

Research Article

Potential Antibacterials of Merang Padi Extract (*Oryza sativa*) on the Growth of Gram Positive and Gram Negative Bacteria

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ABSTRACT

ARTICLE INFO

Submitted : 21 Feb. 2023

Revised : 03 Mar. 2023

Accepted : 09 Mar. 2023

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Abstract: Padi straw (*Oryza sativa*) is one type of stem produced from padi tree trunks. Antibacterial activity testing was carried out by agar diffusion method using paper disc as a backup by observing the formation of a clear zone indicating the presence of antibacterial activity. Variations in the concentration of the padi straw extract test solution were made with a %w/v ratio between 100% pure padi straw extract and DMSO adjusted at various test concentrations, namely, 5%, 10%, 15%, 20%, and 25%. The results showed that the n-hexane fraction of padi straw did not have antibacterial activity because there was no inhibition zone or kill zone indicated by the fraction at both low and high concentrations. While the ethanol extract showed antibacterial activity at a concentration of 15%, the ethyl acetate fraction and the n-butanol fraction had antibacterial activity indicated by the presence of an inhibitory zone at a concentration of 7%. The results of the t-test on the ethanol extract of the test bacteria obtained a Tcount value of 14,687 which means it is very significant or very significantly different because Tcount is greater than Ttable (5% and 1%). This shows that the oxytetracycline antibiotic is better than the potency of the ethanol extract of straw on the test bacteria.

Keywords: Antibacterial; *Oryza sativa*; merang padi

INTRODUCTION

Indonesia is a country rich in biodiversity (a Mega Biodiversity Country) where there are approximately 30,000 species of plants spread throughout the country, around 9,600 species have medicinal properties and approximately 300 species are used as ingredients for traditional medicine by the traditional medicine industry. Therefore, the biodiversity in Indonesia is an asset and resource that must be maintained and managed in order to become an ancestral heritage and benefit the community for health maintenance.

Nowadays the use of traditional medicine is increasingly widespread, one of the alternative treatments that can be used is natural ingredients derived from plants. This is corroborated by data from the WHO (World Health Organization) issued in July 2002 which states that in Indonesia currently around 40% of the Indonesian population uses traditional medicine and 70% resides in rural areas (Nuryani and Jhunnison 2017). Skin diseases in Indonesia are generally caused by bacterial, fungal, viral infections, and because of an allergic basis, in contrast to western countries which are heavily influenced by degenerative factors (Agustina, Mustafidah, and Purbowati 2016). Infection is the process of entry of microorganisms in the form of viruses or bacteria into body cells that cause an immune reaction.

Infectious diseases are a group of disease types that easily attack children and are caused by viral infections, bacterial infections, and parasitic infections. Disease infection is one of the most common health problems in developing countries, including Indonesia (Noor Mutsaqof and Suryani 2016). The host fights infection with the immune system which is an innate response, in the form of an inflammatory process followed by an adaptive response. To fight infectious diseases, drugs can be used (Soedarto 2015). Infectious diseases in Indonesia that are influenced by climate are also supported by several other factors, such as lack of public awareness of hygiene, dense population, lack of knowledge and implementation of most people regarding the basis of infection, unsafe procedures, inappropriate use of antibiotics, and lack of guidelines and policies from the government regarding the use of antibiotics (Nursidika, Saptarini, and Rafiqua 2014). These infectious diseases according to Irianto (2014) are mostly caused by pathogenic bacteria. Pathogenic bacteria are microorganisms or other larger organisms capable of causing disease. The ability of pathogenic bacteria to cause disease is not only influenced by the components present in the microorganism, but also by the ability of the host to fight infection. Infectious diseases are also still a major health

problem. Some of the pathogenic bacteria that cause infection in Indonesia are *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Padi straw (*Oryza sativa*) is one type of stem produced from padi tree trunks. The benefits of padi straw have actually long been known in Indonesia. Since ancient times, padi straw have been widely used by royal princesses to treat hair (Suriani 2018). Straw or rice waste can be used as a preservative because it contains potassium sodium, an anti-microbial substance that can inhibit decay (Yulia 2017). There are still many studies related to the activity of padi straw extract (*Oryza sativa*) that have not been explored and researched. So this research was carried out with the aim of determining the potential of padi straw extract as an antimicrobial on the growth of gram positive and gram negative bacteria.

