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# JOURNAL OF BASIC AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

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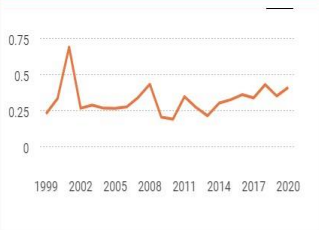
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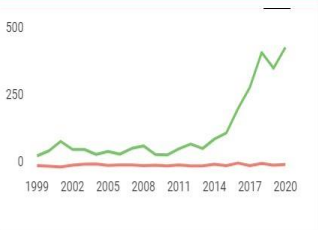
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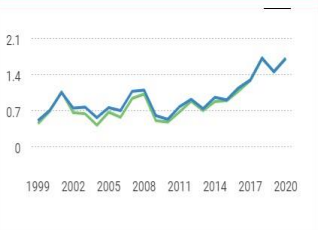
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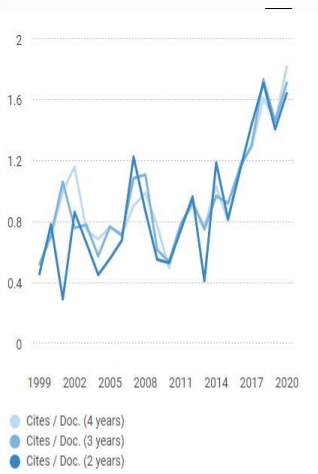
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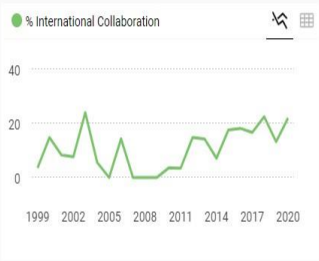
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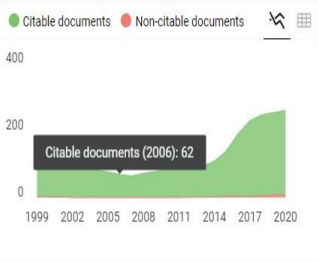
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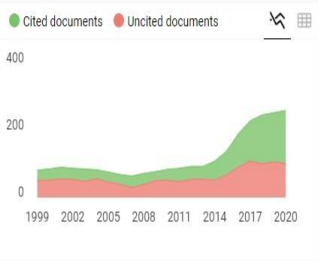
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# Artikel JBCPP.

*by* Iif Hanifah Nurrosyidah

I.H.Nurrosyidah [1,2], N.M. Mertaniasih[3], and I. Isnaeni[1]*

# The effect of red passion fruit (Passiflora edulis Sims.) fermentation time on its activity against Extended Strain Methicillin-Resistant (ESBL) Escherichia coli and Methicillin-Resistant Staphylococcus aureus (MRSA)

https://doi.org/10.1515/jbcpp-2020-0408
**Received** December 14, 2020 **Accepted** February 20, 2021

**Abstract**

**The purpose of this study was to determine the effect of fermentation techniques on the inhibitory activity of red passion fruit (Passiflora edulis Sims.) fermentation filtrate in De Man Rogosa Sharpe broth (MRSB) media against Eschericia coli Extended Spectrum Betalactamase (ESBL) and Methicillin Resistant *Staphylococcus aureus* (MRSA). Wrapping the fruit pulp in banana leaves before was compared to direct fermentation processes. This study was divided into three treatment groups. The first group was the fruit pulp (5 grams) fermented in 45 mL MRSB medium for 24 hours. The second group was the fruit wrapped in banana leaves for three days before fermentation in MRSB for 24 hours. The third group was the fruit pulp wrapped in banana leaves for three days before fermentation in MRSB for 48 hours. Fermentation broth of each condition was taken and then filtered using millipore (0,2 μm). As many as 50 μL filtrates were tested for their inhibitory activity against *E.coli* ESBL and MRSA using kirby bauer method. The second group showed the best antibacterial activity against *E.coli* ESBL and MRSA with average zone of inhibition 38.3 mm and 37.6 mm respectively. These values were higher than the first and third groups activities. The inhibitory activity of the first groups against ESBL and MRSA was categorized as a moderate potency with diameter of growth inhibition zone 16-20 mm, whereas the other groups were categorized as strong potency with diameter higher than 20 mm.**

