

A Healthy Indonesian Traditional Food Against *Methicillin Resistant Staphylococcus aureus* Bacteria (MRSA)

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ABSTRAK

Pejang Pangi merupakan makanan tradisional yang terbuat dari komposisi bawang putih (Allium sativum), sereh (Cymbopogon citratus), pangi (Pangium edule). Makanan ini diyakini memiliki khasiat dalam bidang kesehatan namun belum ada bukti secara ilmiah. Oleh karena itu, pada penelitian ini, pejang wangi diuji kemampuan anti bakterinya terhadap bakteri Staphylococcus aureus yang resisten terhadap metisilin (MRSA). Pejang pangi diekstrak dengan menggunakan 3 pelarut yaitu n-heksan, etanol dan methanol, masing masing pada konsentrasi 5%, 10%, dan 15%. Ekstrak Pejang Pangi kemudian diuji menggunakan metode difusi dengan kertas cakram di atas media tumbuh dan diinkubasi selama 72 jam. Hasil Penelitian menunjukkan bahwa ekstrak makanan pejang wangi dengan pelarut metanol 15% yang diinkubasi selama 24 jam, 48 jam dan 72 jam memiliki kemampuan antibakteri dengan rata-rata zona hambat yang terbentuk adalah 15 mm, 13.67 mm, dan 13 mm. Sedangkan pelarut n-heksan dan etanol tidak memperlihatkan aktivitas anti bakteri. Daya hambat yang dibentuk ekstrak Pejang Pangi terhadap bakteri patogen uji tergolong sangat aktif, yaitu di atas 8 mm sehingga dapat diklaim bahwa makanan tradisional tersebut memiliki kemampuan anti bakteri.

Kata kunci: Aktivitas antibakteri, Pejang Pangi, Bakteri Methicillin Resisten, Staphylococcus aureus.

ABSTRACT

Pejang Pangi is a traditional food made from the composition of garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), pangi (*Pangium edule*). This food is believed to have health benefits but there is no scientific evidence. Therefore, in this study, pejangwangi was tested for its antibacterial ability against methicillin-resistant *Staphylococcus aureus* (MRSA). Pejang pangi was extracted using 3 solvents, namely n-hexane, ethanol and methanol, at concentrations of 5%, 10, respectively. %, and 15%. The extract of Pejang Pangi was then tested using the diffusion method with paper disc on growth media and incubated for 72 hours. The results showed that pejang fragrant food extract with 15% methanol solvent which was incubated for 24 hours, 48 hours and 72 hours had antibacterial ability with the average inhibition zones formed were 15 mm, 13.67 mm, and

13 mm. Meanwhile, n-hexane and ethanol did not show antibacterial activity. The inhibitory power formed by the extract of Penjang Pangi against the tested pathogenic bacteria was classified as very active, which was above 8 mm so it can be claimed that the traditional food had antibacterial ability.

Keywords: Antibacterial activity, Penjang Pangi, Methicillin Resistant, *Staphylococcus aureus*.

INTRODUCTION

Infectious diseases are the biggest problems in public health (Castelli & Sulis, 2017) to stop the spreading of infectious diseases especially caused by bacteria, antibiotics are often applied (Gao & Zhang, 2020). However, antibiotics are often used inappropriately for diseases that are not actually need to treat to antibiotics (Rowe & Linder, 2019) and could rise the resistance of some bacteria against antibiotics. The emergence of resistant bacteria such as *Methicillin Resistant Staphylococcus aureus* (MRSA) is caused by the use of antibiotics that are not based on indications (Turner et al., 2019). Sari et. al (2020) reported that endophytic fungi from elephant ginger rhizome were able to inhibit the growth of MRSA. In addition, ceplukan leaf extract was also reported to have antimicrobial activity of MRSA in vitro (Fitrianti et al. 2011). However, it has not been reported regarding the antimicrobial activity of traditional foods.

Penjang Pangi is a traditional food from the Regency of Soppeng, Province of South Sulawesi, Indonesia, which is made of Pangi (in english or latin), Garlic, Lemongrass, and Salt. *Penjang Pangi* has a slightly soft texture similar to tempeh texture. The characteristic of pejang pangi are (color, aroma, texture). Pangi is the main composition containing polyphenolic compounds such as cyanide and tannin, which can damage bacterial cell membranes (Dubey et al., 2018; Piekarska-Radzic & Klewicka, 2020). Garlic also contains allicin that can kill gram-positive and gram negative bacteria (Furner-Pardoe et al., 2020). Lemongrass (*Cymbopogon* sp) contains essential oils, namely geraniol, citronellal, vanillin, limonene, and kamfen (Kaur et al., 2021). Based on this composition, this traditional food is believed to have health benefits, but no scientific research has been conducted. Therefore, a study on traditional long food is needed to add to the scientific value of this food.

