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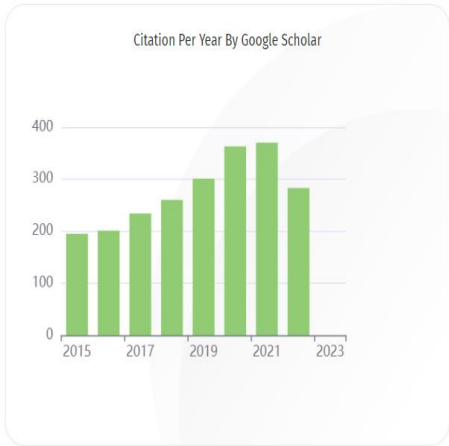
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## Original Article

**Inhibitory activity of fermentation filtrate of red passion fruit pulp (*Passiflora edulis sims.*) against *Escherichia coli* extended-spectrum beta-lactamase (esbl) and methicillin resistant *staphylococcus aureus* (MRSA)**Iif Hanifa Nurrosyidah<sup>1,3</sup>, Ni Made Mertaniasih<sup>2</sup>, Isnaeni<sup>1\*</sup><sup>1</sup>Department of Pharmaceutical Chemistry, Universitas Airlangga, Mulyorejo, Surabaya 60115, Indonesia<sup>2</sup>Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Tambaksari, Surabaya 60268, Indonesia<sup>3</sup>Doctoral Program of Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Mulyorejo, Surabaya 60115, Indonesia

## Abstract

This study aims to determine the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) of the fermentation filtrate (FF) of red passion fruit pulp (*Passiflora edulis Sims.*) against *Escherichia coli* Extended Spectrum Beta Lactamase (ESBL) and Methicillin Resistant *Staphylococcus aureus* (MRSA). The method used was the fermentation of red passion fruit pulp for 24 h using De Man Rogosa Sharpe Broth (MRS- broth) media, and the result showed that the FF of red passion fruit pulp made a dilution series concentration of 50%, 25%, 12.5%, 6.25%. Considering this result, the MIC FF of red passion fruit cells against ESBL and MRSA has a 25% dilution concentration, while MBC has a 50% dilution concentration. This shows that red passion fruit has the potential to be developed as antibacterial material, especially to fight bacteria that are already resistant.

**Keywords:** Inhibitory, red passion fruit, fermentation filtrate, ESBL, MRSA

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## Introduction

Infectious diseases are still a major health problem in developing tropical countries like Indonesia, and death caused by infectious diseases is about 51% (Sardjono and Le, 2007). Many bacteria are resistant to some antibiotics, such as Extended Spectrum Beta Lactamase (ESBL) and Methicillin Resistant *Staphylococcus aureus* (MRSA), therefore, irrational use of antibiotics worsens this condition. Bacterial resistance to antibiotics has become a serious global problem. Around 440 thousand new cases of MDR-TB (Tuberculosis-Multi-Drug Resistance) are reported every year, thereby causing 150 thousand deaths worldwide (WHO, 2014).

The use of natural ingredients as raw materials in drug development is increasingly demanded by the pharmaceutical industry community. Meanwhile, according to the World Health Organization (WHO) data, about 80% of the world's population uses herbal-based products (Djorjevic, 2017). Passion fruit (*Passiflora edulis Sims.*), a member of the Passifloraceae family, has more than 500 species (Duarte and Robert, 2015), it was originated from Brazil and has spread to other countries in Asia, Australia, Africa, India, South America, and the Caribbean. Furthermore, passion fruit has other variants which are identified by the color of the fruit (Singh and Debojyuti, 2013) such as yellow (*P. edulis* var. *Flavicarpa*), purple (*P. edulis* var. *Edulis*), and

orange (*P. edulis* var. *Caerulea*). Passion fruit contains phytochemical compounds of glycosides including flavonoids luteolin-6-C-chinovoside, luteolin-6-C-fucoside, cyanogenic glycosides passiflorin, epipassiflorin, passicapsin, passicoriacin, routine-cipassicogen, epipassicogen epitraphilin B, amygdalin, prunacin, triterpenoid glycosides, and salicylic glycosides. Other chemical compounds such as harman alkaloid b-carbolin, harmine, harmaline and harmalol, phenol, carotene, and g-lactone are also found in passion (Bernes et al., 2007). Therefore, passion fruit has high nutritional value with multiminerals content such as magnesium and phosphorus, various vitamins, as well as high carbohydrate and water (Zibadi et al., 2020).

