Antimicrobial Activity Test of Mangosteen Leaves Ethanol Extract (Garcinia mangostana Linn) Against Pseudomonas aeruginosa Bacteria

R Suhartati¹*, F Apriyani¹, Khusnul¹, D P Virgianti¹, M Fathurohman¹

¹Departement of Medical Laboratory Technology STIKes Bakti Tunas Husada Tasikmalaya, 46115 Indonesia.

rsuhartati@yahoo.com

Abstract. Pseudomonas aeruginosa causes various infections such as skin infections. Mangosteen leaves contains active compounds that are useful in inhibiting bacterial growth. The purpose of this research is to examine the antibacterial activity of mangosteen leaves ethanol extract (Garcinia mangostana Linn) on the growth of Ps. aeruginosa bacteria. The experiment was conducted by an experimental method of Ps. aeruginosa bacteria using the Kirby-Bauer method. The concentration of ethanol extract from mangosteen leaves studied is 10% to 100% concentration with a bacterial density of 0.5 Mc Farland. The results showed that the ethanol extract of mangosteen leaves could inhibit bacteria from a concentration of 10% with an average inhibitory diameter is 13.20 mm, 20% is 14.00 mm, 30% is 14.65 mm, 40% is 15.85 mm, 50% is 16.05 mm, 60% is 16.90 mm, 70% is 17.55 mm, 80% is 18.75 mm, 90% is 19.25 mm and 100% equal to 24.80 mm. Based on the results of research and analysis date that has been carried out, it can be concluded that the ethanol extract of mangosteen leaves (Garcinia mangostana Linn) has antibacterial activity against Ps. aeruginosa, with a minimum inhibitory concentration of 10% with diameter inhibitor zone is 13.20 mm.

1. Introduction

Medicinal ingredients derived from medicinal plants can be in the form of leaves, stems, fruit, flowers and roots which have benefits as a medicine and are used as raw materials in the manufacture of modern medicine and traditional medicine [1]. One plant that has efficacy as a treatment is mangosteen.

Mangosteen (Garcinia mangostana Linn) is a functional plant because most of the plants can be used as medicine [2]. Xanthone compounds can be found on fruit peel, fruit, bark and mangosteen leaves. The first xanthone compounds that have been isolated are alpha mangostin and beta mangostin [3].

Mangosteen plants that have been widely used are parts of the fruit, fruit peel and stems, while mangosteen leaves have not been widely used as ingredients of traditional medicine, but mangosteen leaves contain antibacterial compounds. Mangosteen leaves have four active compounds, namely: flavonoids, tannins, alkaloids and saponins [4]. Study showed that ethanol extract of yellow mangosteen leaves (Garcinia dulcis) at a concentration of 80% had a strong inhibitory response to the...
activity of *Escherichia coli* and *Staphylococcus aureus* bacteria with inhibition zone diameters of 20.30 mm and 18.00 mm respectively [5].

Mangosteen leaf extract (*Garcinia mangostana* Linn) has an activity in inhibiting the growth of *E. coli* bacteria and the optimal concentration of mangosteen leaf extract in inhibiting the growth of *E. coli* bacteria is 80% concentration with an average diameter of 32.75 mm. Mangosteen peel extract (*Garcinia mangostana* Linn) had activity in inhibiting the growth of *Pseudomonas aeruginosa* bacteria and at a concentration of 80% mangosteen peel extract gave an inhibitory response with an average diameter of 11.75 mm [6].

Research on the antibacterial test of mangosteen leaves (*Garcinia mangostana* Linn) against *Pseudomonas aeruginosa* bacteria has never been done, *Pseudomonas aeruginosa* is a opportunistic bacterial pathogen. These bacteria can cause urinary tract infections (UTI), respiratory infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections, various systemic infections and are one of the bacteria that cause nosocomial infections [7].

Based on the description above, the researchers are interested in conducting research on the antibacterial activity test of mangosteen leaves ethanol extract (*Garcinia mangostana* Linn) against *Pseudomonas aeruginosa* bacteria.

2. Experimental and Methode

The methodology of this research is experimental research.

2.1 Tools and materials

The tools used are autoclave, stirring glass, blender, plastic spray bottle, bulp, petri dish, clinipet (10μL, 100μL and 1000μL), funnel, dry sterilizer, Erlenmeyer, measuring glass, beaker glass, microbiological incubator, electric balance, ose Ni Cr, oven, pipette, measuring pipette, tube rack, cotton swab, test tube, turbidimeter, laminar air flow, rotary evaporator.

