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LAMPIRAN

Lampiran 1. Determinasi Tanaman Kucai



HERBARIUM UNIVERSITAS ANDALAS (ANDA)

Departemen Biologi FMIPA Universitas Andalas Kampus Limau Manih Padang
Sumbar Indonesia 25163 Telp. +62-751-777427 e-mail: herbariumanda@yahoo.com

Nomer : 172/K-ID/ANDA/II/2025
Lampiran : -
Perihal : Hasil Identifikasi

Kepada yth,
apt. Suci Fitriani Sammulia, M. Sc
Di
Tempat

Dengan hormat,
Sehubungan dengan surat permohonan determinasi sampel dari Institut Kesehatan Mitra Bunda No. 040/K/S1-Farmasi/IKMB/1/2025 tanggal 18 Februari 2025 di Herbarium Universitas Andalas Departemen Biologi FMIPA Universitas Andalas, kami telah membantu mengidentifikasi tumbuhan yang dibawa, dari:

Nama : apt. Suci Fitriani Sammulia, M. Sc
Instansi : Institut Kesehatan Mitra Bunda

Berikut ini diberikan hasil identifikasi yang dikeluarkan dari Herbarium Universitas Andalas.

No	Family	Spesies
1.	Amaryllidaceae	<i>Allium tuberosum</i> Rotter ex Spreng.
2.	Asteraceae	<i>Blumea balsamifera</i> (L.) DC.

Demikian surat ini dibuat untuk dapat digunakan seperlunya.

Padang, 26 Februari 2025

Kepala,

Dr. Nurainas
NIP. 196908141995122001

Lampiran 2. Surat Etik Penelitian



KOMITE ETIK PENELITIAN
INSTITUT KESEHATAN MITRA BUNDA
THE RESEARCH ETHICAL COMMITTEE INSTITUT KESEHATAN MITRA BUNDA

SURAT KETERANGAN
ETHICAL APPROVAL
 No. 110/K/KEP/IKMB/VIII/2025

Komite Etik Penelitian Institut Kesehatan Mitra Bunda, menyatakan dengan ini bahwa penelitian dengan judul :
The Research Ethical Committee of Institut Kesehatan Mitra Bunda states hereby that the following proposal :

“Uji Aktivitas Antibakteri Ekstrak Etanol Daun KUCAI (*Allium tuberosum* Rottler ex Spreng) Terhadap bakteri *Streptococcus pyogenes* dan *Pseudomonas aeruginosa*”
“Test of Antibacterial Activity of Thick Leaf Extract Fraction (Glochidion superbum) Against Pseudomonas aeruginosa and Staphylococcus aureus”

Peneliti Utama : Dea Anggraini
Principal Investigator

Lokasi Penelitian : Laboratorium Mikrobiologi Institut Kesehatan Mitra Bunda
Research Location

Waktu Penelitian : Juni – September 2025
Time Schedule

Responden/Subjek Penelitian : 2 Produk
Respondent/Research Subject

Telah melalui prosedur kaji etik dan dinyatakan layak untuk dilaksanakan
Has proceeded the ethical assessment procedure and been approved for implementation

Batam, 26 Agustus 2025
 Ketua / Chairman,

Dr. Ibnu Rushd, M.K.M

Lampiran 3. COA Kloramfenikol disk

ThermoFisher
SCIENTIFIC

OXOID

Page 1 of 1

CERTIFICATE OF ANALYSIS

PRODUCT	CT9813B C34 CHLORAMPHENICOL	Delivery/ Customer information
LOT NUMBER	4298173	Date Period
EXPIRY DATE	2027.12.18	2025.03.28
DATE OF MANUFACTURE	2024.12.18	Delivery No.
		Customer
		Customer Order Number

Microbiological Performance

Assay (mpn/ml) 32.1

Tested in accordance with current CLSI methodology

	Zone Size (mm)	Limits (mm)
<i>Staphylococcus aureus</i> ATCC25923	23	19 - 26
<i>Escherichia coli</i> ATCC25922	25	21 - 27
<i>Pseudomonas aeruginosa</i> ATCC27962	32	31 - 40
<i>Streptococcus pneumoniae</i> ATCC49619	27	23 - 27

