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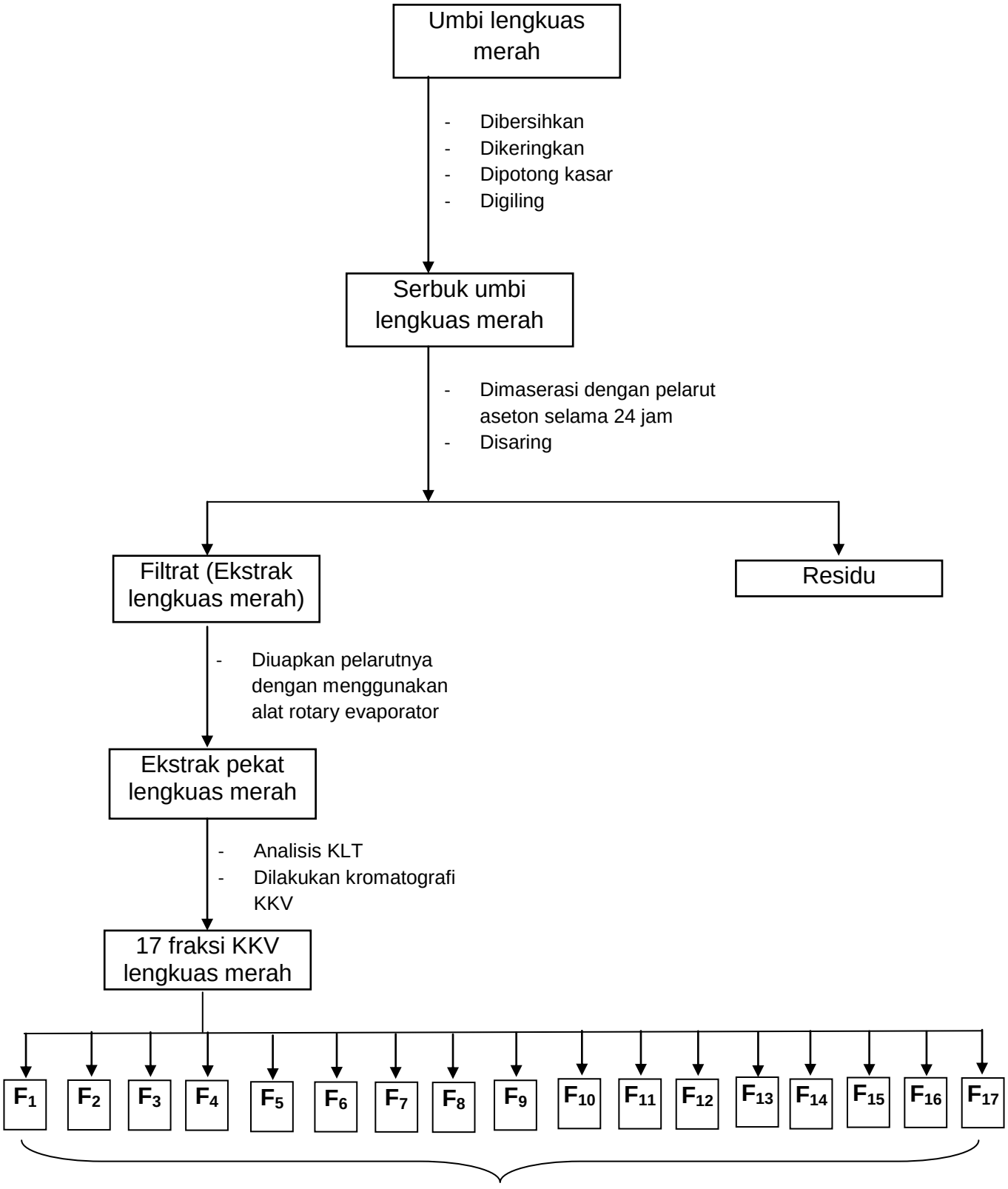
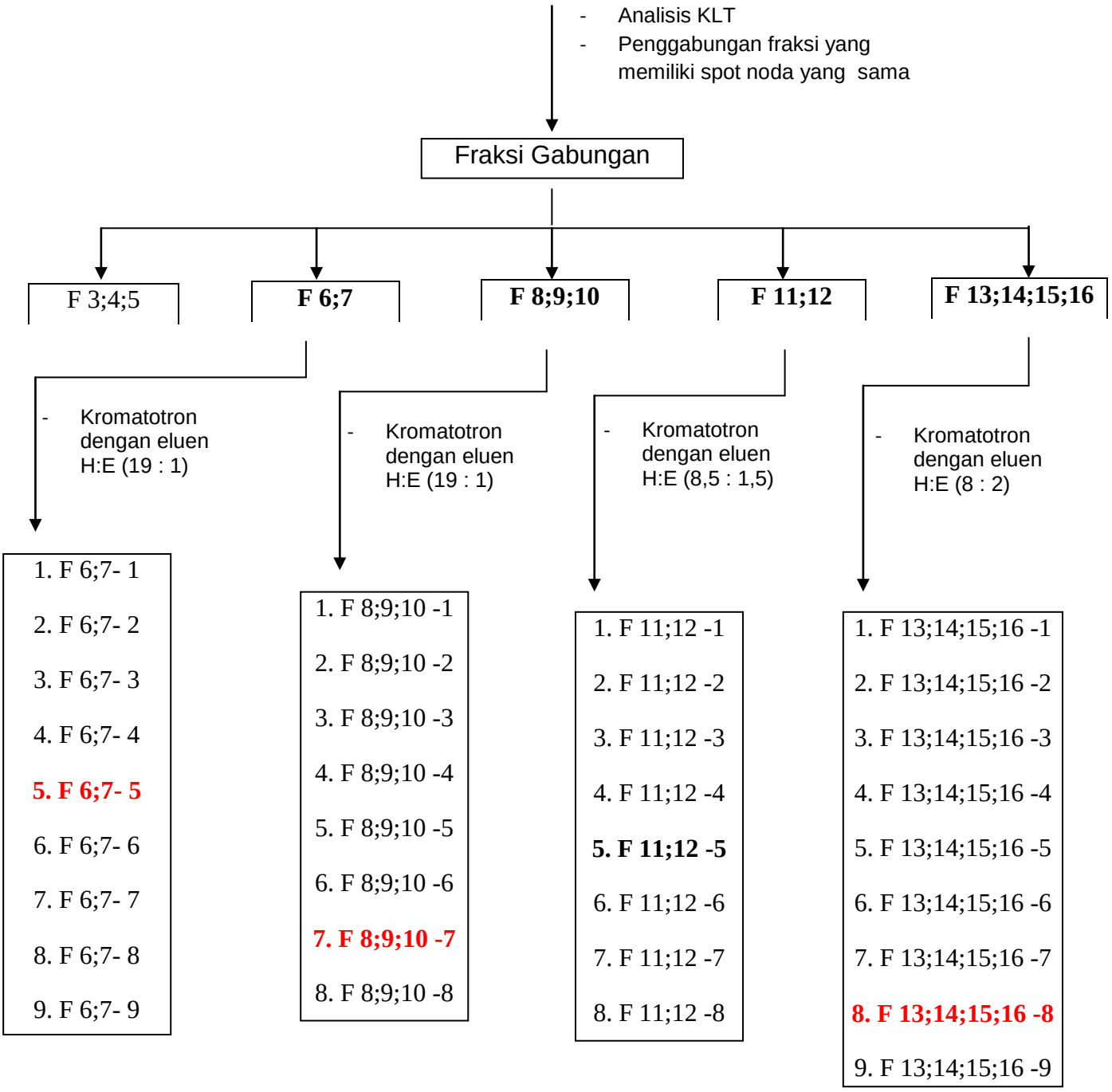
LAMPIRAN