The ingredients are mangosteen leaves, aquadest, BaCl$_2$, H$_2$SO$_4$, disc paper, Muller-Hinton Agar, physiological NaCl, 70% ethanol. The test bacteria used were *Pseudomonas aeruginosa* bacteria obtained from the Laboratory of Microbiology, Medical Laboratory Technology Diploma Study Program, STIKes BTH Tasikmalaya.

2.2 Preparation of mangosteen leaf ethanol extract

The dried mangosteen leaves that have been mashed as much as 100 grams and put in a glass chemistry added 70% ethanol 1 L into the beaker, then macerated for 24 hours. Maserat produced is separated by filtration. All the resulting maserat is collected, and then evaporated with rotary evaporator with low temperature (78°C) until obtained 100% thick extract then made various extract concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

2.3 Preparation bacterial suspension

Before making bacterial suspensions first, it needs to be standardized Mc. Farland 0, 5 is 9.95 ml of 1% H$_2$SO$_4$ added 0.05 ml of 1% BaCl (Mc Farland 1, 1907). After making the Mc. Farland Standart, just made a bacterial suspension described by the Departmen Kesehatan of Indonesia (1989) [8], which provided a clean and sterile test tube filled with 10 mL of 0.85% sterile NaCl. The suspension is made from pure strains of *Pseudomonas aeruginosa* colony in a way taken several colonies using ose. Then mixed in the tube until turbidity is the same as Mc. Farland 0.5. The suspension that has been made is estimated to have 1.5x10$^8$ / mL bacteria.

2.4 Antimicrobial Activity Test

Bacterial isolates from Nutrient Agar use antibacterial activity test is *Pseudomonas aeruginosa* with the Kirby-Bauer method. Muller-Hinton Media For a temperature of 45°C which is still liquid is poured as much as 12 mL (thickness ± 4-5 mm) into a sterile petri dish, then left to cool and freeze. Bacterial suspension *Pseudomonas aeruginosa* with a bacterial density of 1.5x10$^8$ / mL spread with a stirring glass into Muller Hinton medium. 50 μL extract on disc paper soaked in
mangosteen leaf ethanol extract for 30 minutes at various concentrations. Then put the disc paper that has been soaked on the agar which has been planted with bacteria and then incubated at 37°C for 24 hours. Processing, carried also making positive test (Media Muller Hinton + antibiotics chloramphenicol), negative control (Aquadest steril), and control media (Media Muller-Hinton). After incubation the observed presence of obstacles in the form inhibition zone (mm) around disc paper.

3. Results and discussion

Based on the results of the research on the activity test of mangosteen leaf ethanol extract (*Garcinia mangostana Linn*) on the growth of *Pseudomonas aeruginosa* bacteria by diffusion method on Muller-Hinton media planted for 24 hours, the results are as shown in Table 1.

![Image of inhibition zones for different concentrations](image)

**Figure 1.** The inhibitory zone formed in testing the antimicrobial activity of mangosteen leaves against the *Pseudomonas aeruginosa* bacteria in various concentration.
Table 1. Results of Antibacterial Activity Test of Mangosteen Leaf Ethanol Extract (Garcinia mangostana Linn) on Pseudomonas aeruginosa Bacterial Growth

| No. | Concentration (%) | Overall Inhibitory Zone Diameter (mm) | Average (mm) |
|-----|------------------|---------------------------------------|--------------|
| 1   | 10               | 13.10 13.30                           | 13.20        |
| 2   | 20               | 13.90 14.10                           | 14.00        |
| 3   | 30               | 15.30 14.00                           | 14.65        |
| 4   | 40               | 16.20 15.50                           | 15.85        |
| 5   | 50               | 16.50 15.60                           | 16.05        |
| 6   | 60               | 17.10 16.70                           | 16.90        |
| 7   | 70               | 17.20 17.90                           | 17.55        |
| 8   | 80               | 18.40 19.10                           | 18.75        |
| 9   | 90               | 19.90 18.60                           | 19.25        |
| 10  | 100              | 25.00 24.60                           | 24.80        |
| 11  | Positive Control (Cloramfenicol) | 19.20 18.60 | 18.90 |
| 12  | Negative Control (Sterile Aquadest) | - - | - |

Calculation of inhibitory power including diameter of disc paper (6 mm)

Based on data from Table 1. above can be seen that at all concentrations of ethanol extract of the leaves of mangosteen (Garcinia mangostana Linn) can inhibit the growth of Pseudomonas aeruginosa at a density of $1, 5 \times 10^8$ bacterial cell / mL. Based on the inhibition zone measurement analysis that 10% concentration of mangosteen leaf (Garcinia mangostana Linn) ethanol extract can inhibit the growth of Pseudomonas aeruginosa bacteria with an average inhibition zone diameter of 13.20 mm, followed by a concentration of 20% to 100%.