Tested in accordance with current EUCAST methodology

<i>Escherichia coli</i> ATCC25922	25	21 - 27
<i>Staphylococcus aureus</i> ATCC25923	24	20 - 28
<i>Streptococcus pneumoniae</i> ATCC49619	28	24 - 32

The above have been tested in our Product Performance Laboratory using conventional procedures and control ATCC and NCTC cultures, where appropriate, and have met the specified test parameters. Additional challenging strains may also be employed.

The accuracy of the information provided in this certificate of analysis / conformity is considered reliable. However, it is important to note that (i) the information is only applicable, and (ii) the product is only approved, for the product's intended use as specified on the label, specification, and website. No warranty is hereby implied. This certificate may only be reproduced in its entirety and cannot be replicated partially or in any altered form.

Lot Accepted: 2025.01.06

This certificate is produced electronically and valid without a signature.

ATCC is a registered trade mark of the American Type Culture Collection.
NCTC and National Collection of Type Cultures are registered trade marks of the Health Protection Agency.

OXOID LIMITED
Wade Road, Basingstoke, Hampshire RG24 0PU, England
www.thermofisher.com

Lampiran 4. COA Bakteri *Streptococcus pyogenes*


Thermo Fisher Scientific
 Microbiology
 12076 Santa Fe Trail Drive
 12230 Santa Fe Trail Drive
 Lenexa, KS 66215
 800.255.6730
 800.447.5761 fax
 www.thermofisher.com

Certificate of Analysis

Product Name: *S. pyogenes* ATCC 19615 PK/5
Lot Number: 735663

Product Number: R4607000
Expiration Date: 2025-04-02
 (YYYY-MM-DD)

This product has been manufactured, processed and packaged in accordance with Quality Systems Regulation, 21 CFR Part 820. Representative samples were tested per Remel Inc., a part of Thermo Fisher Scientific Quality Control specifications and were found to meet performance criteria for this product.

Purity:

Standardized aliquots of the rehydrated product are inoculated onto nonselective media and examined for pure growth following the appropriate incubation. Selective and Differential media are also tested where applicable.

Viability And Quantification:

Each organism is recovered from the preserved state within the required time frame and at an acceptable level. Passage number is stated as the current preserved state.

Macroscopic And Microscopic Morphology:

Colony morphology is consistent with documented referenced description. Traditional staining is performed.

Characterization:

Organism exhibits characteristic biochemical, enzymatic, genotypical and/or biochemical reactions. Automated and/or conventional testing was performed and results were within established limits. Antimicrobial testing performed where applicable. Results within expected ranges.

CFU/loop: >10(4)

Passage: 3

Gram Reaction: Gram Positive Cocci

Identification Profile: MicroSEQ® or Vitek® 2

Appearance: Preserved Gel Matrix suspended in inoculating loop

pH: N/A

Lampiran 5. COA Bakteri *Pseudomonas aeruginosa*


Thermo Fisher Scientific
Microbiology
12076 Santa Fe Trail Drive
12290 Santa Fe Trail Drive
Lenexa, KS 66215
800.255.6730
800.447.5761 fax
www.thermofisher.com

Certificate of Analysis

Product Name: *P. aeruginosa* ATCC 27853 PK/5
Lot Number: 571191

Product Number: R4607060
Expiration Date: 2026-04-25
(YYYY-MM-DD)

This product has been manufactured, processed and packaged in accordance with Quality Systems Regulation, 21 CFR Part 820. Representative samples were tested per Remel Inc., a part of Thermo Fisher Scientific Quality Control specifications and were found to meet performance criteria for this product.

Purity:

Standardized aliquots of the rehydrated product are inoculated onto nonselective media and examined for pure growth following the appropriate incubation. Selective and Differential media are also tested where applicable.