The Yellow passion fruit pulp extract has been investigated for its antibacterial activity against *Streptococcus mutans*, and its most effective concentration was 40% to 45%. The fermentation filtrate (FF) of yellow passion fruit (*P. edulis* forma *Flavicarpa sims.*) fermented using de Man-Rogosa Sharpe Broth (MRS) media has been reported to have antibacterial activity against *Staphylococcus* spp., MRSA, and *Escherichia coli* ESBL. Previous studies related to the isolation of probiotic bacteria from purple passion fruit showed that passion fruit contains Probiotics *Lactobacillus bulgaricus*, and *Lactobacillus heterohiochii* which produces exopolysaccharides that inhibits the attachment of pathogenic bacteria (Zahro, 2014).

## Methods

## Plant source and determination

The red passion fruits were freshly collected from the local farm in Sidoarjo, on May 2020. It plant was

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determined based on the taxonomy character of leaf, flower, fruit, and stem plant as well as identified as *Passiflora edulis* Sims. by Herbarium Malangensis, Universitas Negeri Malang.

#### Preparation of fermentation media

The MRS broth was prepared by dissolving 52.2 g of MRS broth powder into 1 L of purified water. Also, sterilization was performed by autoclaving for 15 min at 121°C. Nutrient agar (NA) media was prepared by dissolving 20.0 g of the NA powder into 1 L of purified water, and it was further heated in boiling water with continuous stirring to completely dissolve all the powder, and a consistent yellowish liquid was achieved by pouring 10 mL of the mixture into a test tubes using syringe while it is still warm and in liquid form. The test tubes then need to be plugged with cotton and autoclaved for 15 min at 121°C.

#### Sample preparation, fermentation, and characterization

The passion fruits were washed and dried before being divided into two parts and the 5 g of fruit pulps was weighed and put into 50 mL of MRS broth media to be fermented with a rotary shaker at 150 rpm, 37°C for 24 h. After 24 h of fermentation the fermentation broth was removed, centrifuged and the supernatant was collected for characterization.

#### Determination of total plate count (TPC)

The supernatant was then serially diluted 1:10 to 10<sup>7</sup> using sterile normal saline solutio. Each of the serial dilution was inoculated on the MRS agar (Oxoid, UK), and incubated at 37°C for 24 h. Furthermore, cell growth was observed and the plating colonies were counted using a bacteria colony counter.

#### Inoculum preparation

The selected strain of bacteria was transferred aseptically to sterile saline water, vortexed and then the turbidity was measured using a spectrophotometer against the sterile saline water to obtain 25% turbidity or optical density of 25% transmittance (about 10<sup>9</sup> CFU/mL of bacteria) at 580 nm (Safarini et al., 2020).

#### Antibacterial activity test of ff red passion fruit pulp through MIC and MBC determination

This research used agar dilution technique, in which the FF of red passion fruit pulp according to dilution (50%, 25%, 12.5%, 6.25%) was added to agar, hence a seeding that matches the amount of dilution plus one seed was required. It was used as a control without adding FF of red passion fruit pulp, and one seed for positive control with Meropenem (25 µL/mL) and then incubated overnight at 37°C. The lowest concentration of FF of red passion fruit pulp that inhibits bacterial growth is the MIC. Therefore, the minimum concentration of antibiotics that kills bacteria/minimum bactericidal concentration (MBC) is determined by planting bacteria in liquid seedlings which are used for MIC when there is no further growth in agar (Soleha, 2015).

## Results

Red passion fruit (*P. edulis* Sims.), known as sour passion fruit, is one of the species of passion fruit and belongs to the family of *Passifloraceae*. This species is unique and has two different performances namely purple skin (growing in subtropical and tropical highlands) and red skin (growing in lowland tropics) (Karsinah and Mansur, 2010). In this study, the red passion fruits (Fig. 1) from a local farm in Sidoarjo (East Java) were used.



Figure 1. Red Passion Fruit (*Passiflora edulis* Sims).