Based on the inhibition zone formed by testing using the mangosteen leaf ethanol extract (Garcinia mangostana L), when compared with the strength of Chloramphenicol, the inhibition zone concentration of 10% to 70% of mangosteen leaf extract (Garcinia mangostana L) against Pseudomonas aeruginosa bacteria is included in the range of intermediates. While inhibitory zones of 80% to 100% are included in the Sensitive range. Whereas when compared with the standard strength of the inhibitory zone. The mangosteen leaf extract with a concentration of 10% to 90% is in the strong category, the inhibition zone diameter is in the range of 11-20 mm, while the concentration of 100% is very strong because of the diameter. The inhibition zone is in the range of values of 21 mm or more Susanto (2012). Based on this, all concentrations of mangosteen leaf extract (Garcinia mangostana L) can be said to be effective in inhibiting the growth of Pseudomonas aeruginosa bacteria due to antibacterial activity in the strong category.

Similar to the research conducted by Julianti (2017) [4], stated that the mangosteen leaf extract (Garcinia mangostana Linn) has an activity in inhibiting the growth of E.coli bacteria the higher the concentration of mangosteen leaf extract the higher the inhibitory zone formed and the optimal concentration is 80% evenly average diameter of 32.75 mm. Aulia (2013) stated that mangosteen peel extract (Garsinia mangostana Linn) had activity in inhibiting the growth of Pseudomonas aeruginosa bacteria and at a concentration of 80% mangosteen peel extract gave an inhibitory response with an average diameter of 11.75 mm [6]. When compared with the results of the study Mustapa (2011) stated that the ethanol extract of yellow mangosteen leaves (Garcinia dulcis) at a concentration of 80% had a strong inhibitory response to the activity of Escherichia coli and Staphylococcus aureus bacteria with inhibition zone diameters of 20.30 mm and 18.00 mm [5].

The results obtained in this study are in accordance with the statement of Brooks et al (2005), that the effectiveness of an antibacterial agent is influenced by the concentration of substances given the higher the concentration the higher the active ingredient as antibacterial so as to increase the ability of its inhibitory effect on microbes [9].
The formation of the inhibitory zone is thought to have something to do with the active substance contained in the mangosteen leaf (*Garcinia mangostana Linn*) which plays an important role in inhibiting the growth of bacteria. The active substances contained in the mangosteen leaves (*Garcinia mangostana Linn*) are saponins, tannins, flavonoids and alkaloids. The phytochemical test results can be seen in table 2.

### Table 2. Phytochemical Test Results

| Content Test | Alkaloids |
|--------------|-----------|
|              | Favoronoid | Wagner reagent | Tannin | Saponin |
| Mayer reagent| +          | +               | +      | +       |

Flavonoids can perform inhibition mechanism by interfering with the bacterial cell wall synthesis thereby causing plasma leakage resulting in lysis of the bacteria [10]. Alkaloid can disturb the mechanism by means of a constituent component of peptidoglycan in the bacterial cell so that the cell wall layers are not fully formed and cause the death of the bacterial cell [11]. Tannin also has the capability of antibacterial activity by conducting a hydrophobic complex with the protein, inactivates the adhesin, enzymes and transport proteins that disrupt the cell wall of the microorganism growth [12]. Saponin compounds can perform an inhibitory mechanism by forming complex compounds with cell membranes through hydrogen bonds [13], so that they can destroy the permeability of cell walls and eventually lead to cell death [14].

The test bacteria used in this study are *Pseudomonas aeruginosa*, this is because the *Pseudomonas aeruginosa* bacteria causes various infections such as skin infections such as dermatitis, and is one of the bacteria that causes nosocomial infections. These bacteria can cause infection if the body's defense function is abnormal.