Viability And Quantification:

Each organism is recovered from the preserved state within the required time frame and at an acceptable level. Passage number is stated as the current preserved state.

Macroscopic And Microscopic Morphology:

Colony morphology is consistent with documented referenced description. Traditional staining is performed.

Characterization:

Organism exhibits characteristic biochemical, enzymatic, genotypical and/or biochemical reactions. Automated and/or conventional testing was performed and results were within established limits. Antimicrobial testing performed where applicable. Results within expected ranges.

CFU/loop: >10(4)

Passage: 3

Gram Reaction: Gram Negative Rod

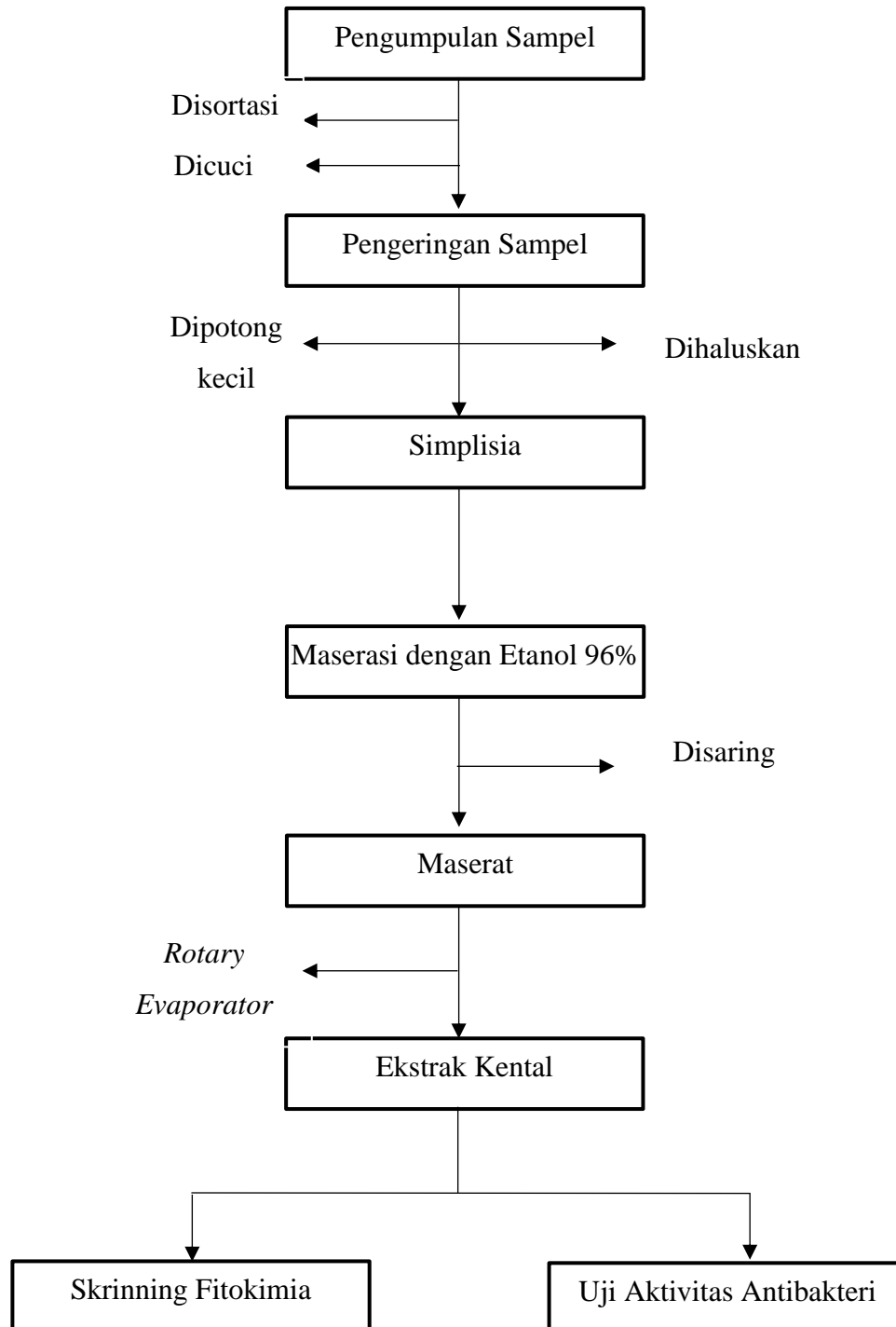
Identification Profile: MicroSEQ® or Vitek® 2