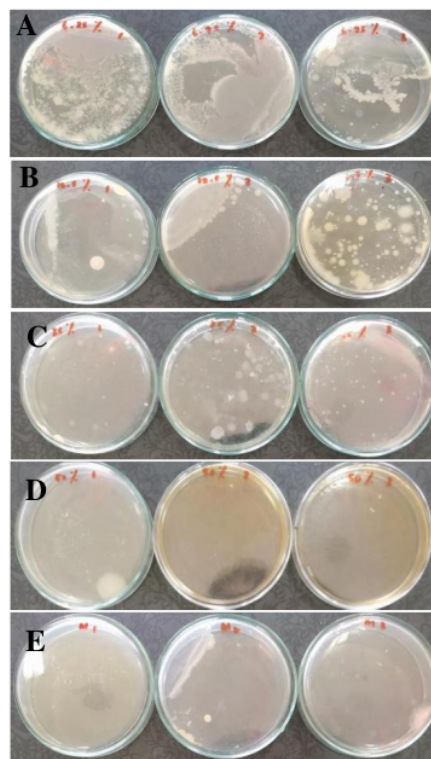
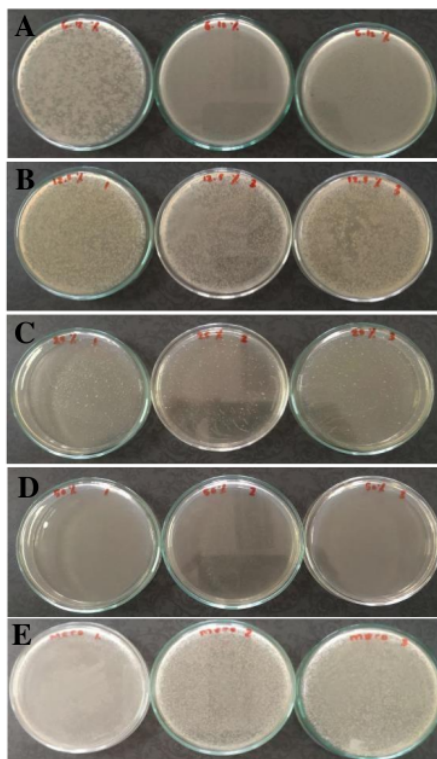


Figure 2. *E. coli* ESBL in the 6.25% Cell-Free Fermentation Supernatant (FF) of red passion fruit (*Passiflora edulis* Sims.) in de Man-Rogosa Sharpe agar (MRS-agar) Media, (a), *E. coli* ESBL in the 12.5% FF of red passion fruit (b), *E. coli* ESBL in the 25% FF of red passion fruit (c), *E. coli* ESBL in the 50% FF of red passion fruit (d), *E. coli* ESBL with 25 µg/mL of meropenem (e)



Based on the results of this research that has been investigated by FF of red passion fruit against *E. coli* ESBL and MRSA with MIC, the dilution concentration was 25%, while minimum bactericidal concentration (MBC) was 50% dilution concentration. The FF of red passion fruit has been shown to inhibit the growth of *E. coli* ESBL and MRSA better than meropenem (Fig. 2 and Fig. 3), where at the MIC of meropenem MRSA growth was still visible.



**Figure 3.** Methicillin-resistant *Staphylococcus aureus* (MRSA) in the 6.25% Cell-Free Fermentation Supernatant (FF) of red passion fruit (*Passiflora edulis* Sims.) in de Man-Rogosa Sharpe Agar (MRS-agar) Media (a), MRSA in the 12.5% FF of red passion fruit (b), MRSA in the 25% FF of fruit (c), MRSA in the 50% FF of red passion fruit (d), MRSA with 25 µg/mL of meropenem (e).

## Discussion

*P. edulis* is a native fruit from Brazil, it has several common names, such as sour passion fruit, yellow passion fruit, black passion fruit, and purple passion fruit, and it presents a wide variability with the different rind colors of its fruits (Bernacci et al., 2008). Passion fruit has many health benefits because its nutritional content. Authentic passion fruit has a sour taste so it's rarely used directly and only made as a beverage ingredient. Though passion fruit is a raw material that produces good citric acid the good one since it contains citric acid in high concentration. The acidity of passion fruit citrate ranges from 2.4-4.8%. Therefore, the citric acid is obtained by fermentation, and it is a weak organic

acid found in the leaves and fruit of certain herbs. This compound is a good natural preservative that is used to regulate the acidity of various processed food and soft drink (Karsinah and Mansur, 2010). Therefore, this compound is thought to provide antibacterial activity from FF of red passion fruit against *E. coli* ESBL and MRSA.

In addition, red passion fruit contains probiotics (Nurrosyidah et al., 2020), which presumably also provide an antibacterial activity against *E. coli* ESBL and MRSA. Further studies related to the isolation of probiotic bacteria from purple passion fruit showed that passion fruit contains Probiotics *Lactobacillus bulgaricus* and *Lactobacillus heterohiochii* which produce exopolysaccharides that inhibits the attachment of pathogenic bacteria (Zahro, 2004). It is supported by the fermentation of probiotic bacteria from the fruit to produce organic acids that further strengthen its antibacterial activity against *E. coli* ESBL and MRSA, since the secondary metabolite content of red passion fruit has antibacterial potential.