**Keywords**: esbl; fermentation; inhibitory activity; mrsa; red passion fruit.

## Introduction

*P. edulis* Sims (Passion Fruit) with the genus Passifloraceae, has 500 distributed species in areas with warm temperatures and tropical regions. This plant comes from Brazil and has spread to other countries (Asia, Australia, Africa, India, South America and the Caribbean). Passion fruit has other variants that can be identified from the color of the fruits, such as yellow (*P. edulis var. Flavicarpa*), purple (*P. edulis var.edulis*), and orange (*P. edulis var. Caerulea*) .Passion fruit is a good source of ascorbic acid (vitamin C) and carotenoids (vitamin A) [1].

Infectious diseases are still a major health problem in developing tropical countries like Indonesia. Deaths caused by infectious diseases are around 51%1. Irrational use of antibiotics worsens this condition. Many bacteria are resistant to some antibiotics, such as Extended Spectrum Beta Lactamase (ESBL) and Methicillin Resistant Staphylococcus aureus (MRSA) [5].

Passion fruit contains glycoside-flavonoids [2], such as luteolin-6-C-chinovoside, luteolin-6-C-fucoside, cyanogenic glycosides passibiflorine, epipassibiflorin, passicapsin, passicoriacin, epipassicoriacin , epitetraphilin B, amygdalin, prunacin, triterpenoid glycosides and salicylic glycosides. Other chemical compounds such as the b-carboline alkaloids harman, harmine, harmaline and harmalol, phenols, carotene and g-lactones are also found in passion fruit. Passion fruit is a fruit that has high nutritional value, many multimineral contents such as magnesium and phosphorus, various vitamins, as well as high carbohydrates and water [3]. Passion fruit is a suitable habitat for the growth of probiotic bacteria, because of its adequate nutritional content. Based on previous research, purple passion fruit (Passiflora edulis Sims. var edulis) contains LAB (*Lactobacillus bulgaricus* dan *Lactobacillus heterohiochii*) [4].

Antibacterial activity of free cell fermentation supernatant (CFFS) yellow passion fruit (*Passiflora edulis forma flavicarpa* Sims.) Fermented in de Man-Rogosa Sharpe Broth (MRS-B) media has been reported for its antibacterial activity *against Staphylococcus spp.*, Methicillin Resistant *Staphylococcus aureus* (MRSA), and *Escherichia coli* Extended Strain Beta Lactamase (ESBL) [6]. Therefore this study will be investigated the effect of fermentation techniques on the inhibitory activity of red passion fruit (*Passiflora edulis* Sims.) fermentation filtrate in De Man Rogosa Sharpe broth (MRSB) media against *Eschericia coli* Extended Spectrum Betalactamase (ESBL) and Methicillin Resistant *Staphylococcus aureus* MRSA). Wrapping the fruit pulp in banana leaves before was compared to direct fermentation processes .

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## Materials and methods

### Plant source and determination

The red passion fruits were collected freshly from local farm in Krembung (Sidoarjo, East Java, Indonesia), harvested on Agustus 2020, and it was identification and determination based on the taxsonomi character of leaf, flower, fruit, steam plant and identificated by Herbarium Malangensis, Univeristas Negeri Malang (East Java, Indonesia) as *Passiflora edulis* Sims.

**Figure 1:** The Red Passion Fruits (*Passiflora edulis* Sims.)

### Preparation of fermentation media

The MRS broth was prepared by dissolving 52.2 gram of the MRS broth powder into 1L of purified water. Sterilization was performed by autoclaving for 15 minutes at 121 °C. Nutrient agar (NA) media was prepared by dissolving 20.0 gram of the NA powder into 1L of purified water. Heating in boiling water with stirring constantly was done to obtain all of the powder to completely dissolve and consistent yellowish liquid was achieved 10 mL of the mixture was then fill into 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 minutes at 121°C. Prepare some banana leaves that have been cleaned and sterilized in the autoclave for 15 minutes at 121°C.