Appearance: Preserved Gel Matrix suspended in inoculating loop

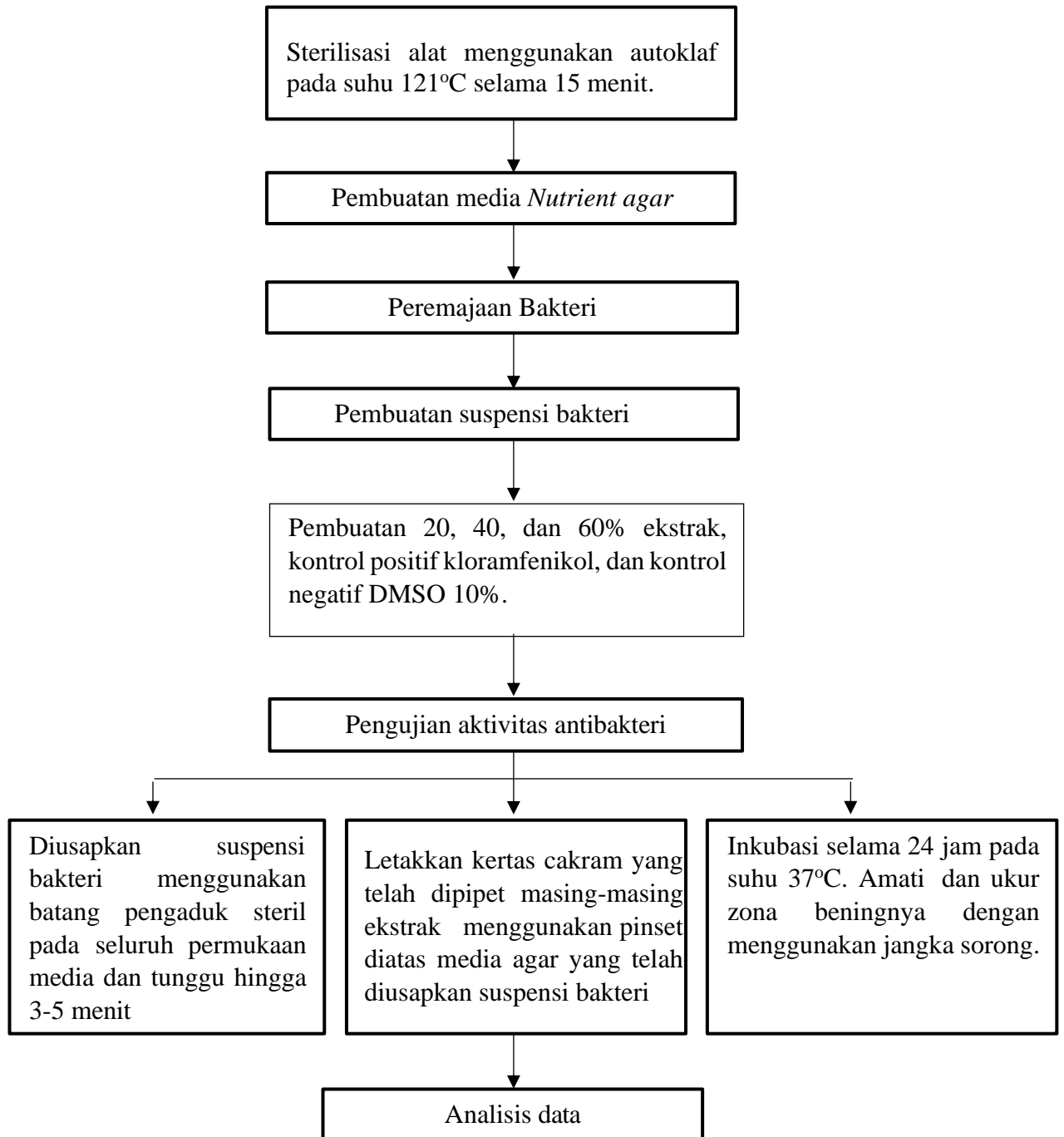
pH: N/A

Morphology: Two colony types may be observed.

Lampiran 6. Pembuatan Ekstraksi Daun Kucai



Lampiran 7. Skema Uji Antibakteri



Lampiran 8. Perhitungan % Rendemen

Berat awal serbuk : 277 g

Berat akhir Ekstrak : 38,90 g

$$\% \text{ Rendeman} = \frac{\text{Berat Akhir}}{\text{Berat Awal}} \times 100\%$$

$$\% \text{ Rendeman} = \frac{38.90 \text{ g}}{277 \text{ g}} \times 100\% = 14,04\%$$

Lampiran 9. Perhitungan Kadar Air Simplisia

Berat Cawan Kosong (A)	Berat Cawan + Sampel Sebelum dikeringkan (B)	Berat Cawan + Sampel Setelah dikeringkan (C)			Kadar Air (%)
		Pengulangan I	Pengulangan II	Pengulangan III	
27.485	29.485	29.640	29.335	29.335	7,5 %



Perhitungan Kadar Air Simplisia:

$$\% \text{ Kadar Air} = \frac{(B - C)}{(B - A)} \times 100\%$$

Keterangan:

- A : Berat cawan kosong
 B : Berat cawan kosong + sampel sebelum pemanasan
 C : Berat cawan kosong + sampel setelah pemanasan

$$\% \text{ Kadar Air} = \frac{(29.485 - 29.335)}{(29.485 - 27.485)} \times 100\% = 7,5 \%$$

Lampiran 10. Perhitungan Kadar Abu Total Simplisia

