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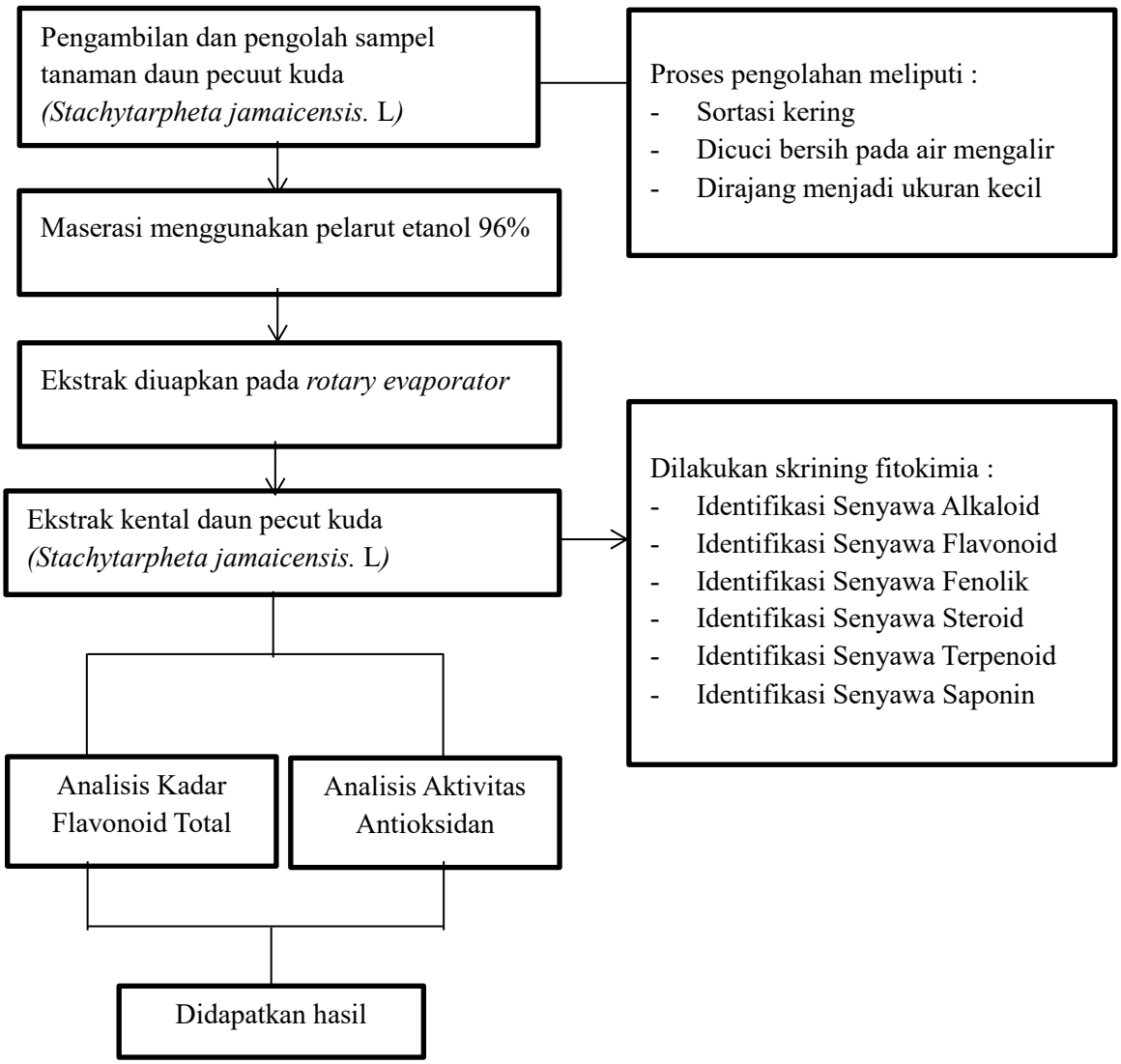