### Sampel preparation, fermentation and characterization

The passion fruits were washed and dried before it was devided into two parts and the 5 gram of passion fruit pulps was weighed put into 50 mL of MRS broth media to be fermented with rotary shaker at 150 rpm, 37°C for 12 hours (group one/ MMB 24). The passion fruits were washed and dried before it was devided into two parts and the 5 gram of fruit pulps was weighed put into banana leaves sterile and wrapped. Once wrapped put in a container in the form of an impermeable jar air and light. The decay is carried out during 72 hours at room temperature. After that 24 hours (group two/ MDP 24) and 48 hours (group three/ MDP 48). The fermentation broth centrifuged and The supernatant was filtered using 0.2 micron millipore sieve to remove bacteria [7].

### Inoculum preparation

The selected bacteria strain was transferred aseptically to sterile saline water, vortex and then the turbidity was measured using spectrophotometer against the sterile saline water to obtain 25% Transmittance (about $10^9$CFU/ml of bacteria) turbidity or optical density at 580 nm [6].

### Antibacterial activity test

The Sterilized nutrient agar media are poured into a sterile petri disk, wait for it to solidify. Then, the inoculum are swabbed onto the nutrient agar surface slowly using a sterile swab. Place the paper disc slowly over the nutrient agar surface. then drop the test solution each as much as 30 µL, and put the vancomycin antibiotic paper disc slowly as a positive control (comparison standard). Then incubated for 24 hours at 37°C. Observe the resulting inhibition zone diameter [8].

## Results

The inhibition zone againts *E.coli* ESBL is obtained which can be seen in table 1. And table 2. For the inhibition zone againts Methicillin Resistant *Staphylococcus aureus* (MRSA). The inhibition zone againts E.coli ESBL of the red passion fruit pulps was fermented with MRS broth media (group one/ MMB 24) classified as moderate (16 mm). While the treated group was wrapped in banana leaves during 72 hours at room temperature before fermentation with MRS broth, group two (MDP 24) and group three (MDP 48) have the inhibition zone againts *E.coli* ESBL was classified as strong (more than 20 mm). The inhibition zone againts Methicillin Resistant *Staphylococcus aureus* (MRSA) of group one (MMB 24) classified as weak (13 mm). While group two (MDP 24) and group three (MDP 48) have the inhibition zone was classified as strong (more than 20 mm).

**Table 1.** Inhibition Zone Diameter For Each Treatment Group againts *E.coli* Extanded Spectrum Betalactamase (ESBL)

| Treatment Group | Average Inhibition Zone Diameter (mm) | Inhibition Classification |
|---|---|---|
| MMB 24 | 16 | Moderate |
| MDP 24 | 38 | Strong |
| MDP 48 | 33 | Strong |

**Table 2.** Inhibition Zone Diameter For Each Treatment Group againts E.coli Extanded Spectrum Betalactamase (ESBL)

| Treatment Group | Average Inhibition Zone Diameter (mm) | Inhibition Classification |
|---|---|---|
| MMB 24 | 13 | Weak |
| MDP 24 | 38 | Strong |
| MDP 48 | 38 | Strong |

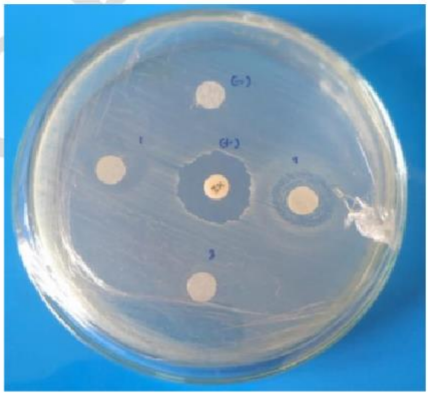

**Figure 4:** Antibacterial Activity of Fermentation Filtrate in MRS-Broth of Red Passion Fruit Pulp Againts *Eschericia coli* Extended Spectrum Betalactamase (ESBL) with Vancomycin 10 $\mu g$ as positive control

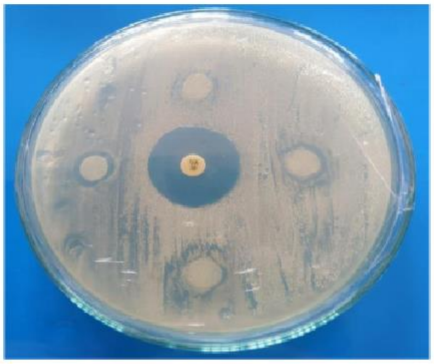

**Figure 5:** Antibacterial Activity of Fermentation Filtrate in MRS-Broth of Red Passion Fruit Pulp Againts Methicillin Resistant *Staphylococcus aureus (MRSA)* with Vancomycin 10 $\mu$g as positive control