Berat Cawan Kosong (A)	Berat Cawan + Sampel Sebelum dikeringkan (B)	Berat Cawan + Sampel Setelah dikeringkan (C)			Kadar Abu (%)
		Pengulangan I	Pengulangan II	Pengulangan III	
61.340	63.340	62.354	61.520	61.520	9 %



Perhitungan Kadar Abu Simplisia:

$$\% \text{ Kadar Abu Total} = \frac{(C - A)}{(B - A)} \times 100\%$$

Keterangan:

- A : Berat krush kosong
 B : Berat krush kosong + sampel sebelum pemanasan
 C : Berat krush kosong + sampel setelah pemanasan

$$\% \text{ Kadar Abu Total} = \frac{(61.520 - 61.340)}{(63.340 - 61.340)} \times 100\% = 9,00 \%$$

Lampiran 11. Perhitungan Susut Pengeringan

Berat Cawan Kosong (A)	Berat Cawan + Sampel Sebelum dikeringkan (B)	Berat Cawan + Sampel Setelah dikeringkan (C)			Susut Pengeringan (%)
		Pengulangan I	Pengulangan II	Pengulangan III	
49.700	51.725	53.470	51.550	51.550	8,64 %



Perhitungan Susut Pengeringan Simplisia:

$$\% \text{ Susut Pengeringan} = \frac{(B - A) - (C - A)}{(B - A)} \times 100\%$$

Keterangan:


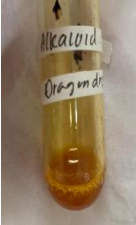


A : Berat cawan kosong





B : Berat cawan kosong + sampel sebelum pemanasan

C : Berat cawan kosong + sampel setelah pemanasan

$$\% \text{ Susut Pengeringan} = \frac{(51.725 - 49.700) - (51.550 - 49.700)}{(51.725 - 49.700)} \times 100\% = 8,64 \%$$