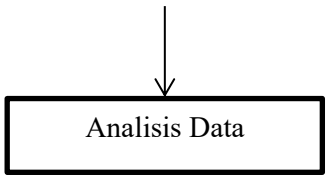
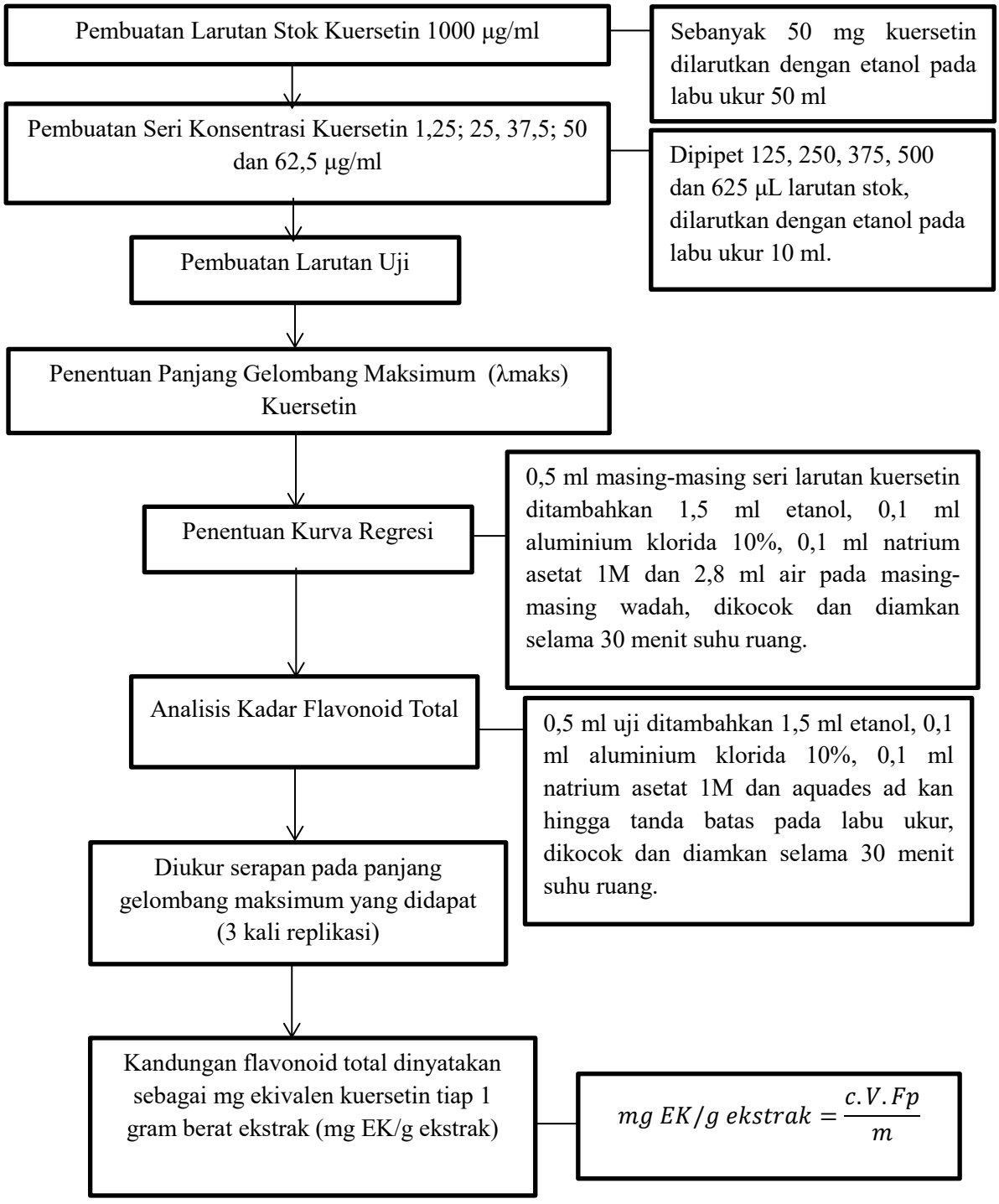
LAMPIRAN