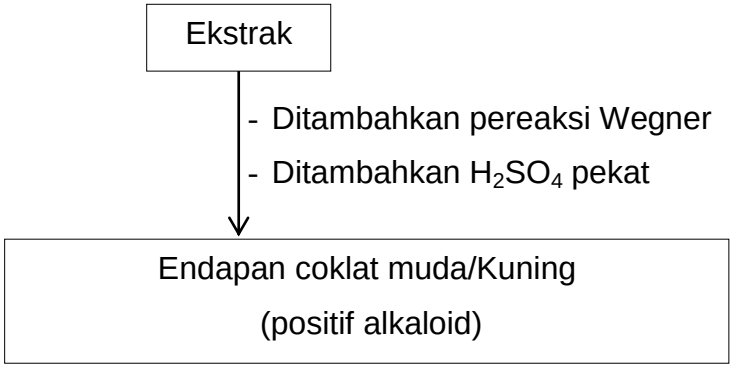
Lampiran 1. Bagan kerja isolasi metabolit sekunder dari ekstrak aseton rimpang tumbuhan lengkuas merah



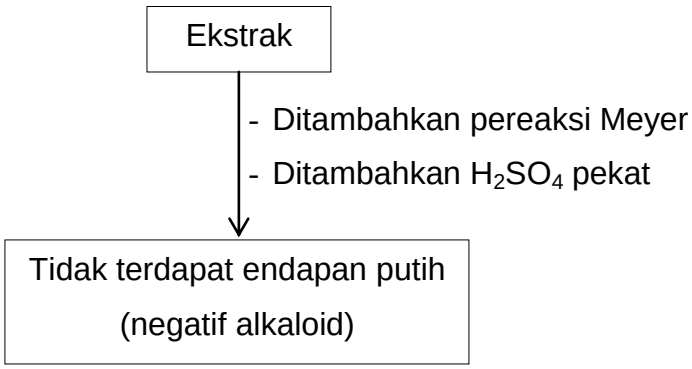

- Analisis KLT
- Penggabungan fraksi yang memiliki spot noda yang sama
Fraksi Gabungan
F 3;4;5
F 6;7
F 8;9;10
F 11;12
F 13;14;15;16
- Kromatotron dengan eluen H:E (19 : 1)
- Kromatotron dengan eluen H:E (19 : 1)
- Kromatotron dengan eluen H:E (8,5 : 1,5)
- Kromatotron dengan eluen H:E (8 : 2)
1. F 6;7- 1
2. F 6;7- 2
3. F 6;7- 3
4. F 6;7- 4
5. F 6;7- 5
6. F 6;7- 6
7. F 6;7- 7
8. F 6;7- 8
9. F 6;7- 9
1. F 8;9;10 -1
2. F 8;9;10 -2
3. F 8;9;10 -3
4. F 8;9;10 -4
5. F 8;9;10 -5
6. F 8;9;10 -6
7. F 8;9;10 -7
8. F 8;9;10 -8
1. F 11;12 -1
2. F 11;12 -2
3. F 11;12 -3
4. F 11;12 -4
5. F 11;12 -5
6. F 11;12 -6
7. F 11;12 -7
8. F 11;12 -8
1. F 13;14;15;16 -1
2. F 13;14;15;16 -2
3. F 13;14;15;16 -3
4. F 13;14;15;16 -4
5. F 13;14;15;16 -5
6. F 13;14;15;16 -6
7. F 13;14;15;16 -7
8. F 13;14;15;16 -8
9. F 13;14;15;16 -9

Lampiran 2. Uji fitokimia ekstrak aseton rimpang lengkuas merah

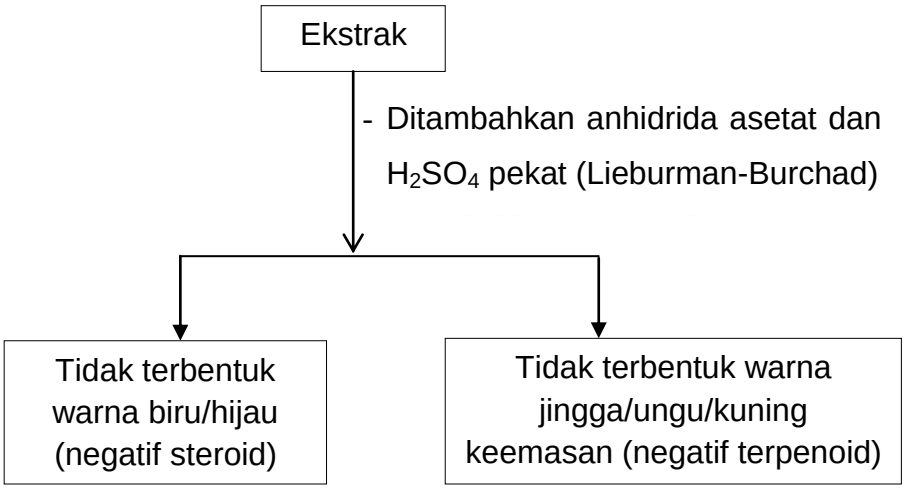
a. Uji Alkaloid (Pereaksi Wegner)