MATERIALS AND METHODS

Material

The tools used were a set of glassware, maceration vessel, rotary evaporator (Buchi), chromatography tools were chromatography chamber, oven (Mettler), analytical balance (Fujitsu), waterbath (Mettler).

The materials used are rice straws that have been burned, ethanol 96% merck solvent, n-hexane solvent, ethyl acetate and n-butanol merck, Dimethyl sulfoxide as an additive in the manufacture of extract preparations of various concentrations. This study used gram positive bacteria, namely *Staphylococcus aureus* and gram negative, namely *Pseudomonas aeruginosa* licensed ATCC 25923 which had been rejuvenated on Saboraud Dextrose Agar (SDA) media.

Method

This type of research is experimental research in the laboratory. The research includes padi straw that has been burned, made into ethanol extract, dried. The results were fractionated with n-hexane, ethyl acetate, and n-butanol. The crude extract and fraction were dried and continued with a post test only control group design, in which the experimental group was treated with different concentrations of padi straw extract (*Oryza sativa*) and the control group was not. Antibacterial potential testing was carried out by observing the presence of an inhibitory zone formed in the experimental group which was only carried out once after treatment.

Preparation of Padi Straw Extract

Padi straw simplicia was macerated in 96% ethanol with the ratio of simplicia and solvent 1:7.5. Maceration was carried out for 3x24 hours with stirring every 4 hours for 15 minutes, the ethanol solvent was changed

every 24 hours. The extraction results were then filtered with filter paper to obtain filtrate and residue, filtering was carried out 3 times so that no residual was included. The extraction results obtained for three days are mixed together and then evaporated using a rotary vacuum evaporator or can also use a water bath at a temperature of 60°C to obtain a thick extract.

Fractionation

The fractions were made by liquid-solid extraction successively from n-hexane, ethyl acetate, and n-butanol each 200 ml.

Making Variations of Extract Concentration

Variations in the concentration of the padi straw extract test solution were made with a %w/v ratio between 100% pure padi straw extract and DMSO adjusted at various test concentrations, namely, 5%, 10%, 15%, 20%, and 25%.

Table 1. Variation of Padi Straw Extract Concentration

Extract Concentration	Extract Weight	DMSO (ml)
5%	0.1	2
10%	0.2	2
15%	0.3	2
20%	0.4	2

Antibacterial Activity Test

The most widely used method in antimicrobial testing is the agar diffusion method. In this method, the antimicrobial substance will diffuse on the agar plate which is planted with the test microbe and affect the growth of the test microbe. The basis for the observation is to see whether or not a zone of inhibition (radical zone) is formed around the paper disc containing antimicrobial substances with various test levels after incubation.

RESULT AND DISCUSSION

Merang padi (*Oryza sativa*) is one of the plants that we often encounter, as a staple food for humans, growing in the tropics. This study aims to determine the yield of the extract, the antibacterial activity, the effective concentration and which compounds have antibacterial potential against gram-positive *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa*.

Antibacterial activity testing was carried out by agar diffusion method using paper disc as a backup by observing the formation of a clear

zone which indicated the presence of antibacterial activity both killing and inhibiting the growth of the test bacteria. The data obtained were then analyzed using the one-way ANOVA method to determine the effect of variations in the concentration of the test in killing or inhibiting bacterial growth, then followed by an appropriate BNT analysis to determine the concentration of the extract which was effective as an antibacterial.

Staphylococcus is a gram positive, catalase positive, nonmotile aerobic or facultative aerobic bacterium, and cannot form spores. This microorganism is in the form of a coccus with a diameter varying from 0.7 to 1.2 μm . *Staphylococcus aureus* forms clusters, like a bunch of grapes, when seen by inspection microscopic. There are 32 species of *staphylococcus*, but only 17 species affect humans. *Staphylococcus aureus* is the only one that forms coagulase (Garna, Shahroddji, and Anggraini 2012). *Staphylococcus aureus* is heat resistant up to 50°C, with a high salt content and is resistant to drought. Colonies of *Staphylococci* are large, it has a diameter 6-8 mm and clear in color. Many strains of this bacterial colony form pigments that are ivory or orange in color. *Pseudomonas aeruginosa* is a very adaptable organism and can use 80 different organic groups for its growth and ammonia as a nitrogen source (Entjang 2003). The taxonomy of *Pseudomonas aeruginosa* bacteria is kingdom *Bacteria*, phylum *Protobacteria*, order *Pseudomonadales*, family *Pseudomonadaceae*, genus *Pseudomonas*, species *Pseudomonas aeruginosa* (Irianto 2014).