In conclusion The MIC of red passion fruit fermentation filtrate against *E. coli* ESBL and MRSA was 25% dilution concentration, while MBC was 50% dilution concentration. This showed that red passion fruit has the potential to be developed as a candidate of antibacterial material, especially to overcome resistant bacteria.

## Acknowledgement

This scientific paper was presented at The Third International I Conference on Agromedicine & Tropical Diseases (ICATD) on September 12-13, 2020 at Jember University, Jember, East Java, Indonesia.

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## Original Article

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## Methods

**Plant source and determination**

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determined based on the taxonomy character of leaf, flower, fruit, and stem plant as well as identified as *Passiflora edulis* Sims. by Herbarium Malangensis, Universitas Negeri Malang.

#### Preparation of fermentation media

The MRS broth was prepared by dissolving 52.2 g of MRS broth powder into 1 L of purified water. Also, sterilization was performed by autoclaving for 15 min at 121°C. Nutrient agar (NA) media was prepared by dissolving 20.0 g of the NA powder into 1 L of purified water, and it was further heated in boiling water with continuous stirring to completely dissolve all the powder, and a consistent yellowish liquid was achieved by pouring 10 mL of the mixture into a test tubes using syringe while it is still warm and in liquid form. The test tubes then need to be plugged with cotton and autoclaved for 15 min at 121°C.

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The supernatant was then serially diluted 1:10 to 10<sup>7</sup> using sterile normal saline solution. Each of the serial dilution was inoculated on the MRS agar (Oxoid, UK), and incubated at 37°C for 24 h. Furthermore, cell growth was observed and the plating colonies were counted using a bacteria colony counter.

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Red passion fruit (*P. edulis* Sims.), known as sour passion fruit, is one of the species of passion fruit and belongs to the family of *Passifloraceae*. This species is unique and has two different performances namely purple skin (growing in subtropical and tropical highlands) and red skin (growing in lowland tropics) (Karsinah and Mansur, 2010). In this study, the red passion fruits (Fig. 1) from a local farm in Sidoarjo (East Java) were used.



Figure 1. Red Passion Fruit (*Passiflora edulis* Sims.).

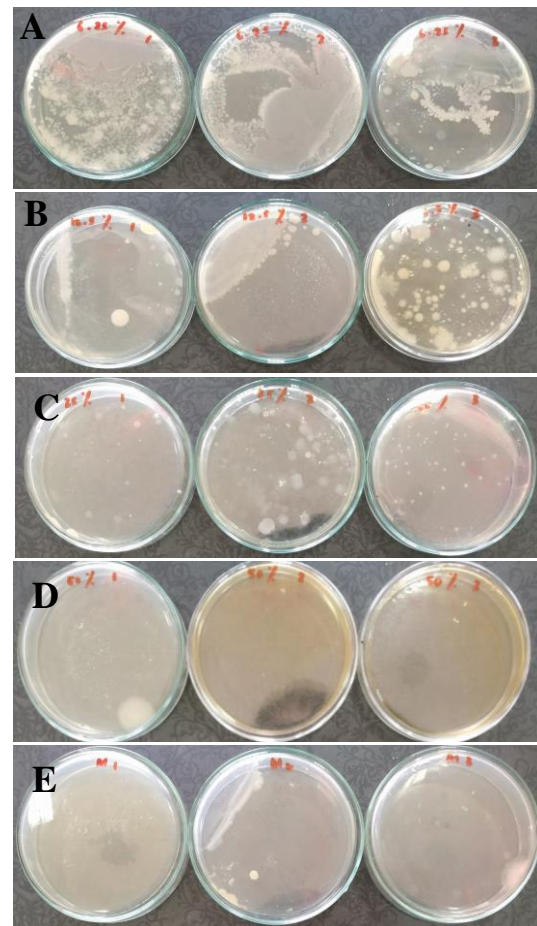
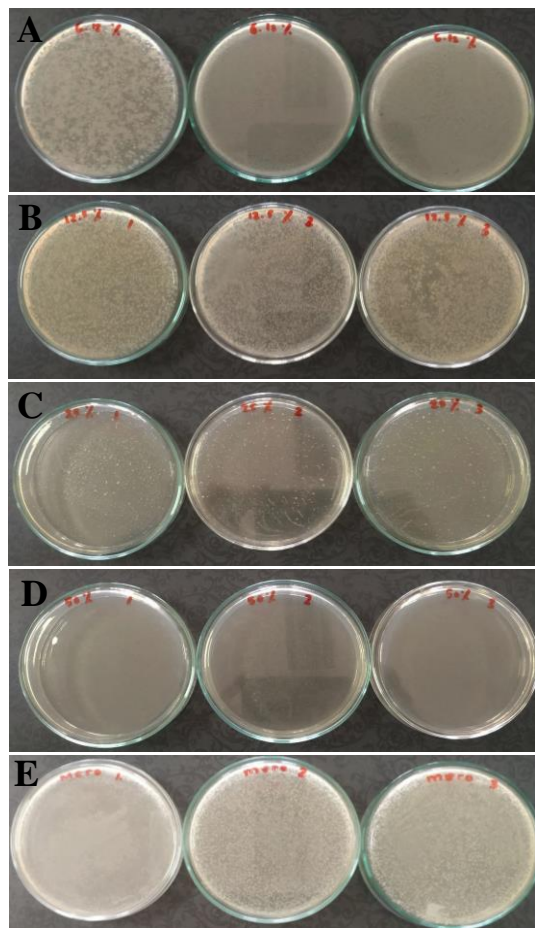