Lampiran 1. Skema Alur Penelitian

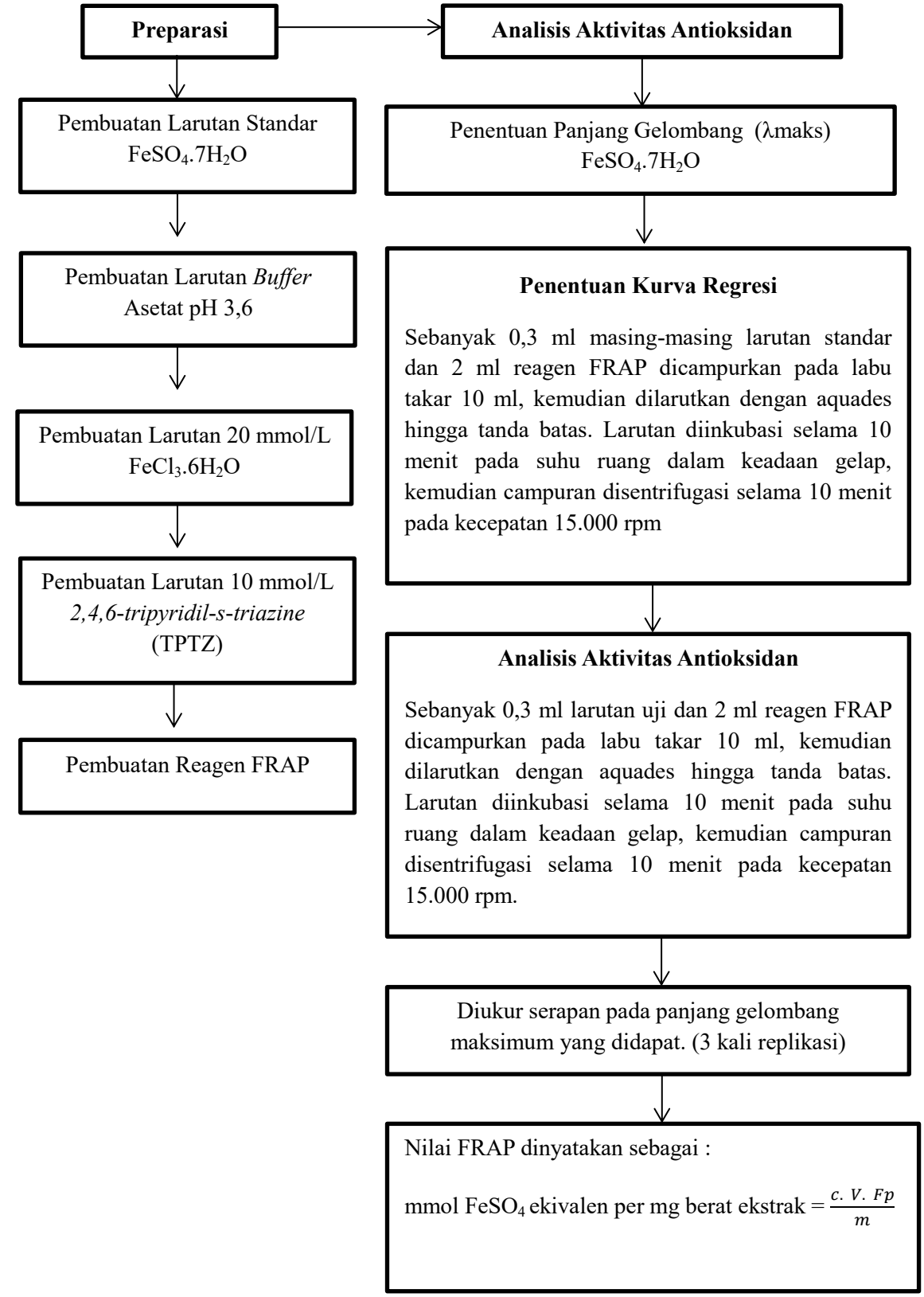



Analisis Data

Lampiran 2. Skema Kerja Flavonoid Total



Lampiran 3. Skema Kerja Analisis Aktivitas Antioksidan



Lampiran 4. Hasil Determinasi Tanaman

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

Nomor : 167/K-ID/ANDA/II/2025
Lampiran : -
Perihal : Hasil Identifikasi

Kepada yth,
Suhaera, S.Farm.,M.Pharm.Sci
Di
Tempat

Dengan hormat,
Sehubungan dengan surat permohonan determinasi sampel dari Institut Kesehatan Mitra Bunda No. 030/K/S1-Farmasi/IKMB/II/2025 tanggal 17 Februari 2025 di Herbarium Universitas Andalas Departemen Biologi FMIPA Universitas Andalas, kami telah membantu mengidentifikasi tumbuhan yang dibawa, dari:

Nama : Suhaera, S.Farm.,M.Pharm.Sci
Instansi : Institut Kesehatan Mitra Bunda

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

No	Family	Spesies
1.	Verbenaceae	*Stachytarpheta jamaicensis* (L.) Vahl

Demikian surat ini dibuat untuk dapat digunakan seperlunya.

Padang, 26 Februari 2025
Kepala,

Dr. Nurainas
NIP. 196908141995122001

Lampiran 5. Surat Etik

YAYASAN HARAPAN BUNDA BATAM
INSTITUT KESEHATAN MITRA BUNDA
KOMITE ETIK PENELITIAN
Jl. Seraya No 1 KOTA BATAM Telp/Fax (0778) 429431, website : http:\\ikmb.ac.id
SURAT KEPUTUSAN MENTERI PENDIDIKAN DAN KEBUDAYAAN REPUBLIK INDONESIA No. 284/M/2020

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

SURAT KETERANGAN
ETHICAL APPROVAL
No. 124/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 Antioksidan Ekstrak Etanol Daun Pecut Kuda (*Stachytarpheta jamaicensis.L*) Dengan Metode FRAP (*Ferric Reducing Antioxidant Power*)"
"Antioxidant Activity Test of Ethanol Extract of Horsetail Leaves (Stachytarpheta jamaicensis.L) Using the FRAP (Ferric Reducing Antioxidant Power) Method"

Peneliti Utama : Apriyanti Nanda Intan
Principal Investigato

Lokasi Penelitian : Laboratorium Kimia Bahan Alam Institut Kesehatan Mitra Bunda Batam
Research Location

Waktu Penelitian : Juli – Agustus 2025
Time Schedule

Responden/Subjek Penelitian : 1 Sampel Daun Pecut Kuda
Respondent/Research Subject

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

Batam, 26 Agustus 2025
Ketua / *Chairman*,

dr. Ibnu Rushd, M.K.M

Lampiran 6. COA 2,4,6-*Triphenyl-1,3,5-triazine* (TPTZ)

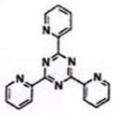
Supelco

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
2,4,6-Tris(2-pyridyl)-s-triazine - for spectrophotometric det. (of Fe), ≥98%

Product Number:	T1253
Batch Number:	BCCL8423
Brand:	SIGMA
CAS Number:	3682-35-7
Formula:	C18H12N6
Formula Weight:	312,33 g/mol
Quality Release Date:	13 JUN 2024