Lampiran 12. Skrinning Fitokimia Daun Kucai

Pemeriksaan	Reagen	Hasil Uji	Gambar
Alkaloid	Pereaksi Mayer	(+) Endapan putih	
Alkaloid	Pereaksi Dragendroff	(+) Endapan jingga	
Flavonoid	HCl Pekat, serbuk Mg, amil alkohol	(+) Berwarna kuning/jingga	
Fenolik	FeCl_3	(+) Coklat kehijauan	

Saponin	Aquadest panas dan HCl pekat	(+) Busa stabil	
Steroid	CH ₃ COOH dan H ₂ SO ₄	(-) Cincin biru kehijauan	
Triterpenoid	CH ₃ COOH dan H ₂ SO ₄	(+) Cincin kecoklatan	
Tanin	FeCl ₃	(+) Hijau Kehitaman	

Lampiran 13. Pembuatan Larutan Uji

Perhitungan konsentrasi larutan ekstrak ditampilkan berdasarkan definisi per 1 ml untuk menunjukkan dasar konsentrasi. Dalam penelitian laboratorium, larutan stok dibuat dalam volume 10 ml untuk mempermudah penimbangan (2,0 g untuk 20%, 4,0 g untuk 40%, 6,0 g untuk 60% dalam 10 ml) dan kemudian dibagi ke volume 1 ml sesuai kebutuhan uji.

Pembuatan konsentrasi ekstrak:

$$\% \text{ konsentrasi} = \frac{m}{v} \times 100 \%$$

Keterangan :

% konsentrasi : Konsentrasi ekstrak yang diketahui

m : Berat/massa ekstrak (gram)

v : Jumlah volume pelarut

PERHITUNGAN	
<p>• Konsentrasi 20%</p> $\% \text{ konsentrasi} = \frac{m}{v} \times 100 \%$ $20\% = \frac{m}{1ml} \times 100 \%$ $20\% = \frac{100\% \times m}{1 ml}$ $100\% \times m = 20\% \times 1 ml$ $100 \times m = 20$ $m = \frac{20}{100}$ $m = 0,2 \text{ Gram}$ <p>Ditimbang 0,2 gram ekstrak kental kemudian dilarutkan dengan DMSO sampai 1 ml, lalu homogenkan.</p>	<p>• Konsentrasi 40%</p> $\% \text{ konsentrasi} = \frac{m}{v} \times 100 \%$ $40\% = \frac{m}{1ml} \times 100 \%$ $40\% = \frac{100\% \times m}{1 ml}$ $100\% \times m = 40\% \times 1 ml$ $100 \times m = 40$ $m = \frac{40}{100}$ $m = 0,4 \text{ Gram}$ <p>Ditimbang 0,4 gram ekstrak kental kemudian dilarutkan dengan DMSO sampai 1 ml, lalu homogenkan.</p>
<p>• Konsentrasi 60%</p> $\% \text{ konsentrasi} = \frac{m}{v} \times 100 \%$ $60\% = \frac{m}{1ml} \times 100 \%$ $60\% = \frac{100\% \times m}{1 ml}$ $100\% \times m = 60\% \times 1 ml$ $100 \times m = 60$ $m = \frac{60}{100}$ $m = 0,6 \text{ Gram}$ <p>Ditimbang 0,6 gram ekstrak kental kemudian dilarutkan dengan DMSO sampai 1 ml, lalu homogenkan.</p>	

Lampiran 14. Perhitungan Media *Nutrient Agar*

$$\begin{aligned}\text{Jumlah media} &= \frac{\sum \text{Cawan petri} \times \sum \text{volume yang dituang} \times 28 \text{ g}}{1000} \\ &= \frac{6 \times 15 \text{ ml} \times 28 \text{ g}}{1000} \\ &= 2,52 \text{ gram}\end{aligned}$$