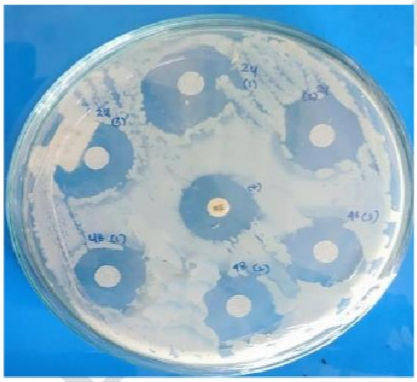

**Figure 6:** Antibacterial Activity of Fermentation Filtrate of Red Passion Fruit Pulp wrapped in Banana Leaves for Three Days then Fermented with MRS-Broth Againts *Eschericia coli* Extended Spectrum Betalactamase (ESBL) with Vancomycin 10 $\mu$g as positive control

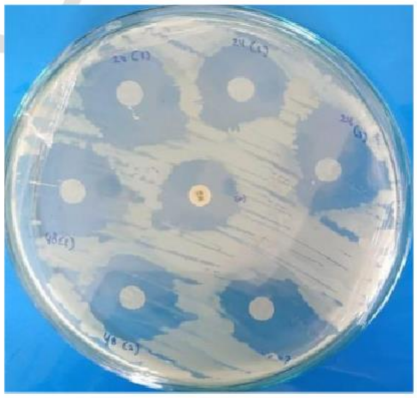

**Figure 7:** Antibacterial Activity of Fermentation Filtrate of Red Passion Fruit Pulp wrapped in Banana Leaves for Three Days then Fermented with MRS-Broth Againts Methicillin Resistant *Staphylococcus aureus (MRSA)* with Vancomycin 10 $\mu$g as positive control

## Discussion

Extended spectrum beta-lactamase (ESBL) is an enzyme that has the ability in hydrolyzing the antibiotics of the penicillin class, cephalosporin generation one, two, and three and the monobactam class and cause resistance to all antibiotics. Extended spectrum beta-lactamase does not hydrolyze cephamycin, which has its own family close to cephalosporin, it is inhibited by beta-lactamase inhibitors such as clavulanate, sulbactam and tazobactam. Extended spectrum beta-lactamase is generally inactive against carbapenem (imipenem, meropenem, ertapenem) [9].

Beta-lactamase is an enzyme that is produced by some bacteria (eg. *Eschericia coli*) which results in resistance to beta-lactam antibiotics. Beta-lactam ring in antibiotics have an important role in inhibiting cell wall synthesis. Beta-lactam rings attached to penicillin binding proteins (PBPs) will top the cell wall synthesis process. The process of cell wall synthesis is at a standstill causes cell death. This occurs due to an osmotic imbalance synthesis failure. Bacterial resistance There are 3 ways to beta-lactam, namely: destruction of the enzyme beta-lactamepada antibiotics, change in target to antibiotics, decreased intracellular uptake antibiotic. All of these pathways have an important role to play in antibiotic resistance. However, bacteria that produce beta-lactamase and destroy betalactam are the main causes of resistance [9].

The cause of widespread nosocomial infection is Methicillin Resistant *Staphylococcus aures* (MRSA). Methicillin Resistant Staphylococcus aures is a strain of Staphylococcus aures that are resistant to β-lactam antimicrobials, among them are from the penicillin group.The mechanism of MRSA resistance occurs due to *Staphylococcus aureus* produces the gene-encoded Penicillin Binding Proteins (PBP2 and PBP2a) mecA against all classes of antibiotics. The function of the PBP2 is stopped because giving β-lactam is compensated by PBP 2a resulting in wall synthesis cells on MRSA take place [10].

