

ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT FROM INGGILI FRUIT (*Xylocarpus granatum*) AGAINST *Propionibacterium acnes* AND *Escherichia coli* USING WELL DIFFUSION METHOD

Wahyu Isnaini Nurhidayanti • Achmad Kadri Ansyori • Reksi Sundu

Received: 04 August 2023 | Revised: 14 September 2023 | Accepted: 23 September 2023 | Published: 15 December 2023
UPT Publication and Journal Management Uniska-JST 2023

Abstract Inggili is a type of mangrove plant that has been traditionally used by the community for medicine. It contains many active compounds that function as antibacterials. Based on empirical data, inggili fruit is used to remove acne on the skin and diarrheal diseases. This study aims to determine the antibacterial activity of methanol extract of inggili pulp in inhibiting *Propionibacterium acnes* and *Escherichia coli* bacteria using the sumuran diffusion method. The research conducted is experimental. The object under study was the antibacterial activity of inggili fruit extract against *Propionibacterium acnes* and *Escherichia coli* bacteria. Antibacterial activity was tested using the sumuran diffusion method with extract concentrations of 10%, 15%, and 20%. The negative control used was 1% DMSO and the positive control used was 0.1% chloramphenicol.

Keywords:: Inggili • *Propionibacterium acnes* • *Escherichia coli* • well diffusion method



This is an open-access article under a Creative Commons Attribution 4.0 International (CC-BY 4.0) License. Copyright © 2023 by authors.

✉ Achmad Kadri Ansyori
achmad.kadri.ansyori@gmail.com

Prodi Sarjana Farmasi, Sekolah Tinggi Ilmu
Kesehatan Samarinda, Indonesia

Introduction

Mangrove plants are one of the plants that can live in extreme environments, where the physical and chemical properties of their habitats always change as a result of tides, freshwater or rivers, silt deposition, decomposition of organic matter, and others so that this plant has excellent potential for research on secondary metabolite compounds it contains (Fahlifi et al., 2018).

Secondary metabolites are defined as compounds synthesized by organisms such as microbes, plants, insecticides, and so on (Maradona, 2013). *Xylocarpus granatum* mangroves have seeds, fruits, and tree bark that are useful as drugs for various types of diseases because they contain secondary metabolite compounds (Kumoro, 2015). The content of flavonoids, tannins, and saponins prevents the risk of skin hyperpigmentation due to exposure to ultraviolet radiation from sunlight. Phytochemical components in bark and stem extracts, both consist of alkaloids, flavonoids, and tannins (Hendrawan et al., 2015; Adelberg et al., 2008).

Propionibacterium acnes is a normal bacterial flora of the skin but can be the cause of opportunistic infections in the form of acne (Nakase et al., 2017). According to the Indonesian cosmetic dermatology study, there was an increase in the incidence of acne which showed 60% of acne sufferers in 2014, 80% in 2015, and 90% in 2016 (Zahrah et al., 2019).

Escherichia coli is a gram-negative bacterium, a normal flora germ found in the

human large intestine. *Escherichia coli* often causes infections of the urinary tract, bile ducts, and other places in the abdominal cavity. *Escherichia coli* is also a cause of diarrhea and urinary tract infections (Erlangga, 2016).

The use of good natural materials as topical therapy, one of which is mangrove plants, namely *inggili* plants. Based on the research of (Gabariel et al., 2019), the results of the inhibitory test of *inggili* plant extracts (leaves, fruits, stems) have antibacterial inhibitory against the growth of pathogenic bacteria (*P.aeruginosa*, *E.coli*, and *V.alginolyticus*). Although the resistance zone formed differs markedly for each extract concentration, the resulting inhibitory power is directly proportional to the increase in concentration. The higher the concentration, the higher the bacterial resistance zone formed. This is thought to be because with every increase in concentration, the amount of bioactive compounds increases so that the ability to inhibit bacterial growth increases.

Based on the above background, it is necessary to do a study testing the antibacterial activity of methanol extract of *inggili* fruit against *Propionibacterium acnes* and *Escherichia coli* bacteria using the sumuran diffusion method.

Materials and Methods

The peel of the fruit as much as 2 kg obtained is carried out wet sorting, aiming to separate the skin of the fruit from the seed. Washed using running water to separate impurities attached to the fruit, then cut to reduce the sample size, so that the drying process is faster than dried by aerating the aim is to reduce the moisture content so that simplisia is not easily damaged and can be used in the long term. Simplisia that has been dried is ground into coarse powder using a blender and sifted using a mesh 60 sieve and 400 mg of fine powder is obtained. This research stage starts with determination, collection of raw materials, making simplisia, and making extracts with methanol solvents, to determine antibacterial activity. The study was conducted in the laboratory of the Samarinda

College of Health Sciences from April to June 2023.