Test	Specification	Result
Appearance (Color)	White to Light Yellow to Light Beige	Off White
Appearance (Form)	Powder	Powder
Purity (HPLC)	≥ 98.0 %	99.8 %
Solubility (Color)	Green-Yellow to Very Dark	Dark Brown-Yellow
	Green-Yellow to Very Dark Brown	
Solubility (Turbidity) 100mg/ml Methanol	Clear to Slightly Hazy	Clear
Infrared Spectrum	Conforms to Structure	Conforms

Dr. R. Schwenninger

Dr.Reinhold Schwenninger
Quality Assurance
Buchs,Switzerland CH

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Version Number: 1

Lampiran 7. COA $FeSO_4.7H_2O$

Certificate of Analysis

1.03965.1000 Iron(II) sulfate heptahydrate for analysis EMSURE® ACS,ISO,Reag. Ph Eur
Batch A2032365

	Spec. Values		Batch Values	
Assay (manganometric)	99.5 - 102.0	%	100.2	%
Identity	passes test		passes test	
Appearance of solution	passes test		passes test	
Insoluble matter	≤ 0.01	%	≤ 0.01	%
pH-value (5 %; water)	3.0 - 4.0		3.4	
Chloride (Cl)	≤ 0.0005	%	≤ 0.0005	%
Phosphate (PO_4)	≤ 0.001	%	≤ 0.001	%
Total nitrogen (N)	≤ 0.001	%	≤ 0.001	%
Heavy metals as Pb	≤ 0.005	%	≤ 0.005	%
As (Arsenic)	≤ 0.0002	%	≤ 0.0002	%
Ca (Calcium)	≤ 0.005	%	≤ 0.005	%
Cr (Chromium)	≤ 0.0050	%	≤ 0.0050	%
Co (Cobalt)	≤ 0.0025	%	≤ 0.0025	%
Cu (Copper)	≤ 0.001	%	≤ 0.001	%
Fe III (Iron III)	≤ 0.02	%	≤ 0.02	%
K (Potassium)	≤ 0.002	%	≤ 0.002	%
Mg (Magnesium)	≤ 0.002	%	≤ 0.002	%
Mn (Manganese)	≤ 0.05	%	≤ 0.05	%
Na (Sodium)	≤ 0.02	%	≤ 0.02	%
Ni (Nickel)	≤ 0.0050	%	≤ 0.0050	%
Pb (Lead)	≤ 0.0005	%	≤ 0.0005	%
Zn (Zinc)	≤ 0.0050	%	≤ 0.0050	%
Substance not precipitated by ammonium hydroxide	≤ 0.1	%	≤ 0.1	%

Corresponds to ACS,ISO,Reag. Ph Eur

Date of release (DD.MM.YYYY) 09.02.2024
Minimum shelf life (DD.MM.YYYY) 15.02.2027

Kristin Haufschildt
Responsible laboratory manager quality control

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Corporation with General Partners
Frankfurter Straße 250
64293 Darmstadt, Germany

SALSA Version 1378288/990001081665// Date: 09.02.2024

Lampiran 8. COA FeCl3.6H2O

Certificate of Analysis

1.03943.0250 Iron(III) chloride hexahydrate for analysis EMSURE® ACS,Reag. Ph Eur
Batch B2033343

	Spec. Values		Batch Values	
Assay (iodometric, $FeCl_3 \cdot 6H_2O$)	99.0 - 102.0	%	99.5	%
Identity	passes test		passes test	
Insoluble matter	≤ 0.01	%	≤ 0.01	%
Free chlorine	passes test		passes test	
Acidic substances	passes test		passes test	
Nitrate (NO_3)	≤ 0.01	%	≤ 0.01	%
Sulfate (SO_4)	≤ 0.01	%	≤ 0.01	%
Total nitrogen (N)	≤ 0.001	%	≤ 0.001	%
Total phosphorus (as PO_4)	≤ 0.01	%	≤ 0.01	%
Heavy metals (as Pb)	≤ 0.005	%	≤ 0.005	%
Ca (Calcium)	≤ 0.01	%	≤ 0.01	%
Cu (Copper)	≤ 0.003	%	≤ 0.003	%
Fe II (Iron II)*	≤ 0.002	%	≤ 0.002	%
K (Potassium)	≤ 0.005	%	≤ 0.005	%
Mg (Magnesium)	≤ 0.005	%	≤ 0.005	%
Na (Sodium)	≤ 0.05	%	≤ 0.05	%
Zn (Zinc)	≤ 0.003	%	≤ 0.003	%

* The Fe(II)-content increases if the recommended storage conditions (+15 to +25 °C) are not observed.

Date of release (DD.MM.YYYY) 10.12.2021
Minimum shelf life (DD.MM.YYYY) 31.12.2024

Dr. Dimitrij Ryvlin
Responsible laboratory manager quality control

This document has been produced electronically and is valid without a signature.

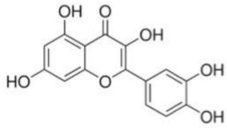
Lampiran 9. COA Kuersetin

Sigma-Aldrich.