The antibacterial activity of the fermented red passion fruit filtrate is probably derived from organic acids and bacteriocins. based on previous research that red passion fruit contains probiotics [11]. Organic acids have been used for many years preservation of food and feed. In addition, the bacteriocin produced by probiotics also has a bactericidal effect bacteriostatic effect at similar or near related bacterial strains, have already put into use as an alternative to antibiotics in livestock. The combination of the use of organic acids and bacteriocins is able to produce an optimum inhibitory power against pathogenic bacteria [12]. In this study, the red passion fruit pulp fermented in banana leaves first, and then fermented in MRS broth resulted in stronger inhibition against ESBL and MRSA compared to fermentation of passion fruit pulp in MRS broth without fermented in banana leaves..

## Conclusions

The second group showed the best antibacterial activity against E.coli ESBL and MRSA with average zone of inhibition 38.3 mm and 37.6 mm respectively. These values were higher than the first and third groups activities. The inhibitory activity of the first groups against ESBL and MRSA was categorized as a moderate potency with diameter of growth inhibition zone 16-20 mm, whereas the other groups were categorized as strong potency with diameter higher than 20 mm.

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Iif Hanifa Nurrosyidah, Ni Made Mertaniasih and Isnaeni*

# The effect of red passion fruit (*Passiflora edulis* Sims.) fermentation time on its activity against Extended Strain Methicillin-Resistant (ESBL) *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA)

https://doi.org/10.1515/jbcpp-2020-0408
Received December 14, 2020; accepted February 20, 2021

## Abstract

**Objectives:** The purpose of this study is to determine the effect of fermentation techniques on the inhibitory activity of red passion fruit (*Passiflora edulis* Sims.) fermentation filtrate in De Man Rogosa Sharpe-broth (MRS-B) media against Extended Strain Methicillin-Resistant (ESBL) *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA).

**Methods:** The fruit pulp was wrapped in banana leaves before compared to direct fermentation processes. This study was divided into three treatment groups. Group 1 was the fruit pulp (5 g) fermented in 45 mL of MRS-B medium for 24 h. Group 2 was the fruit pulp wrapped in banana leaves for 3 days before fermented in MRS-B for 24 h. Group 3 was the fruit pulp wrapped in banana leaves for 3 days before fermentation in MRS-B for 48 h. Fermentation broth of each condition was taken and then filtered using millipore (0.2 μm). As many as 50 μL of filtrates was tested for its inhibitory activity against *E. coli* ESBL and MRSA using the Kirby Bauer method.

**Results:** Group 2 showed the best antibacterial activity against *E. coli* ESBL and MRSA with the average zone of inhibition of 38.3 and 37.6 mm respectively. These values were higher than the first and group 3s activities.

**Conclusions:** The inhibitory activity of group 1s against ESBL and MRSA is categorized as a moderate potency with a diameter of growth inhibition zone of 16–20 mm, whereas the other groups are categorized as strong potency with a diameter higher than 20 mm.

**Keywords:** ESBL; fermentation; inhibitory activity; MRSA; red passion fruit.

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**Iif Hanifa Nurrosyidah,** Department of Pharmaceutical Chemistry, Universitas Airlangga, Surabaya, Indonesia; and Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
**Ni Made Mertaniasih,** Department of Medical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

## Introduction

*Passiflora edulis* Sims (Passion Fruit) of the Passifloraceae family, has 500 distributed species in areas with warm temperatures and tropical regions. This plant comes from Brazil and has spread to other countries (Asia, Australia, Africa, India, South America, and the Caribbean). Passion fruit has other variants that can be identified from the color of the fruits, such as yellow (*P. edulis* var. *Flavicarpa*), purple (*P. edulis* var. *edulis*), and orange (*P. edulis* var. *Caerulea*). Passion fruit is a good source of ascorbic acid (vitamin C) and carotenoids (vitamin A) [1].

Infectious diseases are still a major health problem in developing tropical countries like Indonesia. Deaths caused by infectious diseases are around 51% [1]. Irrational use of antibiotics worsens this condition. Many bacteria are resistant to some antibiotics, such as Extended Strain Methicillin-Resistant (ESBL) *Escherichia coli* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) [2].