Tools include measuring cups, stirring rods, erlenmeyer, test tubes, test tube racks, drip pipettes, autoclaves, blenders, incubators, cameras, spirit lamps, ose needles, analytical scales, vortexes, calipers, Laminar Air Flow, label paper, tube tongs, petri dishes, perforators and tweezers. Materials include Inggili fruits, Nutrient Agar (NA), clindamycin 1%, bred *Propionibacterium acnes* and *Escherichia coli*, DMSO 1,175%, 95% methanol, screen paper, gauze, *chloramphenicol* 0,1%, cotton, H₂SO₄, BaCl₂, aluminium foil, and cotton bud.

Extraction

The skin of the *inggili* fruit is taken on a large fruit. The fruit skin used in this study was obtained from Tepian Village, Sembakung District, Nunukan Regency, North Kalimantan Province. Weighing fruit simplisia 300 g of simplisia powder was extracted by maceration using 2 L of methanol solvent. Stirring was carried out for the first 2 hours, then soaked for 24 hours and filtered. Remaceration is carried out with 1 L of methanol stirred and soaked for 24 hours. All the maserat obtained is evaporated until a viscous extract is obtained (Mukhriani, 2014).

Phytochemistry Screening

Alkaloid

Meyer reagent of 5 drops of filtrate, put into a test tube added 2-3 drops of HCl 2N and 2 drops of Meyer reagent. When a white or yellow precipitate is formed, it indicates the presence of alkaloid compounds. Bouchardat reagent of 5 drops of filtration, put into the test tube added 2-3 drops of HCl 2N and 2 drops of Bouchardat reagent. If a brown-to-black precipitate is formed, it indicates the presence of alkaloid compounds. Dragendrof reagent as much as 5 drops of filtrate, put into a test tube added 2-3 drops of HCl 2N and 2 drops of dragendrof reaction. If an orange-to-red-brown precipitate is formed, it indicates the presence of alkaloids (Pardede et al., 2013).



Flavonoid

A total of 5 drops of filtrate were added 0.1 g Mg powder and concentrated hydrochloric acid 1 mL, amyl alcohol 2 mL, shaken and left to separate. 28 Flavonoids are positive if red, yellow, and orange colors form in the amyl alcohol layer (Pardede et al., 2013).

Tanin

A total of 10 drops of filtrate, added aquades until the color fades, added 2-3 drops of FeCl reagent, if a blue or blackish-green color is formed, it shows the presence of tannin compounds in the extract (Dewatisari et al., 2018).

Saponin

A total of 10 drops of filtrate, and 5 drops of hot water are added and then cooled, then shaken vigorously for ± 10 seconds. If a lot of foam is formed for 10 minutes as high as 1 cm to 10 cm does not disappear with the addition of hydrochloric acid 2N 1 drop, then it indicates the presence of saponin compounds (Pardede et al., 2013).

Antibacterial activity

Antibacterial activity testing used several concentrations with two kind of bacteria, which include *Propionibacterium acnes* and *Escherichia coli*. Antibacterial activity method following the previous reported research (Mpila et al., 2012; Nugroho and Widayati, 2013).

Results and Discussion

Extraction has been carried out and produced methanol extract from the skin of the inggili fruit. Data from phytochemical screening showed that the extract contained flavonoid compounds and alkaloids, but specifically, alkaloids were only positive using dragendrof tests while alkaloid tests with other reagents gave negative test results. This indicates that the alkaloid content in the extract is not able to be detected properly because the intensity or quantity of alkaloids contained in the extract is only small. Other tests such as tannins and saponins showed negative results or the extract did not contain these compounds (table 1). This phytochemical screening data shows that the compounds responsible for antibacterial activity are flavonoids because alkaloids are difficult to detect due to their small intensity or quantity in the extract.

Flavonoids are polar compounds that dissolve in polar solvents such as methanol (Marjoni, 2016). Flavanoids have effective properties to inhibit bacterial growth by damaging the bacterial cell membrane in the phospholipid section to reduce permeability which causes bacteria to be damaged so that it affects the inhibitory zone formed from antibacterial activity tests.