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigma-aldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name : **Quercetin ≥95% (HPLC), solid**
Product Number : **Q4951-10G**
Batch Number : **0000270512**
Source Batch : SLCQ4754
CAS Number : 117-39-5
Molecular Formula : $C_{15}H_{10}O_7$
Formula Weight : 302.24 g/mol
Quality Release Date : 03 FEB 2023



Test	Specification	Result
Appearance (Color) Yellow	Conforms	Conforms
Appearance (Form)	Powder	Powder
1H NMR Spectrum	Conforms to Structure	Conforms
Loss on Drying	≤ 4 %	3 %
Purity (HPLC)	≥ 95 %	97 %

Brian Dulle, Supervisor
Quality Assurance
St. Louis, Missouri
US

Sigma-Aldrich warrants, that at the time of the quality release or subsequent retest date this product conformed to the information contained in this publication. The current Specification sheet may be available at Sigma-Aldrich.com. For further inquiries, please contact Technical Service. Purchase must determine the suitability of the product for its particular use. See reverse side of website or packing slip for additional terms and conditions of sale.

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For further information please contact mlsbranding@sial.com

Lampiran 10. Karakteristik Simplisia dan Ekstrak Daun Pecut Kuda

- Uji Organoleptis Simplisia

Pengujian	Hasil	Gambar
Bau	Bau khas	
Warna	Hijau kecoklatan	
Bentuk	Serbuk kasar	
Rasa	Pahit	

- Uji Organoleptis Ekstrak

Pengujian	Hasil	Gambar
Bau	Bau khas	
Warna	Hijau kecoklatan	
Bentuk	Kental seperti pasta	

- Hasil Data Perhitungan Kadar Air Simplisia Daun Pecut Kuda

  Perhitungan % Kadar air :

  % Kadar air $= \frac{(B-C)}{(B-A)}\ x\ 100\ \%$

  $= \frac{(77.450-77.287)}{(77.450-75.475)}\ x\ 100\ \%$

  $= \frac{(163)}{(1.975)}\ x\ 100\ \%$

  $= 8.25\ \%$

- Hasil Data Susut Pengeringan Simplisia Daun Pecut Kuda

  Perhitungan % Susut pengeringan :

  % Susut pengeringan $= \frac{(B-A)-(C-A)}{(B-A)}\ x\ 100\ \%$

  $= \frac{(31.025-29.025)-(30.867-29.025)}{(31.025-29.025)}\ x\ 100\ \%$

  $= \frac{(2000)-(1.824)}{(2.000)}\ x\ 100\ \%$

= 7,9 %

- Hasil Data Kadar Abu Total Simplisia Daun Pecut Kuda

  Perhitungan % kadar abu total :

  % Kadar abu total $= \frac{(C-A)}{(B-A)}\ x\ 100\ \%$

  $= \frac{(52.254-52.130)}{(54.130-52.130)}\ x\ 100\ \%$

  $= \frac{(124)}{(2.000)}\ x\ 100\ \%$

  = 6,2 %

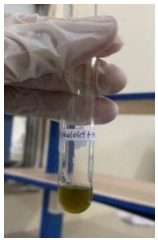
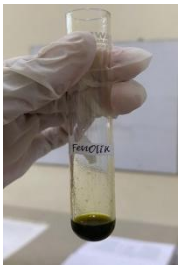
Lampiran 11. Perhitungan % Rendemen Ekstrak Etanol Daun Pecut Kuda

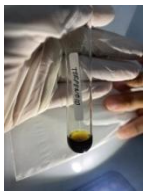
$$\%\ Rendemen\ = \frac{berat\ ekstrak\ kental}{berat\ simplisia}\ \text{x}\ 100\%$$

$$\%\ Rendemen\ = \frac{203\ g}{1.800\ g}\ \text{x}\ 100\%$$

= 11,27 %

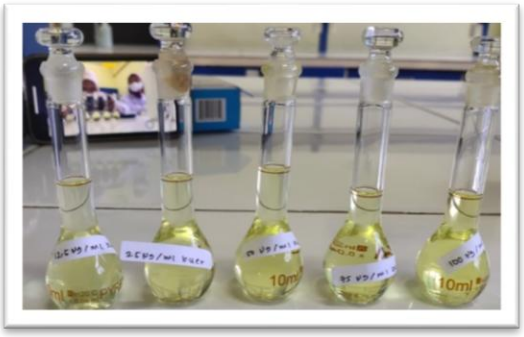
Lampiran 12. Uji Skrining Fitokimia

Pemeriksaan	Reagen	Hasil Uji	Keterangan	Gambar
Alkaloid	Pereaksi Mayer	(-)	Tidak ada endapan berwarna putih	
Alkaloid	Pereaksi Dragendroff	(-)	Tidak ada endapan berwarna merah	
Flavonoid	H_2SO_4	(+)	Adanya perubahan warna menjadi coklat kekuningan	
Fenolik	$FeCl_3$ 1 %	(+)	Adanya perubahan warna menjadi hijau kehitaman	
Saponin	Air panas dan HCl Pekat	(-)	Tidak ada busa yang permanen	
Tanin	$FeCl_3$ 1 %	(+)	Adanya perubahan warna menjadi hijau kehitaman	