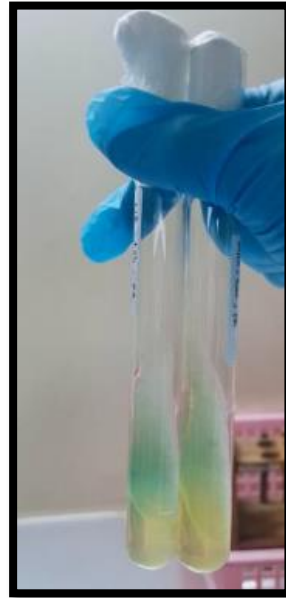
$$\begin{aligned}\text{Volume air} &= \sum \text{Cawan petri} \times \sum \text{volume yang dituang} \\ &= 6 \times 15 \text{ ml} \\ &= 90 \text{ ml}\end{aligned}$$

Jadi, media ditimbang sebanyak 2,52 gram kemudian dilarutkan dengan 90 ml aquadest, dan homogenkan menggunakan *magnetic stirrer*.

Lampiran 15. Hasil peremajaan bakteri









Streptococcus pyogenes




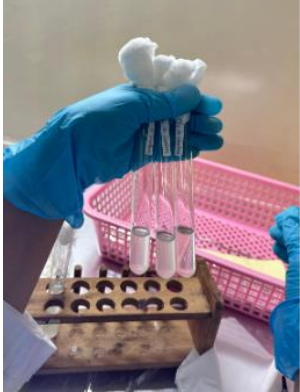


Pseudumonas aeruginosa

Lampiran 16. Dokumentasi Kegiatan

	
Daun Kucai	Pencucian sampel daun Kucai
	
Perajangan sampel daun Kucai	Pengeringan dengan dianginkan
	
Penimbangan simplisia kering	Penghalusan sampel daun Kucai dengan blender

	
Maserasi daun Kucai	Hasil maserat
	
Penimbangan Hasil Kadar Air dan Susut Pengeringan	Penimbangan Hasil Kadar Abu Total
	
Pengentalan sampel daun Kucai dengan rotay evaporator	Ekstrak kental daun Kucai

	
Proses sterilisasi alat	Pembuatan media NA
	
Peremajaan bakteri	Pembuatan suspensi bakteri

Lampiran 17. Analisis Data

- Streptococcus pyogenes

1. Uji Normalitas

Tests of Normality

Perlakuan	Kolmogorov-Smirnov ^a			Shapiro-Wilk			
	Statistic	df	Sig.	Statistic	df	Sig.	
Streptococcus_pyogenes	K.Positif	.236	3	.	.977	3	.710
	K.Negatif	.	3	.	.	3	.
	20%	.188	3	.	.998	3	.911
	40%	.314	3	.	.893	3	.363
	60%	.217	3	.	.988	3	.791

a. Lilliefors Significance Correction

2. Uji Homogenitas

Test of Homogeneity of Variances

Perlakuan	Based on	Levene	df1	df2	Sig.
		Statistic			
Streptococcus_pyogenes	Mean	2.193	4	10	.143
	Median	.836	4	10	.533
	Median and with adjusted df	.836	4	6.381	.547
	trimmed mean	2.080	4	10	.159

3. Uji ANOVA

ANOVA

Streptococcus_pyogenes

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	757.598	4	189.399	463.132	.000
Within Groups	4.090	10	.409		
Total	761.687	14			