Passion fruit contains glycoside-flavonoids [3], such as luteolin-6-C-chinovoside, luteolin-6-C-fucoside, cyanogenic glycosides passibiflorine, epipassibiflorin, passicapsin, passicoriacin, epipassicoriacin, epitetraphilin β, amygdalin, prunacin, triterpenoid glycosides, and salicylic glycosides. Other chemical compounds such as the β-carboline alkaloids harman, harmine, harmaline and harmalol, phenols, carotene, and g-lactones are also found in passion fruit. Passion fruit is a fruit that has high nutritional value, many multimineral contents such as magnesium and phosphorus,

various vitamins, as well as high carbohydrates, and water [4]. Passion fruit is a suitable habitat for the growth of probiotic bacteria because of its adequate nutritional content. Based on previous research, purple passion fruit (*P. edulis* Sims. var. *edulis*) contains LAB (*Lactobacillus bulgaricus* dan *Lactobacillus heterohiochii*) [5].

The cell-free fermentation supernatant from yellow passion fruit (*P. edulis forma flavicarpa* Sims.) fermented in De Man Rogosa Sharpe-Broth (MRS-B) media has been reported can inhibit the growth of *Staphylococcus* spp., MRSA, and *Escherichia coli* Extended Strain Beta-Lactamase (ESBL) [6]. Therefore, this study investigates the effect of fermentation techniques on the inhibitory activity of red passion fruit (*P. edulis* Sims.) fermentation filtrate in MRS-B media against *ESBL E. coli* and MRSA. The fruit pulp was wrapped in banana leaves before compared to direct fermentation processes.

# Materials and methods

## Plant source and determination

The red passion fruits were collected freshly from a local farm in Krembung (Sidoarjo, East Java, Indonesia), harvested in August 2020. They were identified and determined based on the taxonomy character of leaf, flower, fruit, and steam plant and identified on Herbarium Malangensis, Univeristas Negeri Malang (East Java, Indonesia) as *P. edulis* Sims, with the identification number of 'Nomor: 09/25.07.18/herb.malg' (see Figure 1).

## Preparation of fermentation media

The MRS-broth was prepared by dissolving 52.2 g of the MRS-broth powder into 1 L of purified water. 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. Heating in boiling water with stirring constantly was done to obtain all of the powder to completely dissolve and the consistent yellowish liquid was achieved. Ten milliliters of the mixture was then filled into test tubes using a syringe while it was still warm and in liquid form. The test tubes then need to be plugged with cotton and autoclaved for 15 min at 121 °C. Some banana leaves that have been cleaned and sterilized in the autoclave for 15 min at 121 °C were prepared. This NA medium was used to rejuvenate the tested bacterial isolates (ESBL and MRSA) and antibacterial activity test.

**Figure 1:** The red passion fruits (*P. edulis* Sims.).

## Sample preparation, fermentation, and characterization

The passion fruits were washed and dried before they were divided into two parts and the 5 g of passion fruit pulps was weighed and put into 45 mL of MRS-broth media to be fermented with a rotary shaker at 150 rpm, 37 °C for 12 h (group 3/MMB 24). The passion fruits were washed and dried before they were divided into two parts and the 5 g of fruit pulps was weighed and put into banana leaves (the banana leaves were not autoclaved, only cleaned and disinfected with 70% alcohol), and then wrapped in a container in the form of an impermeable jar air and light. The decay was carried out for 3 days at room temperature. After that, group 2 and group 3 were wrapped in banana leaves for 3 days, then the red passion fruit pulp was fermented with MRS-broth media for 24 h (group 2) and 48 h (group 3). The fermentation broth was centrifuged and The supernatant was filtered using a 0.2 μm millipore sieve to remove bacteria [7] (see Figures 2 and 3).

## Inoculum preparation

The selected bacteria strain was transferred aseptically to sterile saline water, vortex and then the turbidity was measured using spectrophotometer against the sterile saline water to obtain 25% Transmittance (about $10^9$ CFU/mL of bacteria) turbidity or optical density at 580 nm [6].