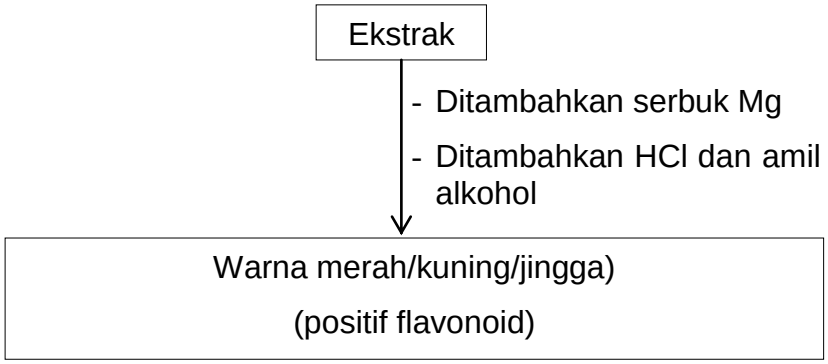
b. Uji Alkaloid (Pereaksi Meyer)



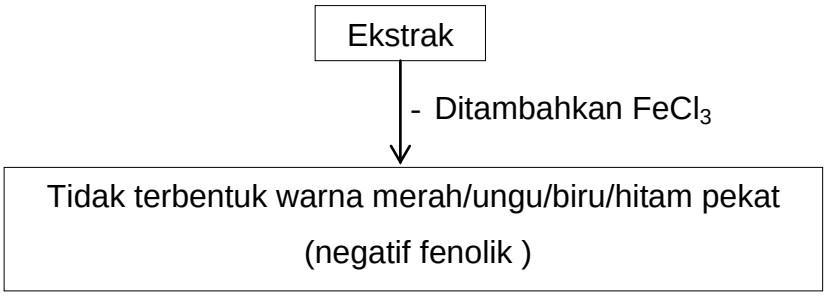
c. Uji Steroid dan Terpenoid (Pereaksi Lieberman-Burchard)