Pseudomonas aeruginosa can grow well at 37-42°C. Growth at these temperatures helps distinguish this species from other *Pseudomonas* species in the fluorescence group. These bacteria are oxidase positive, and not ferment carbohydrates, but many strains can oxidize glucose. Identification is usually the presence of a characteristic pigment, and growth at 42°C. To distinguish *Pseudomonas aeruginosa* from other *Pseudomonas* species based on their biochemical activity, testing with various substrates is required (Jawetz, Melnick, and Adelberg 2005). Humans carry *Staphylococcus aureus* in their nasal cavities and skin (Soedarto 2015).

The results obtained were then analyzed by using the Ttest to compare the antibacterial potential of the padi straw (*Oryza sativa*) extract with a substitute antibiotic, namely oxytetracycline.

Yield

Padi straw (*Oryza sativa*) extract is an extract from the extraction process using the maceration method, namely soaking 400 g dry samples using 3 L of ethanol as a solvent at room temperature. The maceration solution was then concentrated with a rotary evaporator, the concentrated extract was then evaporated in a water bath to obtain an extract with a minimum water content of 84.4 g of coarse padi straw (*Oryza sativa*) extract.

The extract that has been obtained is then weighed 20 g and then liquid-solid fractionation is carried out. The solvents used were n-hexane, ethyl acetate, and n-butanol, respectively. The results obtained were 2.91 g of n-hexane fraction, 4.26 g of ethyl acetate fraction, and 6.14 g of n-butanol fraction.

Table 2. Yield Data of Padi Straw Extract

Sample	Yield (%) Padi Straw Against					
	Fresh Sample (3200g)	Dry Sample (400g)	Crude Extract (84.4g)	Fraction of n-hexane (2.91g)	Ethyl acetate fraction (4.26g)	Fraction of n-butanol (6.14g)
Fresh Sample	-	-	-	-	-	-
Dry sample	12.5	-	-	-	-	-
Extract ethanol	2.63	21.1	-	-	-	-
n-hexane fraction	0.09	0.72	3.44	-	-	-
Ethyl acetate fraction	0.13	1.06	5.04	-	-	-
Fraction of n-butanol	0.19	1.53	7.27	-	-	-

Based on the table 2, the results show that the crude padi straw extract contains more polar compounds than non-polar compounds.

Antibacterial Activity of Padi Straw Extract

Determination of the concentration series of the antibacterial activity test was carried out by the agar diffusion method. The concentration series made are 5%, 10%, 15%, and 20%. Based on the results of the tests that have been carried out, the following results are obtained:

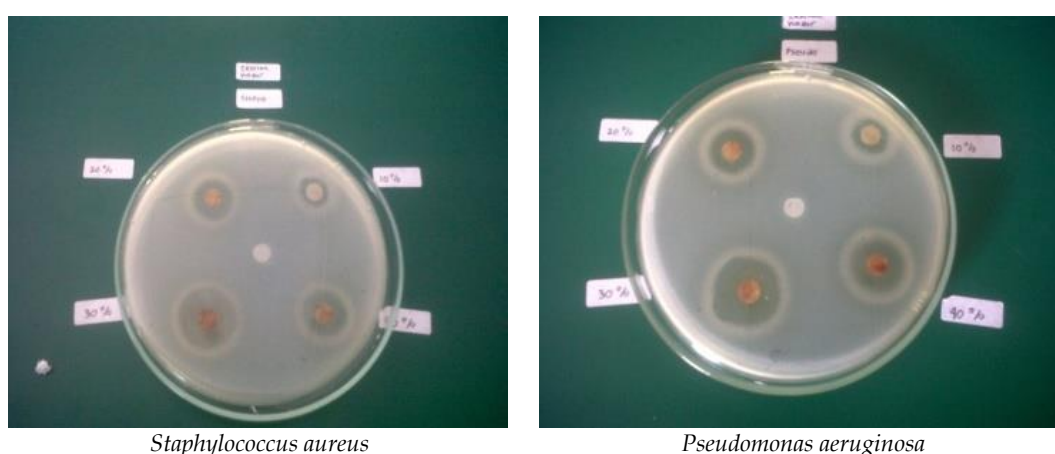
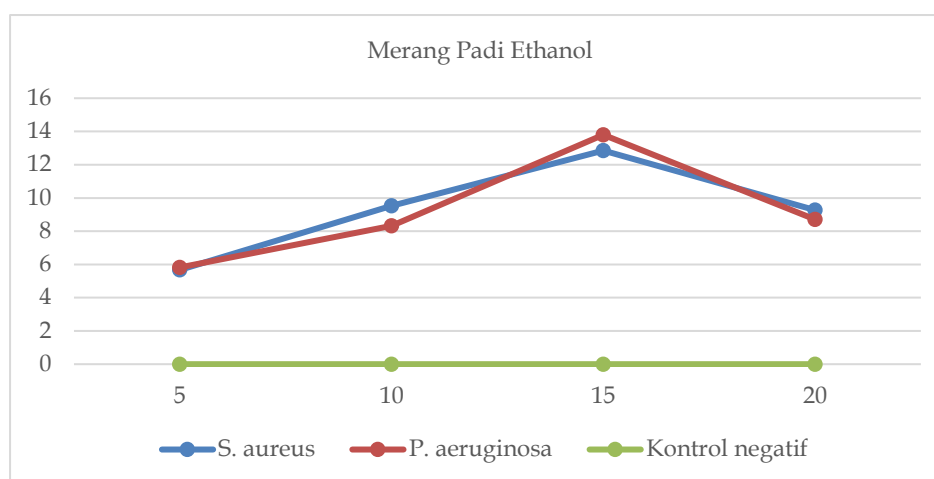


Figure 1. Ethanol Extract

Table 3. Clear zone data for Merang Padi Ethanol Extract

Test Bacteria	Concentration	Average Clear Zone Diameter (mm)	
		Ethanol	Control (-)
<i>S. Aureus</i>	5%	5.67	0
	10%	9.52	0
	15%	12.85	0
	20%	9.27	0
<i>P. aereuginosa</i>	5%	5.82	0
	10%	8.33	0
	15%	13.80	0
	20%	8.71	0

The ethanol extract of padi straw (*Oryza sativa*) had antibacterial activity in inhibiting or killing *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria, while water as a negative control did not have an inhibitory effect on both gram-positive and gram-negative bacteria.

**Figure 2.** Graph of Antibacterial Activity of Merang Padi Ethanol Extract

Merang Padi Fraction

The results of antibacterial testing of the fractions of n-hexane, ethyl acetate, and n-butanol of padi straw compared with negative controls can be seen in the following table:

Table 4. Clear Zone Data of Merang Padi Fraction

Test Bacteria	Concentration	Average Clear Zone Diameter (mm)				
		n-hexane	ethyl acetate	n-butanol	control (-)	control (+)
<i>S. aureus</i>	3%	0	5.39	3.98	0	20.52
	5%	0	8.09	7.86	0	21.85
	7%	0	12.83	4.75	0	22.35
	9%	0	5.51	1.71	0	23.24
<i>P. aeruginosa</i>	3%	0	2.95	3.09	0	20.52
	5%	0	4.69	7.14	0	21.85
	7%	0	12.63	5.17	0	22.35
	9%	0	4.88	4.72	0	23.24

Based on the results of the tests that have been carried out, the results of the n-hexane fraction of padi straw (*Oryza sativa*) have no antibacterial activity because there is no inhibition zone or kill zone indicated by the fraction at both low and high concentrations. According to (Rorong 2015) this is because the padi straw extract has a polar nature so that in the non-polar n-hexane fraction, the antibacterial activity produced is a small inhibition zone or even no killing zone at all. While the ethanol extract, ethyl acetate fraction, and n-butanol fraction had antibacterial activity indicated by the presence of an inhibition zone.