Table 1. Phytochemical Screening Results of Methanol Extract of Inggili Fruit

No	Compound Test	Reagent	Result	Detail
1.	Alkaloid	Mayer	-	No precipitate
		Bouchardhat	-	No precipitate
		Dragendrof	+	Orange precipitate
2.	Flavonoid	HCl, Mg, Amil alcohol	+	orange coating on amyl alcohol
3.	Tanin	FeCl 1%	-	blackish green color
4.	Saponin	HCl 2N	-	No foam

(+) Positive results and (-) Negative results

Antibacterial Activity

Antibacterial activity was carried out with several concentrations, which aimed to determine the sensitivity of the methanol extract

of Inggili fruit peel to the growth of *Propionibacterium acnes* and *Escherichia coli*



bacteria. This method was chosen because it has the advantage that the procedure is simple, easy, and practical to perform can also be used to see the sensitivity of various microbes to antimicrobials at certain concentrations and is often used in antibiotic sensitivity tests in quality control programs (Mpila et al., 2012; Melliawati, 2014; Mulia, 2018).

In this study, antibacterial testing used several series of concentrations of methanol extract of *inggili* fruit peel which aimed to determine the effect of each concentration on *Propionibacterium acnes* and *Escherichia coli* bacteria. Methanol extract from *inggili* fruit peel

is made into 3 different concentrations namely 10%, 15%, and 20%. The positive control used in this test was chloramphenicol while the negative control used was DMSO 1%. This antibacterial activity test uses the sumuran diffusion method, the reason for choosing this method is because it has advantages that are easy to do and relatively cheap. The results of the antibacterial activity test of methanol extract of *inggili* fruit against *Propionibacterium acnes* and *Escherichia coli* bacteria with concentrations used namely 10%, 15% and 20% can be seen in Table 2.

Table 2. Results of bacterial inhibition zones of *Propionibacterium acnes* and *Escherichia coli*

Extract Concentration (%)	<i>P. acne</i> (mm)	Average (mm)	<i>Escherichia coli</i> (mm)	Average (mm)	Categories of Inhibition zone
10	15	14,3	15	14,3	Very strong
	14		14		
	14,5		14		
15	15	15	14,5	14,6	Very strong
	15		14,5		
	15		15		
20	14,5	15	15	15,3	Very strong
	15		15,5		
	15,5		15,3		
Control (+)	33		33		Very strong
Control (-)	0		0		None

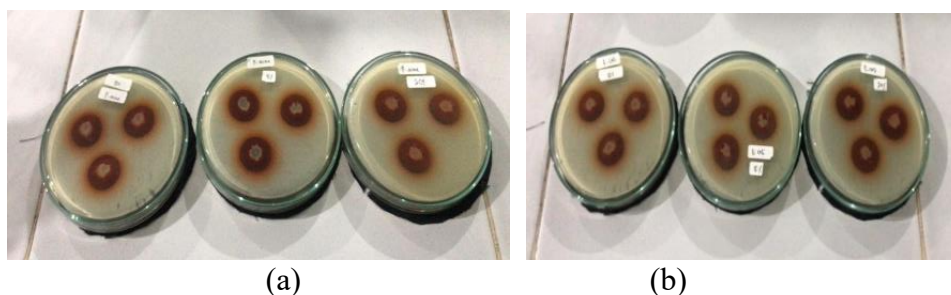


Figure 1. Inhibition Zone of Methanol extract of Inggili Fruit Skin against a. *Propionibacterium acnes* and b. *Escherichia coli*

The parameter measured in the antibacterial activity test is the formation of an inhibitory

zone around the well that has been inserted with a solution of methanol extract

concentration of inggili fruit peel (figure 1). The antibacterial activity test showed that *Propionibacterium acnes* bacteria with an extract concentration of 10% had an inhibitory zone diameter of mm 14.3 and at an extract concentration of 15% an inhibitory zone diameter of 15 mm was formed and at an extract concentration of 20% the diameter of the inhibitory zone obtained was 15 mm. The results of antibacterial activity tests show that the skin of the inggili fruit also has activity as an

inhibitor of *Escherichia coli* bacteria, at a concentration of 10% the extract of the inggili fruit has an inhibitory zone diameter of 14.3 mm, and at a concentration of 15% has an inhibitory zone diameter of 14.6 mm, and at a concentration of 20% the diameter of the inhibitory zone is 15.3 mm. Antibacterial activity increased with increased concentrations from 10%, 15%, to 20% but still below the antibacterial activity of positive controls.

Conclusion

Methanol extract of *inggili* fruit peel (*Xylocarpus granatum*) contains flavonoids responsible for antibacterial activity using bacteria *Propionibacterium acnes* and *Escherichia coli* at concentrations of 10%, 15%, and 20%.