d. Uji Flavonoid

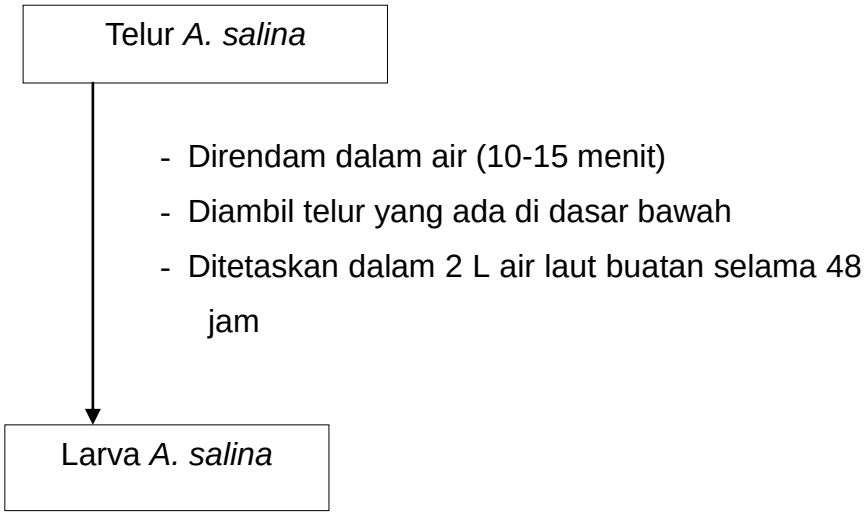


e. Uji Fenolik

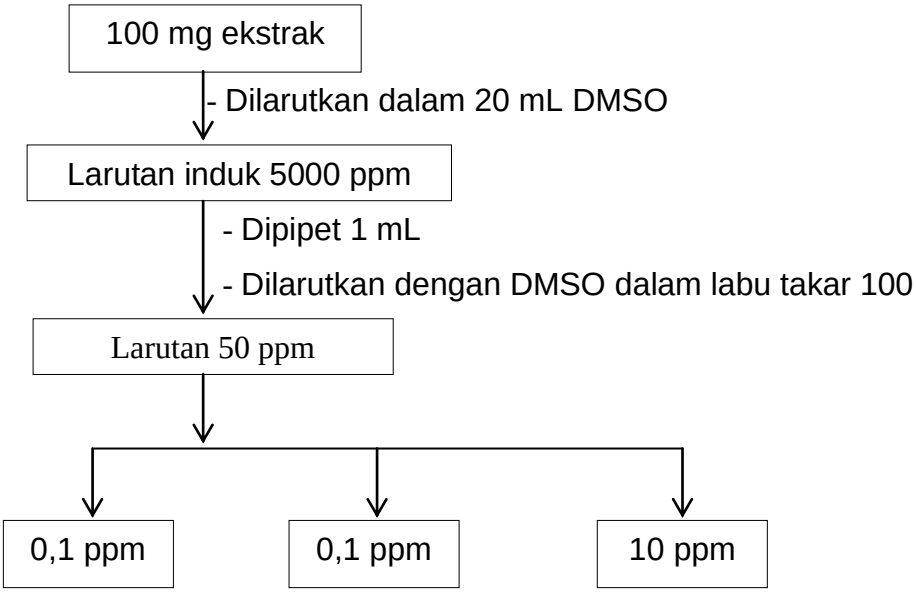


Lampiran 3. Bagan kerja uji toksisitas (BSLT) ekstrak aseton rimpang lengkuas merah