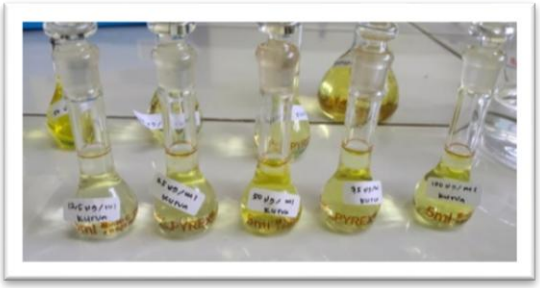
Steroid	Pereaksi Liberman dan H_2SO_4	(-)	Tidak ada perubahan warna	
Terpenoid	Pereaksi Liberman dan H_2SO_4	(+)	Adanya perubahan warna menjadi kecoklatan	

Lampiran 13. Pembuatan Larutan Penetapan Kadar Flavonoid Total

Larutan Stok Kuersetin

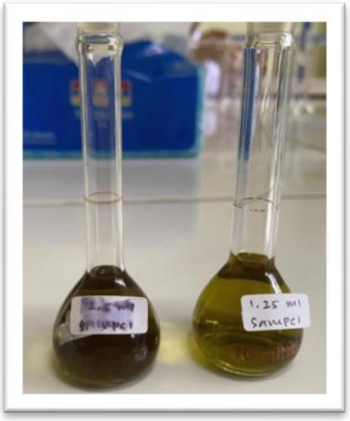


Seri Konsentrasi Kuersetin



Larutan Kurva Kuersetin

Ekstrak Daun Pecut Kuda



Seri Konsentrasi Sampel

Sampel + Kuersetin

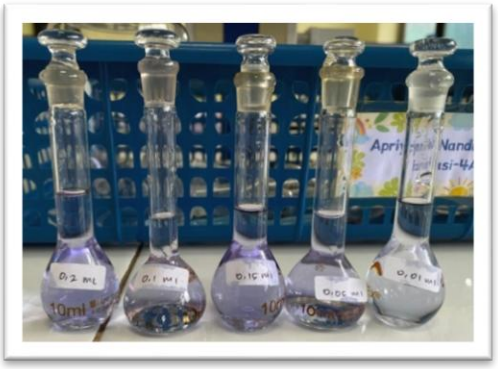
Lampiran 14. Pembuatan Larutan Analisis Antioksidan

Larutan Standar $FeSO_4.7H_2O$

Seri Konsentrasi $FeSO_4.7H_2O$

Larutan HCL 37 % 40 mmol/L

Larutan $FeCl_3.6H_2O$



Larutan Kurva Regresi FRAP

Sampel + Pereaksi FRAP

Lampiran 15. Perhitungan Pembuatan Larutan Kadar Flavonoid Total

- Pembuatan Larutan Stok dan Seri Konsentrasi Kuersetin

  - Konsentrasi Larutan Stok = $\frac{massa\ (mg)}{volume\ (mL)} = \frac{50\ mg}{50\ mL}$

    = 1 mg/ml = 1.000 µg/ml

  - Seri Konsentrasi dibuat dalam labu ukur 10 ml dengan konsentrasi 12,5; 25; 37,5; 50; dan 62,5 µg/ml

    - 12,5 µg/ml = V1. M1 = V2. M2

      V1. 1.000 µg/ml = 10 ml. 12,5 µg/ml

      V1 = $\frac{125}{1000}$ = 0,125 ml ad 10 ml

    - 25 µg/ml = V1. M1 = V2. M2

      V1. 1000 µg/ml = 10 ml. 25 µg/ml

      V1 = $\frac{250}{1000}$ = 0,25 ml ad 10 ml

    - 37,5 µg/ml = V1. M1 = V2. M2

      V1. 1000 µg/ml = 10 ml. 37,5 µg/ml

      V1 = $\frac{375}{1000}$ = 37,5 ml ad 10 ml

    - 50 µg/ml = V1. M1 = V2. M2

      V1. 1000 µg/ml = 10 ml. 50 µg/ml

      V1 = $\frac{500}{1000}$ = 0,5 ml ad 10 ml

    - 62,5 µg/ml = V1. M1 = V2. M2

      V1 . 1000 µg/ml = 10 ml. 62,5 µg/ml

      V1 = $\frac{625}{1000}$ = 0,625 ml ad 10 ml

- Pembuatan Larutan Uji

  - Sampel 100 mg (diubah ke µg) = 100 x 1000 = 100.000 µg
  - Volume Etanol 25 ml = $\frac{100.000\ \mu g}{25\ mL}$ = 4.000 µg/ml
  - Konsentrasi Larutan 1000 µg/ml dan 500 µg/ml didalam labu ukur 10 ml
    - 1000 µg/ml = V1. M1 = V2. M2

      V1. 4.000 µg/ml = 10 ml. 1.000 µg/ml

      V1 = $\frac{10.000}{4.000}$ = 2,5 ml:ad 10 ml
    - 500 µg/ml = V1. M1 = V2. M2

      V1. 4.000 µg/ml = 10 ml. 500 µg/ml

      V1 = $\frac{5.000}{4.000}$ = 1,25 ml ad 10 ml

Lampiran 16. Perhitungan Pembuatan Larutan Analisis Aktivitas Antioksidan

- $FeSO_4.7H_2O$ (1 mmol/ L) dalam 100 ml = 0,1 L
  - mmol/L = $\frac{mg}{Bm.\ V\ (L)}$