Figure 2. *E. coli* ESBL in the 6.25% Cell-Free Fermentation Supernatant (FF) of red passion fruit (*Passiflora edulis* Sims.) in de Man-Rogosa Sharpe agar (MRS-agar) Media, (a), *E. coli* ESBL in the 12.5% FF of red passion fruit (b), *E. coli* ESBL in the 25% FF of red passion fruit (c), *E. coli* ESBL in the 50% FF of red passion fruit (d), *E. coli* ESBL with 25 µg/mL of meropenem (e)

Based on the results of this research that has been investigated by FF of red passion fruit against *E. coli* ESBL and MRSA with MIC, the dilution concentration was 25%, while minimum bactericidal concentration (MBC) was 50% dilution concentration. The FF of red passion fruit has been shown to inhibit the growth of *E. coli* ESBL and MRSA better than meropenem (Fig. 2 and Fig. 3), where at the MIC of meropenem MRSA growth was still visible.



**Figure 3.** Methicillin-resistant *Staphylococcus aureus* (MRSA) in the 6.25% Cell-Free Fermentation Supernatant (FF) of red passion fruit (*Passiflora edulis* Sims.) in de Man-Rogosa Sharpe Agar (MRS-agar) Media (a), MRSA in the 12, 5% FF of red passion fruit (b), MRSA in the 25% FF of red passion fruit (c), MRSA in the 50% FF of red passion fruit (d), MRSA with 25 µg/mL of meropenem (e).

## Discussion

*P. edulis* is a native fruit from Brazil, it has several common names, such as sour passion fruit, yellow passion fruit, black passion fruit, and purple passion fruit, and it presents a wide variability with the different rind colors of its fruits (Bernacci et al., 2008). Passion fruit has many health benefits because its nutritional content. Authentic passion fruit has a sour taste so it's rarely used directly and only made as a beverage ingredient. Though passion fruit is a raw material that produces good citric acid the good one since it contains citric acid in high concentration. The acidity of passion fruit citrate ranges from 2.4-4.8%. Therefore, the citric acid is obtained by fermentation, and it is a weak organic

acid found in the leaves and fruit of certain herbs. This compound is a good natural preservative that is used to regulate the acidity of various processed food and soft drink (Karsinah and Mansur, 2010). Therefore, this compound is thought to provide antibacterial activity from FF of red passion fruit against *E. coli* ESBL and MRSA.

In addition, red passion fruit contains probiotics (Nurrosyidah et al., 2020), which presumably also provide an antibacterial activity against *E. coli* ESBL and MRSA. Further studies related to the isolation of probiotic bacteria from purple passion fruit showed that passion fruit contains Probiotics *Lactobacillus bulgaricus* and *Lactobacillus heterohiochii* which produce exopolysaccharides that inhibits the attachment of pathogenic bacteria (Zahro, 2004). It is supported by the fermentation of probiotic bacteria from the fruit to produce organic acids that further strengthen its antibacterial activity against *E. coli* ESBL and MRSA, since the secondary metabolite content of red passion fruit has antibacterial potential.

In conclusion The MIC of red passion fruit fermentation filtrate against *E. coli* ESBL and MRSA was 25% dilution concentration, while MBC was 50% dilution concentration. This showed that red passion fruit has the potential to be developed as a candidate of antibacterial material, especially to overcome resistant bacteria.

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