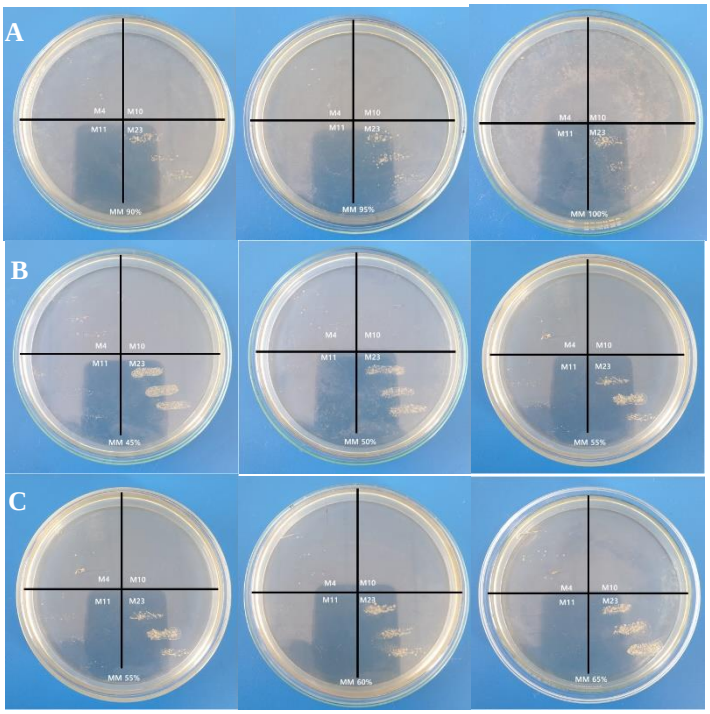

Figure 4: Streak Plate Method. A) MRSA 4 and MRSA 23 in 90%(+), 95%(+), and 100%(+) red passion fruit ferment filtrates; B) MRSA 10 in 45%(+), 50%(+), and 55%(-) red passion fruit ferment filtrates; and C) MRSA 11 in (55%(+), 60%(+), and 65%(-) red passion fruit ferment filtrates

This study used the streak plate method to determine the antibacterial activity of yellow and red passion fruit ferment filtrates. The screening results of red passion fruit ferment filtrate and yellow passion fruit ferment filtrate determined that MRSA 4 and MRSA 23 bacteria were negative or resistant. This is indicated by the growth of both bacteria, which continued up to a concentration of 100%. The MIC value for inhibiting the growth of MRSA and ESBL-producing bacteria was lower in yellow passion fruit ferment filtrate than in red passion fruit ferment filtrate, signifying that yellow passion fruit was more effective in inhibiting MRSA and ESBL-producing bacteria. The MICs of red passion fruit (*Passiflora edulis* Sims) ferment filtrate against MRSA 10 and MRSA 11 were 50% and 60%, respectively; and yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) ferment filtrate against MRSA 10 and MRSA 11 were both 30%. Meanwhile, the MICs of red passion fruit ferment filtrate were 35% against ESBL 41 and ESBL 43 and 25% against ESBL 45 and ESBL 47, whereas the MICs of yellow passion fruit ferment filtrate were 25% against ESBL 41 and ESBL 43 and 12.5% against ESBL 45 and ESBL 47.

This study applied the total plate count method to compare the percentages of inhibition of bacterial growth after 24-hour and 48-hour incubations at concentrations of 100% and 75%. The results indicated that 24 hours was optimal for the ferment filtrate.

Within 48 hours, MRSA 4 and MRSA 23 bacteria entered the death phase, while MRSA 10 and MRSA 11 bacteria experienced a 99.99% decrease in growth. The comparison of 24-hour and 48-hour incubation times showed that MRSA and ESBL-producing bacteria in the ferment filtrates were harvested after 24 hours, not 48 hours, as the bacteria died after 48 hours (entering the death phase).

A comparison of the percentage of inhibition between 100% ferment filtrate and 75% ferment filtrate is shown in Table 1 and Table 2.