    1 mmol/L = $\frac{x}{278.0{,}1} = 1\ x\ 27{,}8 = 27{,}8\ mg$
  - Konsentrasi Larutan dari 0,2; 0,15; 0,1; 0,05 dan 0,01 mmol/L dalam 10 ml Labu Ukur
    - 0,2 mmol/L = V1. M1 = V2. M2

      V1. 1 = 10 ml. 0,2

      V1 = 2 ml ad 10 ml
    - 0,15 mmol/L = V1. M1 = V2. M2

      V1. 1 = 10 ml. 0,15

      V1 = 1,5 ml ad 10 ml
    - 0,1 mmol/L = V1. M1 = V2. M2

      V1. 1 = 10 ml. 0,1

      V1 = 1 ml ad 10 ml
    - 0,05 mmol/L = V1. M1 = V2. M2

      V1. 1 = 10 ml. 0,05

      V1 = 0,5 ml ad 10 ml
    - 0,01 mmol/L = V1. M2 = V2. M2

      V1. 1 = 10 ml. 0,01

      V1 = 0,1 ml ad 10 ml
- Pembuatan Larutan Uji
  - Sampel 200 mg (diubah ke µg) = 200 x 1000 = 200.000 µg

- Volume Etanol 25 ml = $\frac{200.000\ \mu g}{25\ mL}$ = 8.000 µg/ml
- Konsentrasi Larutan 2000 µg/ml dan 1000 µg/ml didalam labu ukur 10 ml
    - 2000 µg/ml = V1. M1 = V2. M2

      V1. 8.000 µg/ml = 10 ml. 2.000 µg/ml

      V1 = $\frac{20.000}{8.000}$ = 2,5 ml:ad 10 ml
    - 1000 µg/ml = V1. M1 = V2. M2

      V1. 8.000 µg/ml = 10 ml. 1.000 µg/ml

      V1 = $\frac{10.000}{8.000}$ = 1,25 ml ad 10 ml

➢ Pembuatan Larutan HCL 37% (40 mmol/L/ 0,04 mol/L = 0,04 M)

- M = $\frac{\rho\ .\%.10}{Mr\ HCL}$ = $\frac{1,19.37\%.10}{36,5}$ = 12,1
- Pengenceran

  V1. M1 = V2. M2

  V1. 12,1 = 500. 0,04

  V1 = $\frac{500.0,04}{12,1}$ = 1,65 ad 500 ml

➢ Pembuatan Larutan TPTZ 10 mmol/L dalam labu ukur 500 ml = 0,05 L

mmol/L = $\frac{mg}{Bm.\ V\ (L)}$

10 mmol/L = $\frac{x}{312,33\ .\ 0,05}$

X = 10 x 15,62 = 156,2 mg

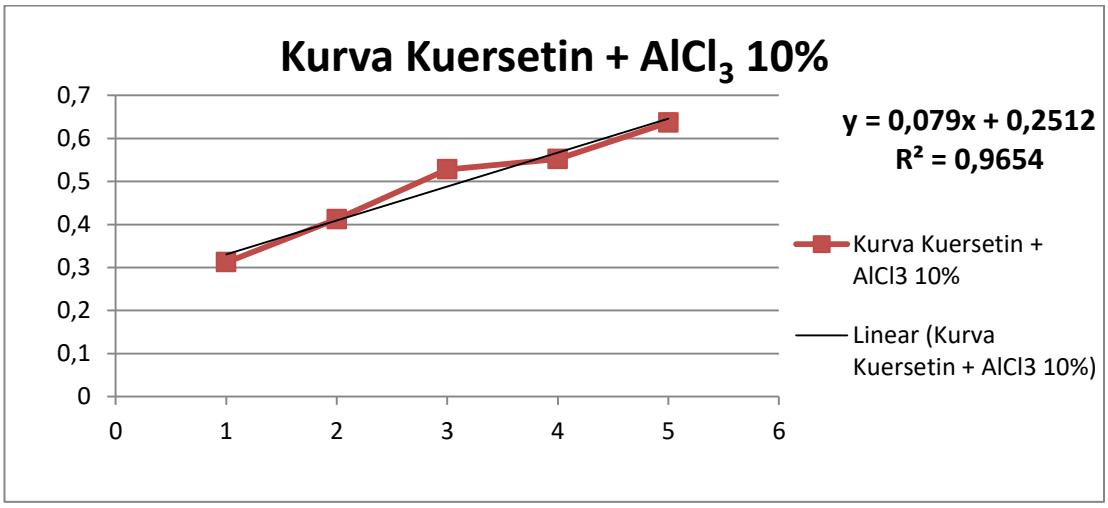
➢ Pembuatan Larutan $FeCl_3.6H_2O$ 20 mmol/L dalam 100 ml = 0,1 L

mmol/L = $\frac{mg}{Bm.\ V\ (L)}$

20 mmol/L = $\frac{x}{27{,}029\ .\ 0{,}1}$

X = 20 x 27,029 = 540,58 mg => 540,6 mg (dibulatkan)