MATERIALS AND METHODS

Materials

The materials used in this study were *Penjang Pangi*, bacteria isolate MRSA, Sodium Agar, Mueller Hinton disc paper, filter paper, cotton, heat-resistant plastic, label paper, gauze,

aluminum foil, sterile distilled water, methanol, 90% ethanol, n-hexane, twen 80, and linezolid antibiotics.

Methods

a. Extraction of *Penjang pangi*

The extract was carried out by maceration using n-hexane, methanol, and ethanol with same manner. Approximately 1200 gr *Penjang pangi* was added n-hexan in a jar and mixed gently then left macerated for 3 days. The macerated mixture was then filtered using filter paper and a funnel, and concentrated using a rotary evaporator at 50 °C. The concentrated suspension was then used for concentration making. *Penjang pangi* extract was suspended into each solvent in concentrations of 5%, 10% and 15% v/v, respectively. The methanol extract was dissolved in sterile distilled water while the n-hexane and ethanol extracts were dissolved in sterile distilled water and 0.2 mL tween 80.

b. MRSA suspension equivalent to 108 CFU/ml

1 ose of bacterial culture was homogenized in 0.9% NaCl and 0.5 McFarland.

c. *Penjang Pangi* Extract against MRSA test.

This test was carried out by the diffusion method. The MRSA bacteria culture was spread with sterile cotton on the surface of the NA medium. Then, several wells were made for NA media and each *Penjang Pangi* extract was inserted into the wells. Then incubated for 24, 48 and 72 hour at 37°C.

RESULT

The extract of *Penjang Pangi* was obtained by maceration method using n-hexane, ethanol, and methanol as solvents. The results of the extract with n-hexane and ethanol as solvents are in the form of oil, while the results of the extracts with methanol as solvents are in the form of liquid. The test results of these extracts against MRSA bacteria are shown in Table 1.

Table 1. The results of inhibition with 36 treatments and 3 replications on MRSA bacteria.

No	Perlakuan	Ulangan			Rerata (mm)
		I	II	III	
1	A0B1C1	30	30	30	30
2	A0B1C2	30	30	30	30
3	A0B1C3	30	30	30	30
4	A0B2C1	30	30	30	30
5	A0B2C2	30	30	30	30
6	A0B2C3	30	30	30	30
7	A0B3C1	30	30	30	30
8	A0B3C2	30	30	30	30
9	A0B3C3	30	30	30	30
10	A1B1C1	0	0	0	0
11	A1B1C2	0	0	0	0
12	A1B1C3	0	0	0	0
13	A1B2C1	0	0	0	0
14	A1B2C2	0	0	0	0
15	A1B2C3	0	0	0	0
16	A1B3C1	0	0	0	0
17	A1B3C2	0	0	0	0
18	A1B3C3	0	0	0	0
19	A2B1C1	0	0	0	0
20	A2B1C2	0	0	0	0
21	A2B1C3	0	0	0	0
22	A2B2C1	0	0	0	0
23	A2B2C2	0	0	0	0
24	A2B2C3	0	0	0	0
25	A2B3C1	0	0	0	0
26	A2B3C2	0	0	0	0
27	A2B3C3	0	0	0	0
28	A3B1C1	0	0	0	0
29	A3B1C2	0	0	0	0
30	A3B1C3	0	0	0	0
31	A3B2C1	0	0	0	0
32	A3B2C2	0	0	0	0
33	A3B2C3	0	0	0	0
34	A3B3C1	16	14	15	15
35	A3B3C2	15	13	13	13.67
36	A3B3C3	13,5	13	12,5	13

Description:

A0 : Control

A1 : n-hexsane extract

A2 : Ethanol extract

A3 : methanol extract

- B1 : 5 % concentration
- B2 : 10 % concentration
- B3 : 15% concentration
- C1 : 24 hours incubation
- C2 : 48 hours incubation
- C3 : 72 hours incubation

Antibacterial activity can be known whether or not the inhibition zone on bacterial growth on solid media. The results showed that the zone of inhibition of the Penjang Pangi extract against MRSA was formed after incubation periods of 24, 48, and 72 hours. Positive control with linezolid antibiotic produced a clear zone of about 30 mm. Longan Pangi extract in ethanol and n-hexane did not show an inhibition zone. The extract of Penjang Pangi with methanol as a solvent did not completely provide an inhibition zone for the growth of the test bacteria. Only at a concentration of 15% has a clear zone. Penjang Pangi in methanol extract with a concentration of 15% at 24 hours incubation with an average diameter of 15 mm, 48 hours incubation 13.67 mm, and 72 hours incubation 13 mm (Figure 1).