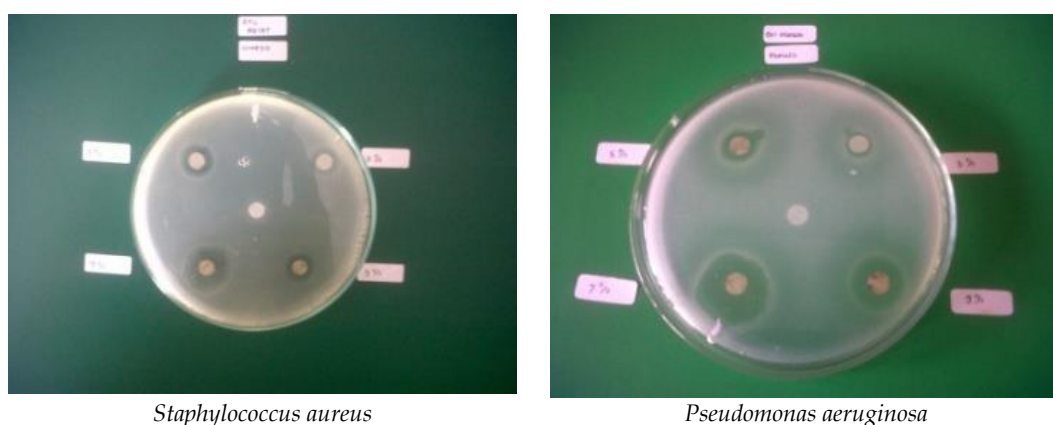


Figure 3. Ethyl Acetate Fraction

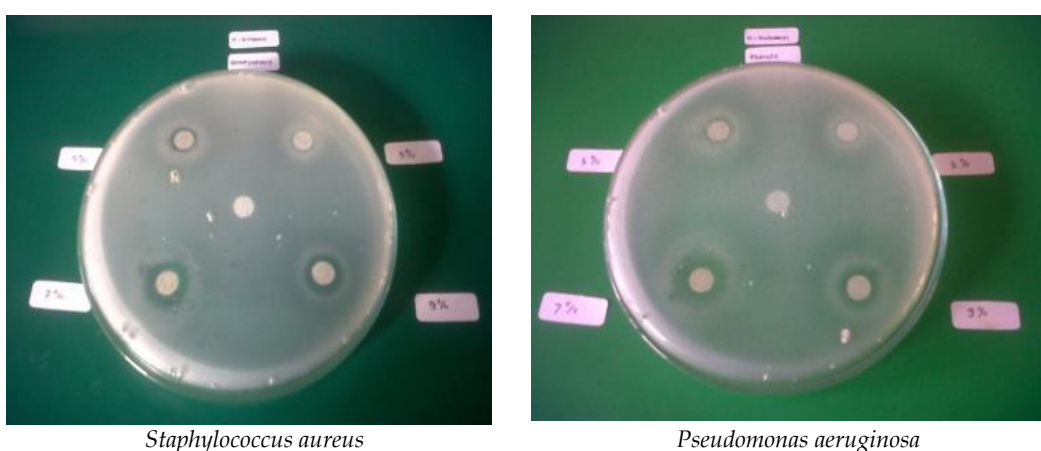


Figure 4. Fraction of n-butanol

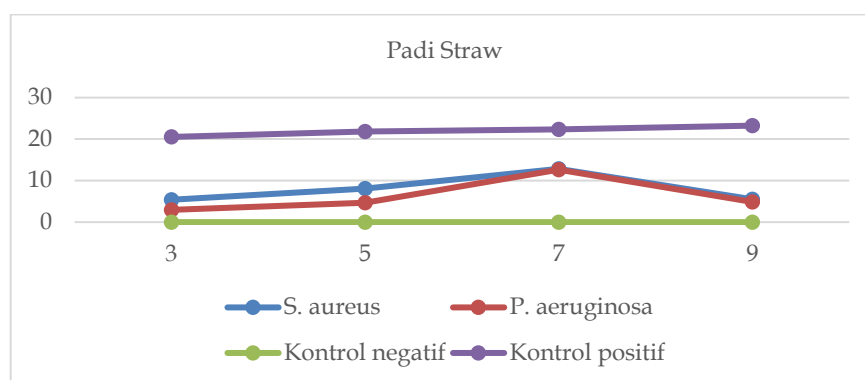


Figure 5. Graph of Merang Padi Ethyl Acetate Fraction

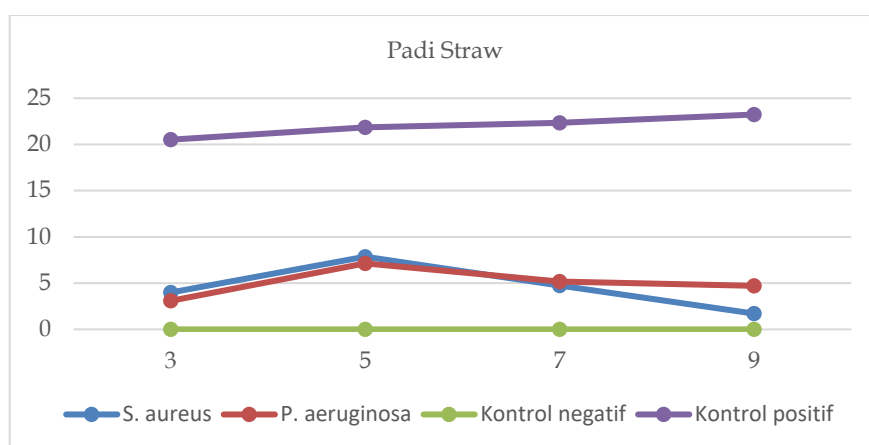


Figure 6. Graph of n-butanol Fraction of Merang Padi

The concentration of ethanol extract used in this study was greater than the concentration of the fraction used. The concentration of padi straw (*Oryza sativa*) ethanol extract used was 5-20%, while the fraction concentration was 3-9%. Due to the ethanol extract, the compound is still in a complex state than the fraction where the compound in the fraction is already in a single state based on its polarity and solubility, so that the fraction already has better antibacterial activity at low concentrations compared to the ethanol extract which is still in a complex state.

Secondary metabolite compounds in padi straw (*Oryza sativa*) are alkaloids, saponins, and phenols. These secondary metabolite compounds can inhibit the growth or kill the bacteria. Alkaloids work as antibacterials by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death (Amalia, Sari, and Nursanty 2017). Saponins are strong surface tension lowering compounds, so they work as antimicrobials by damaging the cytoplasmic membrane (disrupting the stability of cell membranes) causing lysis of microbial cells, for example protodioscin. The