References

- Adelberg, Jawetz, Melnick. (2008). *Medical Biology*. Edisi 23. Jakarta: Penerbit Buku Kedokteran EGC.
- Kumoro, A., C. (2015). Teknologi ekstraksi senyawa bahan aktif dari tanaman obat. Yogyakarta: Plantaxia.
- Dewatisari, W. F., Rumiyan, L. and Rakhmawati, I. (2018). Rendemen dan Skrining Fitokimia pada Ekstrak Daun *Sansevieria sp.* *Jurnal Penelitian Pertanian Terapan*, 17(3), 197.
- Erlangga, S. (2016). Analisis Cemarkan Mikroba Pada Sampel Simplisia Sambilo, Temulawak dan Kunyit di Tiga Tempat Penjualan Simplisia di Purbalingga. *Skripsi*.
- Fahlifi, M. R., Efizon, D. and Adriman. (2018). Analisis Keberlanjutan Ekosistem Mangrove Di Desa Sungai Bela Kabupaten Indragiri Hilir. *Terubuk*, 46(3), 4-47.
- Gabariel, E., Yoswaty, D. and Nursyirwani. (2019). Daya Hambat Ekstrak *Xylocarpus Granatum* terhadap Bakteri Patogen inhibitor of *Escherichia coli* bacteria, at a concentration of 10% the extract of the inggili fruit has an inhibitory zone diameter of 14.3 mm, and at a concentration of 15% has an inhibitory zone diameter of 14.6 mm, and at a concentration of 20% the diameter of the inhibitory zone is 15.3 mm. Antibacterial activity increased with increased concentrations from 10%, 15%, to 20% but still below the antibacterial activity of positive controls.
- Pseudomonas aeruginosa, *Escherichia coli*, dan *Vibrio alginolyticus*’, *Jurnal Perikanan dan Kelautan*, 24(2), 114-118.
- Hendrawan., Zuraida., I. and Pamungkas, B. F. (2015). Preliminary Studies of Antibacterial Activity Methanol Extracts of *Xylocarpus granatum* from the Coastal of Muara Badak’, *Jurnal Ilmu Perikanan Tropis*, 20(2), 15-22.
- Marjoni R, (2016). Dasar-Dasar Fitokimia Untuk Diploma III, Jakarta.
- Maradona, D. (2013). Uji Aktivitas Ekstrak Etanol Daun Durian (*Durio zibethius L.*), Daun Lengkek (*Dimocarpus longan Lour*), Daun Rambutan (*Nephelium lappaceum L*) Terhadap Bakteri *Stertococcus Aureus* ATCC 25925 dan *Escherichia coli* ATCC 25922. *Skripsi*, Fakultas Kedokteran dan Ilmu Kesehatan, UIN Syarif Hidayatullah Jakarta.
- Melliawati, R. (2014). *Escherichia Coli: Escherichia coli*, Encyclopedia of Food Microbiology: Second Edition, 4(1), 688-694.

- Mukhriani (2014). Ekstraksi, Pemisahan Senyawa, Dan Identifikasi Senyawa Aktif, *Jurnal Kesehatan UIN Alauddin*, 7(2), 361-367.
- Mulia, S. (2018). In Vitro Effect of MRSAc in Bacteriocins Produced from MRSA on Propionibacterium Acnes Comparing with Antibiotics, *Journal of Global Pharma Technology*, 10, 214-221.
- Mpila., D. A., et al. (2012). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mayana (*Coleus atropurpureus (l) Benth*) Terhadap *Staphylococcus aureus*, *Escherechia coli* dan *Pseudomonas aeruginosa* Secara In Vitro.
- Nakase, K., et al. (2017). *Propionibacterium acnes* is developing gradual increase in resistance to oral tetracyclines, *Journal of Medical Microbiology*, 66(1), 8-12.
- Nugroho, R., and Widayati, R. (2013). Terapi Topikal Clindamycin Dibandingkan Dengan Niacinamide + Zinc Pada Acne Vulgaris, *Jurnal Kedokteran Diponegoro*, 2(1), 108796.
- Pardede, A., Manjang, Y., & Efdi, M. (2013). Skrining fitokimia ekstrak metanol dari kulit batang manggis (*Garcinia cymosa*). *Media Sains*, 6(2), 60-66.
- Zahrah, H., Mustika, A. and Debora, K. (2019). Aktivitas Antibakteri dan Perubahan Morfologi dari Propionibacterium Acnes Setelah Pemberian Ekstrak Curcuma Xanthorrhiza, *Jurnal Biosains Pascasarjana*, 20(3), 160.