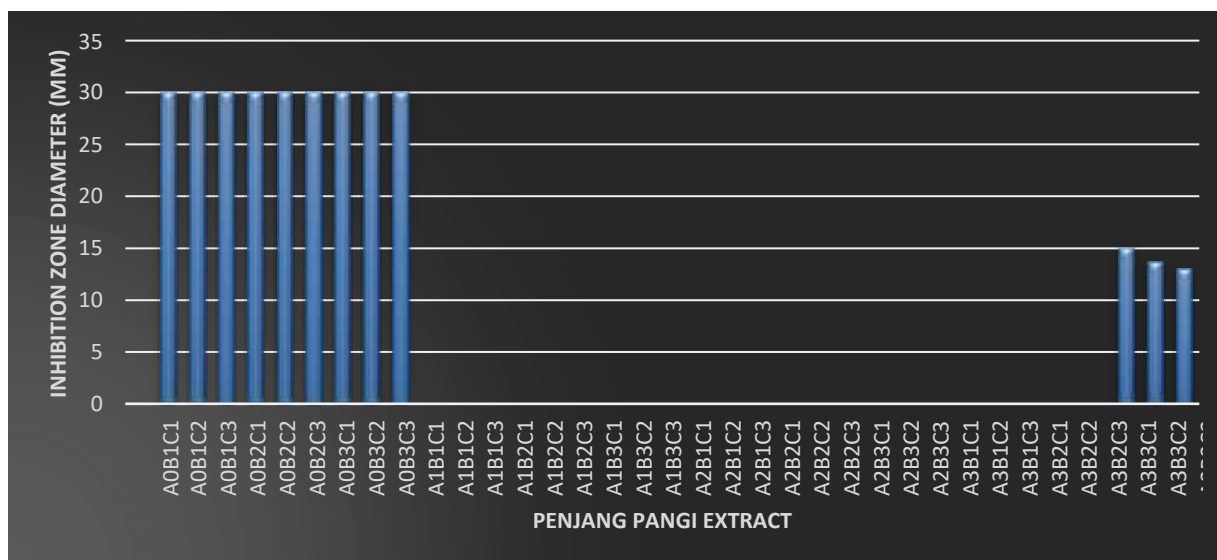


Figure 1. The diameter inhibition zone diameter formed by *Penjang Pangi* extract

DISCUSSION

Antibacterial activity test is a technique for measuring the a compound that can have an effect on growth bacteria. The method used in testing the antibacterial activity of a substance, namely diffusion method (Kourmouli et al., 2018). The method is carried out by using a certain glass which was inserted into solid media to form wells, and solid media has been inoculated with indicator bacteria, then in the well the antibacterial agent is added, then incubated, and a

clear zone will be formed around the well which indicates the formation of an inhibition zone of the substance antibacterial (Palanker et al., 2019) (Kourmouli et al., 2018).

Based on the test, it was known that the solvent extract of n-hexane and ethanol did not show an inhibition zone. This indicates that the two extracts did not have antimicrobial activity. This is presumably because n-hexane and ethanol are non-polar solvents (Escorsim et al., 2018). Thus, the phytochemical compounds contained in the extract are non-polar compounds. This is supported by the results of the second extraction in the form of oil and difficult to dissolve in water. The formation of oil extract in n-hexane solvent was due to n-hexane being a non-polar compound (Gu et al., 2017). al., 2014). So the yield of n-hexane and ethanol must be added with tween 80. When tween 80 was added, the n-hexane extract was homogeneous with distilled water (Akinyele et al., 2017). While the extraction with methanol solvent produces an extract in the form of water, this is because methanol is a polar solvent.

In this test, only the methanol solvent extract showed an inhibition zone. This is presumably because the antibacterial compounds in the *Penjang Pangi* extract are more easily soluble or attracted by polar solvents than non-polar solvents. The zone of inhibition produced by the methanol extract was classified as very active, according to Elgayyar et al (2001) which classified essential oils into three groups of zones of inhibition, namely very active (zone of inhibition >8 mm), active (zone of inhibition >6 and <8 mm), and inactive (zone of inhibition <6 mm) .

The inhibition formed by the methanol extract concentration of 15% of *Penjang Pangi* to MRSA bacteria was bacteriostatic. Bacteriostatic compounds are a group of compounds capable of inhibiting the growth and development of the test bacteria. Therefore, the extract could not kill all methicillin-resistant *Staphylococcus aureus* bacteria, although the greater the concentration of antibacterial compounds, the larger the diameter of the inhibition zone formed (Chakraborty et al., 2018). However, the size of the inhibition zone will decrease by day (Adepu & Khandelwal, 2017).

CONCLUSION

Penjang Pangi have ability on antibacterial activity after being tested in three solvents which are only *penjang pangi* extract in 15% of methanol solvent showing a very active inhibition zone against MRSA

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