## Antibacterial activity test

The Sterilized NA media were poured into a sterile Petri disk and waited to solidify. Then, the inoculum was swabbed onto the NA

**Figure 2:** The fermentation of red passion fruit pulp wrapped in banana leaves for 3 days (for group 2 and group 3).

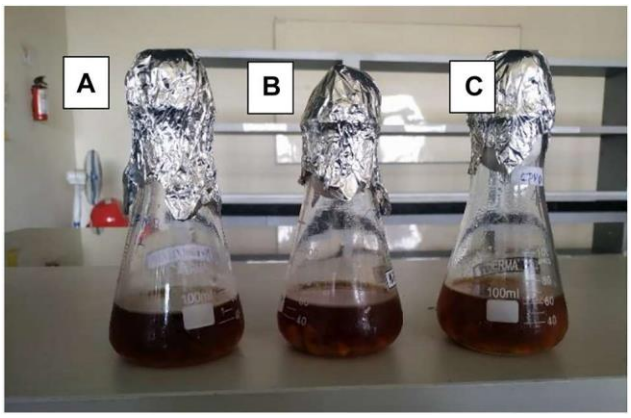


**Figure 3:** (A) Fermented filtrate of red passion fruit pulp with MRS-broth for 24 h (group 1), (B) fermented filtrate of red passion fruit pulp wrapped in banana leaves for 3 days then fermented with MRS-broth for 24 h (group 2), (C) fermented red passion fruit pulp wrapped in banana leaves for 3 days then fermented with MRS-broth for 48 h (group 3).

surface slowly using a sterile swab. The paper disk in the test solution was soaked each as much as 30 µL then the paper disc was placed slowly over the NA surface, and the vancomycin antibiotic paper disc was put slowly as a positive control (comparison standard). There were three times of replication in this study. Then, it was incubated for 24 h at 37 °C. The resulting inhibition zone diameter was observed [8].

## Results

The inhibition zone of *E. coli* ESBL obtained can be seen in Table 1. And Table 2 for the inhibition zone of MRSA. The inhibition zone of *E. coli* ESBL of the red passion fruit pulps was fermented with MRS-broth media (group 3) classified as moderate (16 mm). On the other hand, the treated group was wrapped in banana leaves for 72 h at room temperature before fermented with MRS-broth, group 2 and group 3 had the inhibition zone against *E. coli* ESBL classified as strong (more than 20 mm). The inhibition zone against MRSA of group 3 was classified as weak (13 mm). On the other hand, group 2 and group 3 had the inhibition zone classified as strong (more than 20 mm) (see Figures 4 and 5).

## Discussion

Extended-spectrum beta-lactamase (ESBL) is an enzyme that has the ability in hydrolyzing the antibiotics of the penicillin class, cephalosporin generation one, two, and three, and the monobactam class and cause resistance to all antibiotics. ESBL does not *hydrolyze cephamycin*, which has its own family close to cephalosporin, but it is inhibited by beta-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam. ESBL is generally inactive against carbapenem (imipenem, meropenem, and ertapenem) [9].

The cause of widespread nosocomial infection is MRSA. MRSA is a strain of *S. aures* that is resistant to β-lactam antimicrobials, among them are from the penicillin group. The mechanism of MRSA resistance occurs due to *S. aureus* that produces the gene-encoded Penicillin Binding Proteins

**Table 1:** Inhibition zone diameter for each treatment group against *E. coli* extended-spectrum beta-lactamase (ESBL).

| Treatment group | Inhibition zone diameter, mm | | | | Inhibition classification |
|---|---|---|---|---|---|
| | Replication 1 | Replication 2 | Replication 3 | Average | |
| Group 1 | 17 | 18 | 13 | 16 | Moderate |
| Group 2 | 38 | 39 | 37 | 38 | Strong |
| Group 3 | 33 | 33 | 33 | 33 | Strong |

ESBL, extended spectrum beta-lactamase.

**Table 2:** Inhibition zone diameter for each treatment group against Methicillin-Resistant *S. aureus* (MRSA).

| Treatment group | Inhibition zone diameter, mm | | | | Inhibition classification |
|---|---|---|---|---|---|
| | Replication 1 | Replication 2 | Replication 3 | Average | |
| Group 1 | 9.5 | 13.5 | 16 | 13 | Weak |
| Group 2 | 39 | 37 | 38 | 38 | Strong |
| Group 3 | 39 | 37 | 38 | 38 | Strong |

MRSA, Methicillin-Resistant *S. aureus*.