mechanism of phenol as an antimicrobial is by interfering with the permeability of the cytoplasmic membrane, causing leakage of intracellular materials that inactivate the activity and synthesis of microbial enzymes (Putri and Nurmagustina 2017).

The effective concentration of an extract is the concentration that best inhibits or kills the test bacteria. The effective concentration was obtained from the antibacterial activity test data of the padi straw (*Oryza sativa*) extract which was then analyzed by the method of analysis of variance (ANOVA) to determine the effect of variations in the concentration of the extract on the growth of the test bacteria.

Potency of Padi Straw Extract And Fraction Against Test Bacteria

The difference in potency between oxytetracycline and ethanol extract was very significant or significantly different where the antibacterial potential of oxytetracycline was stronger than that of the ethanol extract against the test bacteria.

Table 5. Clear zone results of Ethanol Extract and Oxytetracycline against *Staphylococcus aureus* bacteria

Concentration (%)	Ethanol Extract (mm)	Concentration (%)	Oxytetracycline (mm)
5	5.67	3	24.29
10	9.52	5	25.33
15	12.85	7	26.45
20	9.27	9	27.07

Table 6. Clear zone results of Ethanol Extract and Oxytetracycline against *Pseudomonas aeruginosa* bacteria

Concentration (%)	Ethanol Extract (mm)	Concentration (%)	Oxytetracycline (mm)
5	5.82	3	20.52
10	8.33	5	21.85
15	13.80	7	22.35
20	8.71	9	23.24