Mangosteen leaf extract (*Garcinia mangostana Linn*) can inhibit the growth of *Pseudomonas aeruginosa* bacteria which are Gram negative (-) bacteria consisting of one or more thin layers of peptidoglycan and membranes divided outside the peptidoglycan layer [15]. Because it contains only a small layer and does not contain acid, the cell wall of the Gram-negative bacteria (-) can be inhibited by antibacterial ingredients such as those contained in the mangosteen leaf ethanol extract (*Garcinia mangostana Linn*).

### 4. Conclusion

Based on the results of the research that has been done, it can be concluded that ethanol extract of mangosteen leaves (*Garcinia mangostana Linn*) has antibacterial activity against the growth of *Pseudomonas aeruginosa* bacteria and The minimum inhibitory concentration of the mangosteen leaf ethanol extract inhibiting the growth of *Pseudomonas aeruginosa* bacteria is a concentration 10% with an average inhibition zone diameter of 13.20 mm.

### 5. References

[1] Agusta 2000 *Minyak Atsiri Tumbuhan Topika Indonesia* (Bandung : ITB)
[2] Darmawansyih 2014 Khasiat Buah Manggis untuk Kehidupan. *Jurnal Al Hikmah* (XV) No 1. Makasar : UIN Alauddin Makasar. P. 61 and 64.
[3] Aldi, dkk 2016 Uji Efek Immunomodulator Dari Ekstrak daun Manggis (*Garcinia Mangostana L.*) dengan Metode Carbon Clearence dan Menghitung Jumlah Sel Leukosit Pada Mencit Putih Jantan. *Jurnal Farmasi Higae* STIFARM. 20-31.
[4] Julianti, Reska 2017 Pengaruh Ekstrak Daun Manggis (Garcinia mangostana L) terhadap Pertumbuhan Bakteri Escherichia coli sebagai Pengayaan Bahan Ajar Praktikum Mikrobiologi Pendidikan Biologi FKIP Universitas Jambi

[5] Mustapa, S.W, Boekoesoe, L dan Mustapa, M.A. 2011 Uji Aktivitas Antibakteri Ekstrak Etanol Daun Manggis Kuning (Garcinia dulcis) terhadap bakteri. KIM Fakultas Ilmu-Ilmu Kesehatan dan Keolahragaan Universitas Gorontalo.3(3) : 1-7.

[6] Aulia, H.Z.H. 2013 Uji Aktivitas antibakteri Ekstrak Etanol Kulit Manggis (Garcinia mangostana Linn) terhadap Pseudomonas aeruginosa Secara In Vitro. Fakultas Kedokteran Universitas Syiah Kuala.

[7] Supardi, H.I. dan Sukamto 1999 Mikrobiologi Dalam Pengolahan dan Keamanan Pangan (Bandung: Alumni)

[8] Departemen Kesehatan. 1989. Bakteriologi Umum. (Jakarta : Pusat Pendidikan Tenaga Kesehatan)

[9] Brooks, G.F., J.S. Butel dan S.A. Moorse 2005 Mikrobiologi Kedokteran (Jakarta:Salemba Medika)

[10] Chusnie, T.P.T. Lamb, AJ. 2005 Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agent. Vol.26, 343-356.

[11] Juliantina, R., Citra D.A., Nirawani, B., Nurmasitoh, T. & Bowo, E.T. 2009 Manfaat Sirih Merah (Piper Crocatum) Sebagai Agen Antibacterial Terhadap Bakteri Gram Positif dan Gram Negatif. J Kedokteran Kesehatan Indonesia, Yogyakarta.

[12] Hashem, F.M. & El-Kiey, M.A. 2002 Nigellasativa seeds of Egypt. Journal Of Pharmaceutical Sciences, 3 (1): 121-133.

[13] Cannell, R.J.P. 1998. Natural Products Isolation. (New Jersey : Human Press)

[14] Noer, I.S. dan L. Nurhayati 2006 Bioaktivitas Ulva reticulate Forsskal. Asal Gili Kondo Lombok Timur Terhadap Bakteri. Jurnal Biotika. Vol. 5 (1) : 45-60.

[15] Radji, M. 2011. Mikrobiologi. (Jakarta: Buku Kedokteran EGC)

Acknowledgment

The authors thank to Bakti Tunas Husada of Health Science College Tasikmalaya for the provision of the facilities to complete our research.