Table 1. Percentage of Inhibition of red passion fruit (MM) and yellow passion fruit (MK) against MRSA with 3 replication

NO	Bacteria	Percentage of Inhibition			
		MM 100%	MM 75%	MK 100%	MK 75%
1	MRSA 10	91,61% + 143,77	100% + 0	97,82% + 133,39	100% + 0
2	MRSA 11	100% + 0	100% + 0	100% + 0	100% + 0

Table 2. Percentage of Inhibition of red passion fruit (MM) and yellow passion fruit (MK) against ESBL-producing bacteria with 3 replication

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NO	Bacteria	Percentage of Inhibition			
		MM 100%	MM 75%	MK 100%	MK 75%
1	ESBL 41	98,16% + 141,01	98,31% + 135,94	99,99% + 108,8	99,99% + 117,84
2	ESBL 43	76,74% + 133,33	97,09% + 137,76	45,98% + 133,63	99,98% + 134,32
3	ESBL 45	85,98% + 137,85	92,94% + 134,63	86,81% + 132,42	100% + 0
4	ESBL 47	57,31% + 138,54	88,01% + 139,40	99,99% + 13,8	99,99% + 94,79

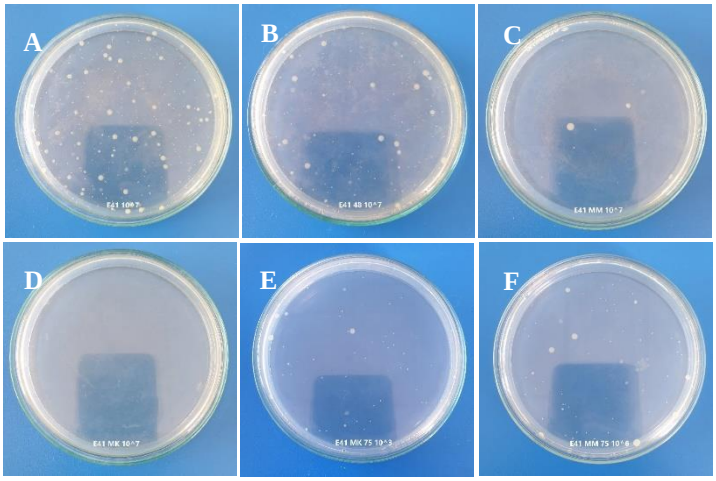

Figure 5: Total Plate Count Method. A) ESBL after 24 hours; B) ESBL 41 after 48 hours; C) 100% red passion fruit (MM) against ESBL 41 after 24 hours; D) 100% yellow passion fruit (MK) against ESBL 41 after 24 hours; E) 75% red passion fruit (MM) against ESBL 41 after 24 hours; and F) 75%yellow passion fruit (MK) against ESBL 41 after 24 hours

The 75% ferment filtrate was more effective for inhibiting the growth of MRSA and ESBL-producing bacteria. As seen in Table 1, the percentage of inhibition of 75% ferment filtrate is higher than 100% ferment filtrate, meaning that 75% ferment filtrate is more effective than 100% ferment filtrate.

In conclusion, the best time and concentration for the ferment filtrates of red passion fruit (*Passiflora edulis* Sims) and yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) as growth inhibitors against the clinical isolates of extended-spectrum beta-lactamase (ESBL)-producing *E.coli* and methicillin-resistant *Staphylococcus aureus* are 24 hours and 75%, respectively. At lower concentrations, many of the phenols formed are undissociated, more hydrophobic, can bind to hydrophobic regions of membrane proteins, and dissolve well in the lipid phase of bacterial membranes. This affects the integration of the cytoplasmic membrane, leading to intracellular leakage, which in turn causes lysis by activating enzymes that require more energy, resulting in less energy for antimicrobial activity.

CONCLUSION

This study examined red passion fruit (*Passiflora edulis* Sims) and yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) ferment filtrate in MRS media. The results may lead to the discovery of new antibacterial agents against methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase (ESBL). Furthermore, characterization and identification of red and yellow passion fruits are absolutely necessary to determine the antimicrobial compounds that can be produced so that they can be used as antibacterials in the future.

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The authors have no conflicts of interest to disclose.

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