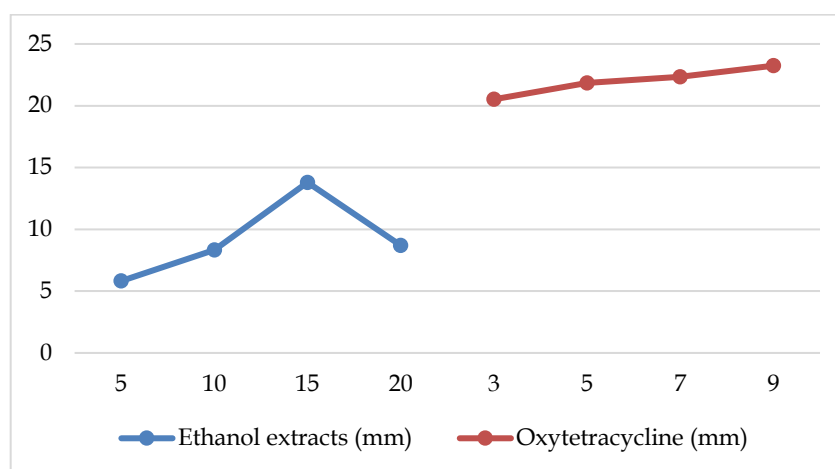
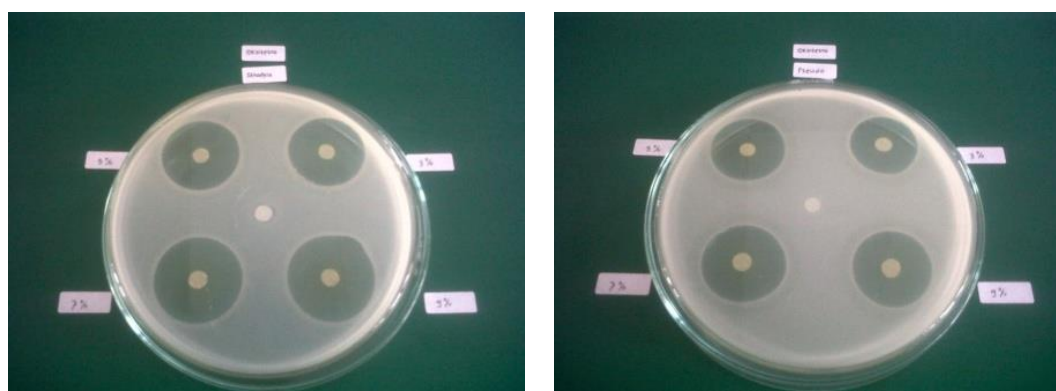


Figure 7. Graph of ethanol extracts and oxytetracycline against test bacteria

Table 7. T-test results Ethanol extract with oxytetracycline

Subject pair (%)		Tcount	Ttable		Information
K	E		5%	1%	
3	5	14.687	3.182	5.841	Tcount > Ttable (5% and 1%) = Very significant
5	10				
7	15				
9	20				



Staphylococcus aureus

Pseudomonas aeruginosa

Figure 8. Positive Control (Oxytetracycline)

Based on table 7., the calculation of the t-test on the ethanol extract of the test bacteria obtained a T count value of 14.687 which means it is very significant or very significantly different because Tcount is greater than Ttable (5% and 1%) (Esti and Irul 2017). This shows that the oxytetracycline antibiotic is better than the potency of the ethanol extract of padi straw (*Oryza sativa*) on the test bacteria.

KESIMPULAN

Ethanol extract of Padi straw has antibacterial activity in inhibiting or killing *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. The n-hexane fraction of Padi straw did not have antibacterial activity because there was no inhibition or killing zone indicated by the fraction at both low and high concentrations. This is because the Padi straw extract has a polar nature so that in the non-polar n-hexane fraction, the antibacterial activity produced is a small inhibition zone or even no killing zone at all. While the ethanol extract, ethyl acetate fraction, and n-butanol fraction had antibacterial activity indicated by the presence of an inhibition zone

ACKNOWLEDGEMENT

Thank you to the ICPHS 2022 committee for facilitating the dissemination of this manuscript.