4. Uji Post-Hoc

Multiple Comparisons						
Dependent Variable: Streptococcus_pyogenes						
Tukey HSD						
(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K.Positif	K.Negatif	20.43000*	.52215	.000	18.7116	22.1484
	20%	12.72333*	.52215	.000	11.0049	14.4418
	40%	8.40000*	.52215	.000	6.6816	10.1184
	60%	3.83333*	.52215	.000	2.1149	5.5518
K.Negatif	K.Positif	-20.43000*	.52215	.000	-22.1484	-18.7116
	20%	-7.70667*	.52215	.000	-9.4251	-5.9882
	40%	-12.03000*	.52215	.000	-13.7484	-10.3116
	60%	-16.59667*	.52215	.000	-18.3151	-14.8782
20%	K.Positif	-12.72333*	.52215	.000	-14.4418	-11.0049
	K.Negatif	7.70667*	.52215	.000	5.9882	9.4251
	40%	-4.32333*	.52215	.000	-6.0418	-2.6049
	60%	-8.89000*	.52215	.000	-10.6084	-7.1716
40%	K.Positif	-8.40000*	.52215	.000	-10.1184	-6.6816
	K.Negatif	12.03000*	.52215	.000	10.3116	13.7484
	20%	4.32333*	.52215	.000	2.6049	6.0418
	60%	-4.56667*	.52215	.000	-6.2851	-2.8482
60%	K.Positif	-3.83333*	.52215	.000	-5.5518	-2.1149
	K.Negatif	16.59667*	.52215	.000	14.8782	18.3151
	20%	8.89000*	.52215	.000	7.1716	10.6084
	40%	4.56667*	.52215	.000	2.8482	6.2851

*. The mean difference is significant at the 0.05 level.

- *Pseudomonas aeruginosa*

1. Uji Normalitas

Tests of Normality							
	Perlakuan	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Pseudomonas_aeruginosa	K.Positif	.353	3	.	.824	3	.174
	K.Negatif	.	3	.	.	3	.
	20%	.253	3	.	.964	3	.637
	40%	.216	3	.	.989	3	.796
	60%	.212	3	.	.990	3	.812

a. Lilliefors Significance Correction

2. Uji Homogenitas

Test of Homogeneity of Variances					
		Levene Statistic			
		Statistic	df1	df2	Sig.
Pseudomonas_aeruginosa	Based on Mean	2.967	4	10	.074
	Based on Median	1.155	4	10	.386
	Based on Median and with adjusted df	1.155	4	5.587	.420
	Based on trimmed mean	2.821	4	10	.084

3. Uji ANOVA

ANOVA

Pseudomonas_aeruginosa

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	543.064	4	135.766	344.252	.000
Within Groups	3.944	10	.394		
Total	547.008	14			

4. Uji *Post-Hoc*

Multiple Comparisons

Dependent Variable: Pseudomonas_aeruginosa
Tukey HSD

(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K.Positif	K.Negatif	17.88333 [*]	.51276	.000	16.1958	19.5709
	20%	7.45000 [*]	.51276	.000	5.7625	9.1375
	40%	5.74000 [*]	.51276	.000	4.0525	7.4275
	60%	3.56333 [*]	.51276	.000	1.8758	5.2509
K.Negatif	K.Positif	-17.88333 [*]	.51276	.000	-19.5709	-16.1958
	20%	-10.43333 [*]	.51276	.000	-12.1209	-8.7458
	40%	-12.14333 [*]	.51276	.000	-13.8309	-10.4558
	60%	-14.32000 [*]	.51276	.000	-16.0075	-12.6325
20%	K.Positif	-7.45000 [*]	.51276	.000	-9.1375	-5.7625
	K.Negatif	10.43333 [*]	.51276	.000	8.7458	12.1209
	40%	-1.71000 [*]	.51276	.047	-3.3975	-.0225
	60%	-3.88667 [*]	.51276	.000	-5.5742	-2.1991
40%	K.Positif	-5.74000 [*]	.51276	.000	-7.4275	-4.0525
	K.Negatif	12.14333 [*]	.51276	.000	10.4558	13.8309
	20%	1.71000 [*]	.51276	.047	.0225	3.3975
	60%	-2.17667 [*]	.51276	.012	-3.8642	-.4891
60%	K.Positif	-3.56333 [*]	.51276	.000	-5.2509	-1.8758
	K.Negatif	14.32000 [*]	.51276	.000	12.6325	16.0075
	20%	3.88667 [*]	.51276	.000	2.1991	5.5742
	40%	2.17667 [*]	.51276	.012	.4891	3.8642