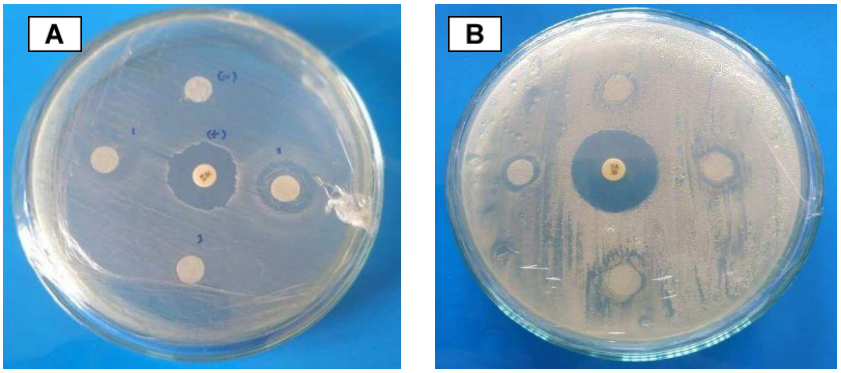


**Figure 4:** Antibacterial activity of group 1 against *Extended Strain Methicillin-Resistant (ESBL) E. coli* with vancomycin 10 µg as a positive control (A), the antibacterial activity of Group 1 methicillin-resistant *S. aureus (MRSA)* with vancomycin 10 µg as a positive control.

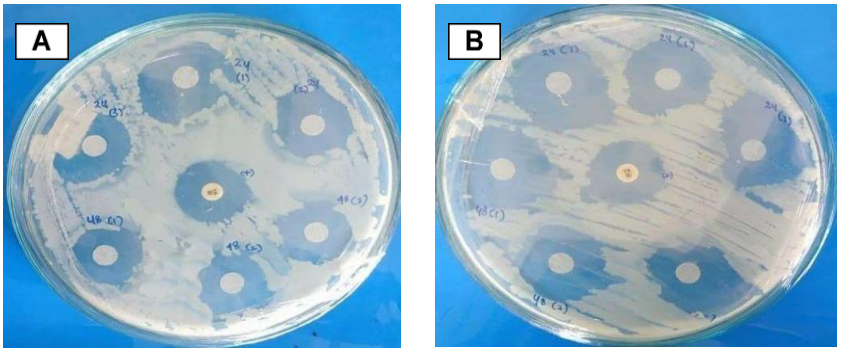


**Figure 5:** Antibacterial activity of group 2 and group 3 against *ESBL E. coli* with vancomycin 10 µg as a positive control (A), the antibacterial activity of group 2 and group 3 against methicillin-resistant *S. aureus (MRSA)* with vancomycin 10 µg as a positive control.

(PBP2 and PBP2a) mecA against all classes of antibiotics. The function of the PBP2 is stopped because giving β-lactam is compensated by PBP 2a resulting in wall synthesis cells on where MRSA takes place [10].

The antibacterial activity of the fermented red passion fruit filtrate is probably derived from organic acids and bacteriocins. It is based on previous research that red passion fruit contains probiotics [11]. Organic acids have been used for many years preservation of food and feed. In addition, the bacteriocin produced by probiotics also has a bactericidal effect. The bacteriostatic effect at similar or near related bacterial strains has already been put into use as an alternative to antibiotics in livestock. The combination of the use of organic acids and bacteriocins is able to produce an optimum inhibitory power against pathogenic bacteria [12]. In this study, the red passion fruit pulp is fermented in banana leaves first and then fermented in MRS-broth resulting in stronger inhibition against ESBL and MRSA compared to fermentation of passion fruit pulp in MRS-broth without fermented in banana leaves. Based on the result from this study, group 2 gives the best inhibition zone compared to group 1 and group 3. The fermentation time in group 3 (48 h) is not directly proportional to the increase in the inhibitory strength of ESBL and MRSA.

## Conclusions

Group 2 shows the best antibacterial activity against *E. coli* ESBL that is higher than group 1 and group 3 with an average inhibition zone of 38 mm classified as strong activity. Group 2 and group 3 show strong activity against MRSA with an average inhibition zone of both 38 mm. The inhibitory activity of group 1 against ESBL is categorized as weak (13 mm) and its activity against MRSA is categorized as a moderate potency with a diameter of growth inhibition zone of 16–20 mm.

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