Lampiran 17. Kurva Regresi Flavonoid Total



Ekstrak	Absorbansi	X	µmol	Rata-rata
1,25	0,493	3,06	3,06	
	0,493	3,06	3,06	
	0,493	3,06	3,06	3,061
2,5	0,737	6,15	6,15	
	0,737	6,15	6,15	
	0,737	6,15	6,15	6,149

- V.Etanol Sampel 1,25 PPM

  $X = \frac{0,493-0,2512}{0,079}$

  = 3.061 µmol/ml dibulatkan (3,06 µmol/ml)

- V.Etanol Sampel 2,5 PPM

  $X = \frac{0,737-0,2512}{0,079}$

  = 6,149 µmol/ml dibulatkan (6,15 µmol/ml)

- % Kuersetin 1,25 ml

  0,493 = 0,079x + 0,2512

  0,2418 = 0,079x

x = 3,06 PPM

% Kuersetin $= \frac{C.V.Fp}{m}$

$= \frac{3{,}06\ PPM.25\ ml.8}{0{,}1\ g}$

= 6,12 mg EK g Ekstrak

- % Kuersetin 2,5 ml

0,737 = 0,079x + 0,2512

0,4858 = 0,079x

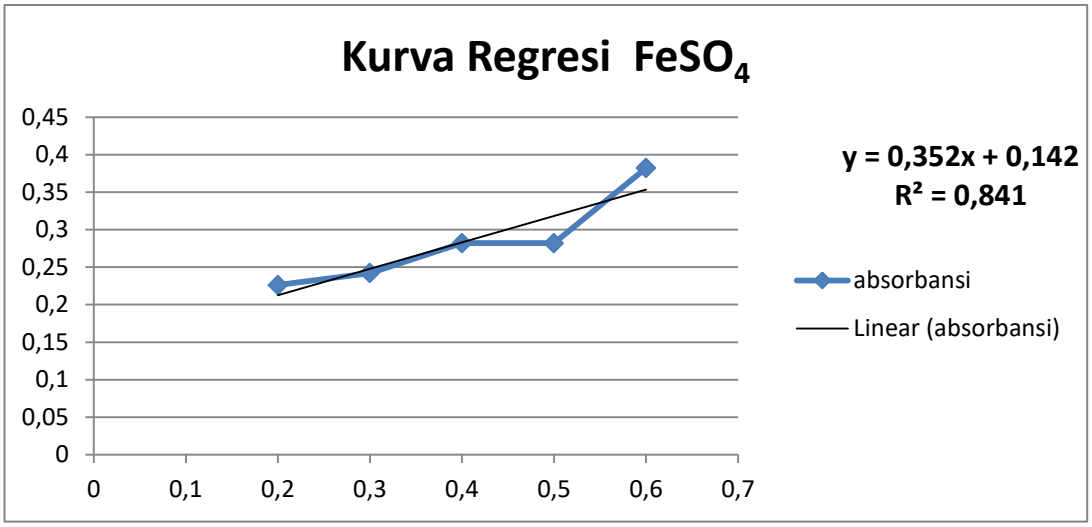
x = 6,15 PPM

% Kuersetin $= \frac{C.V.Fp}{m}$

$= \frac{6{,}15\ PPM.25\ ml.4}{0{,}1\ g}$

= 6,15 mg EK g Ekstrak

Lampiran 18. Kurva Regresi Antioksidan Metode FRAP



Konsentrasi	Absorbansi	X	µmol	Rata-rata
1,25	0,616	1,35	1,350	
	0,616	1,35	1,350	
	0,616	1,35	1,350	1,350
2,5	0,677	1,52	760	
	0,677	1,52	760	
	0,677	1,52	760	760

- 1,25 PPM

  0,616 = 0,352x + 0,142

  0,474 = 0,352x

  x = 1,35 mmol/L

  µmol $FeSO_4$ g Ekstrak = $\frac{1,35 \,.0,025\, .8}{0,2\ g}\ x\ 1000$ ⟶ Dikali 1000 karena ingin mengubah satuannya dari mmol ke µmol

  =1.350 µmol $FeSO_4$ g Ekstrak

- 2,5 PPM

  0,677 = 0,352x + 0,142

  0,535 = 0,352x

x = 1,52 mmol/L

µmol $FeSO_4$ g Ekstrak = $\frac{1,52 \,.0,025 \,.4}{0,2\ g}\ x\ 1000$

= 760 µmol $FeSO_4$ g Ekstrak

Lampiran 19. Larutan Pembanding 1000 PPM (Vitamin C)

Sampel	Absorbansi	Rata-rata
Asam askorbat	0,503	0,503
	0,503	
	0,503	

- Vitamin C

0,503 = 0,352x + 0,142

0,361 = 0,352x

x = 1,025 mmol/L

Vitamin C $= \frac{1,025 \,.0,025 \,.4}{0,2\ g}\ x\ 1000$

= 512,5 µmol $FeSO_4$ g Vitamin C