DAFTAR PUSTAKA

- Agustina, Dini, Hidayati Mustafidah, and Mustika Ratnaningsih Purbowati. 2016. "Sistem Pakar Diagnosa Penyakit Kulit Akibat Infeksi Jamur." *Juita IV* (2): 67-77.
- Amalia, Alfi, Irma Sari, and Risa Nursanty. 2017. "Aktivitas Antibakteri Ekstrak Etil Asetat Daun Sembung (*Blumea balsamifera* (L.) DC.) Terhadap Pertumbuhan Bakteri Methicillin Resistant *Staphylococcus*

- aureus* (MRSA)." *Jurnal UIN Ar-Raniry* 5 (1): 387–91.
- Entjang, I. 2003. "Mikrobiologi & Parasitologi Untuk Akademi Keperawatan." *Bandung: Citra Aditya Bakti*.
- Esti, Tyastirin, and Hidayati Irul. 2017. "'Statistik Parametrik untuk Penelitian Kesehatan."
- <https://files.osf.io/v1/resources/deuxv/providers/osfstorage/5b684afe7e433e00150608d4?action=download&version=1&direct>.
- Garna, Herry, Azhali Manggus Shahrodji, and Alam Anggraini. 2012. "Buku Ajar Divisi Infeksi dan Penyakit Tropis." *Jakarta: Sagung Seto*.
- Irianto, Koes. 2014. "Bakteriologi Medis, Mikologi Medis, dan Virologi Medis." *Bandung: Alfabeta*.
- Jawetz, E, J.L. Melnick, and E.A Adelberg. 2005. "Mikrobiologi Kedokteran." Edited by N. diterjemahkan oleh Mudihardi, E., Kuntaman, Wasito, E. B., Mertaniasih and L. M., Harsono, S., Alimsardjono. XXII. *Jakarta: Salemba Medika*.
- Noor Mutsaqof, Ahmad Aniq, Wiharto -, and Esti Suryani. 2016. "Sistem Pakar untuk Mendiagnosis Penyakit Infeksi Menggunakan *Forward Chaining*." *Jurnal Teknologi & Informasi ITS* 4 (1): 43. <https://doi.org/10.20961/its.v4i1.1758>.
- Nursidika, Perdina, Opstaria Saptarini, and Nurul Rafiqua. 2014. "Aktivitas Antimikrob Fraksi Ekstrak Etanol Buah Pinang (*Areca catechu* L) Pada Bakteri Methicillin Resistant *staphylococcus aureus*." *Majalah Kedokteran Bandung* 46 (2): 94–99. <https://doi.org/10.15395/mkb.v46n2.280>.
- Nuryani, Siti, and Jhunnison Jhunnison. 2017. "Daya Antifungi Infusa Daun Kenikir (*Cosmos caudatus* k.) Terhadap Pertumbuhan Jamur *Candida Albicans* Secara in Vitro." *Jurnal Teknologi Laboratorium* 5 (1): 5–11.
- Putri, Dwi Desmiyeni, and Dwi Eva Nurmagustina. 2017. "Kandungan Total Fenol dan Aktivitas Antibakteri Kelopak Buah Rosela Merah dan Ungu Sebagai Kandidat Feed Additive Alami Pada Broiler." *Jurnal Penelitian Pertanian Terapan* 14 (3): 174–80. <https://doi.org/10.25181/jppt.v14i3.157>.
- Rorong, Johnly Alfreds. 2015. "Analisis Fenolik Jerami Padi (*Oryza sativa*) Pada Berbagai Pelarut sebagai Biosensitizer untuk Fotoreduksi Besi." *Jurnal MIPA* 4 (2): 169. <https://doi.org/10.35799/jm.4.2.2015.10430>.
- Soedarto. 2015. "Mikrobiologi Kedokteran." *Jakarta: Sagung Seto*.
- Suriani, Rini. 2018. "Formulasi Sediaan Shampo dari Merang Padi (*Oryza sativa*)." *Medan*. <http://repository.helvetia.ac.id/id/eprint/748/25/KTI> RINI SURIANI 1515194046.pdf.

Yulia, Yulia. 2017. "Efektivitas Pemanfaatan Sampah Merang Padi (*Oryza sativa*) Sebagai Pengawet Mie Tiaw Basah." *Jurnal Vokasi Kesehatan* 3 (2): 103. <https://doi.org/10.30602/jvk.v3i2.106>.