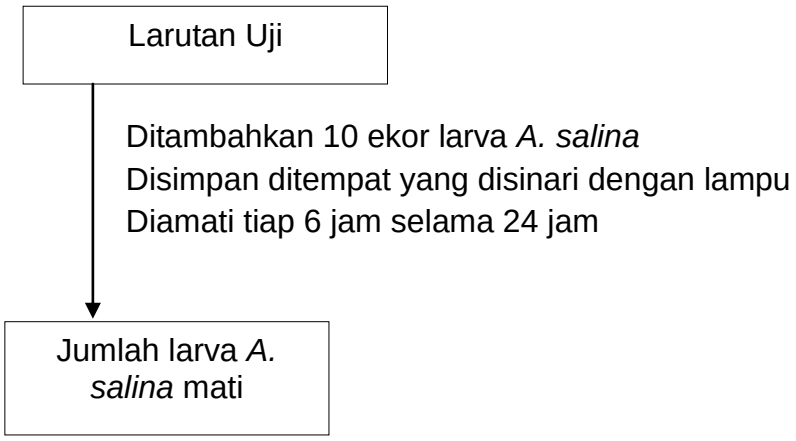
a. Penetasan telur *A. salina*



b. Penyiapan larutan uji



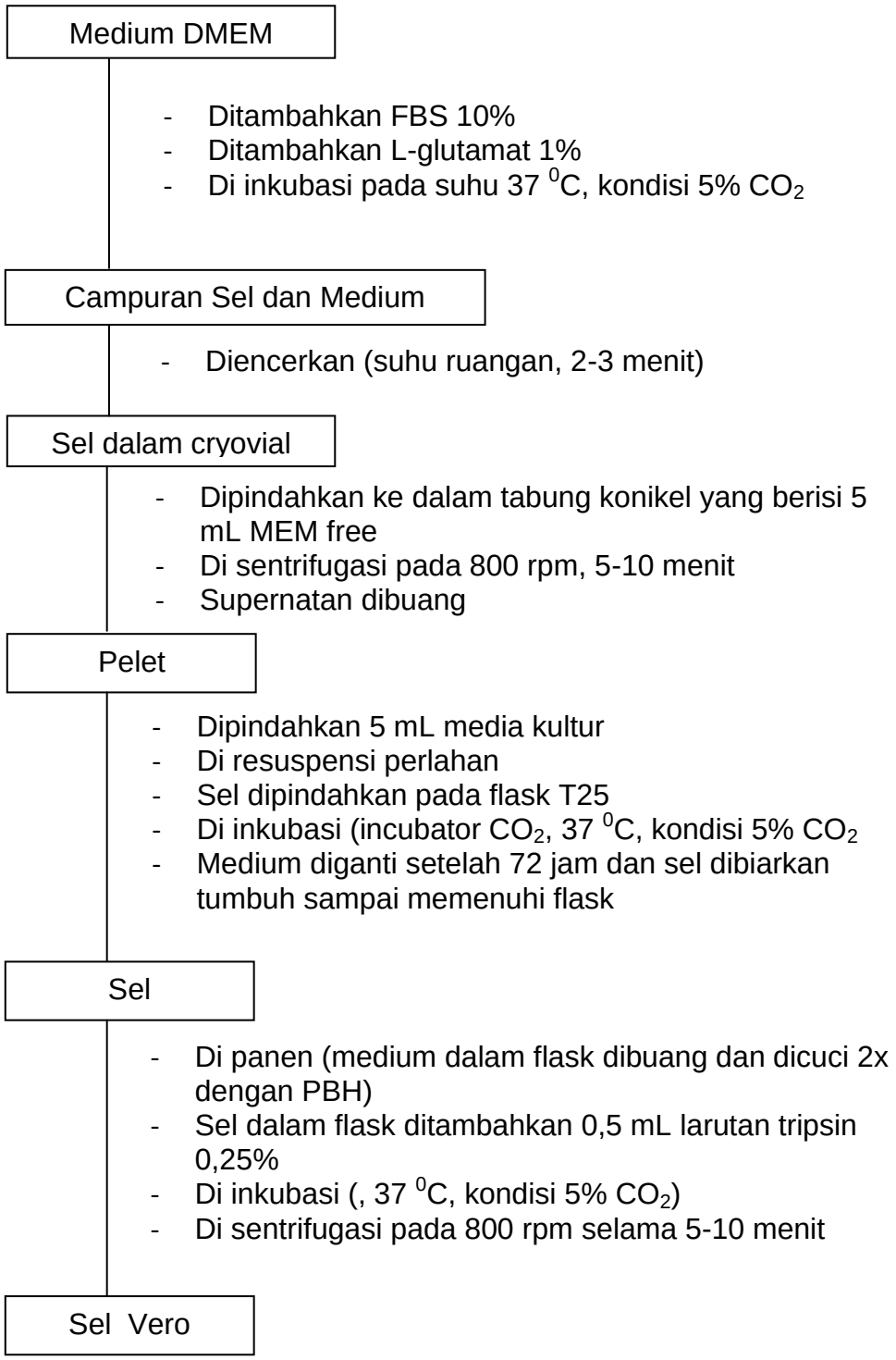
c. Pengujian Toksisitas



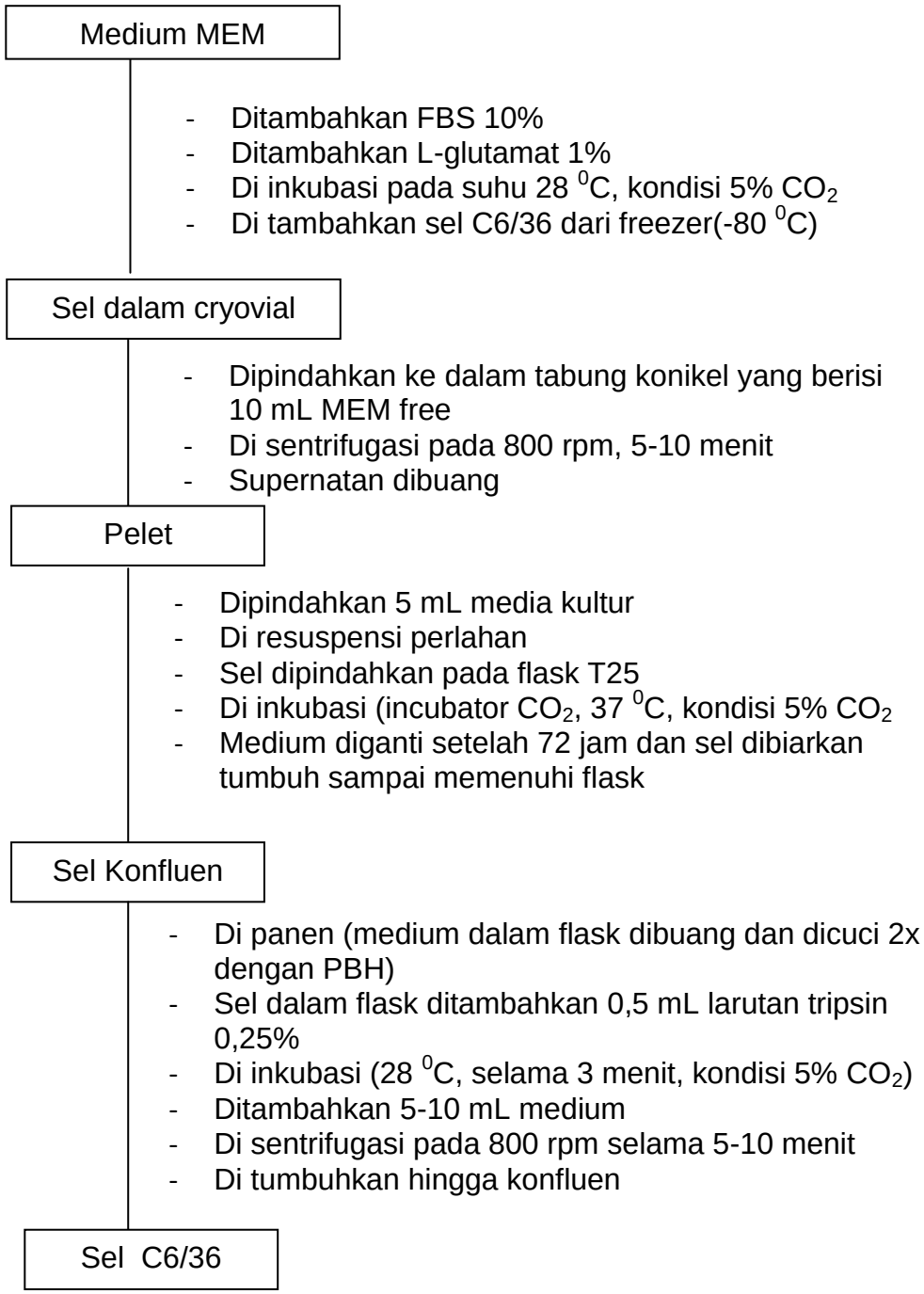
kontrol untuk setiap konsentrasi larutan uji disiapkan menggunakan air laut dan pelarut DMSO dengan perlakuan yang sama dengan sampel.

Lampiran 4. Pengujian antivirus dengue

a. Kultur *cell line* vero



b. Kultur *cell line* C6/36



c. Pembuatan stok virus pada *cell line* C6/36

400 µL virus (stok sebelumnya yang telah diencerkan dengan medium

- Di tambahkan hingga 1 mL ke dalam flask yang berisi sel C6/36
- Di kocok
- Di inkubasi (28 ^{0}C)
- Setiap 15-20 menit dikocok perlahan, selama 1 jam
- Setelah 1 jam ditambahkan medium MEM (2% FBS) 5 mL
- Di inkubasi (sampai terlihat efek sitopatik secara perlahan)
- Di sentrifugasi pada 4000 rpm, 4 ^{0}C, selama 10 menit

Supernatan virus (stok virus disimpan pada

d. Pembuatan stok virus pada *cell line* Vero

Stok virus sel C6/36

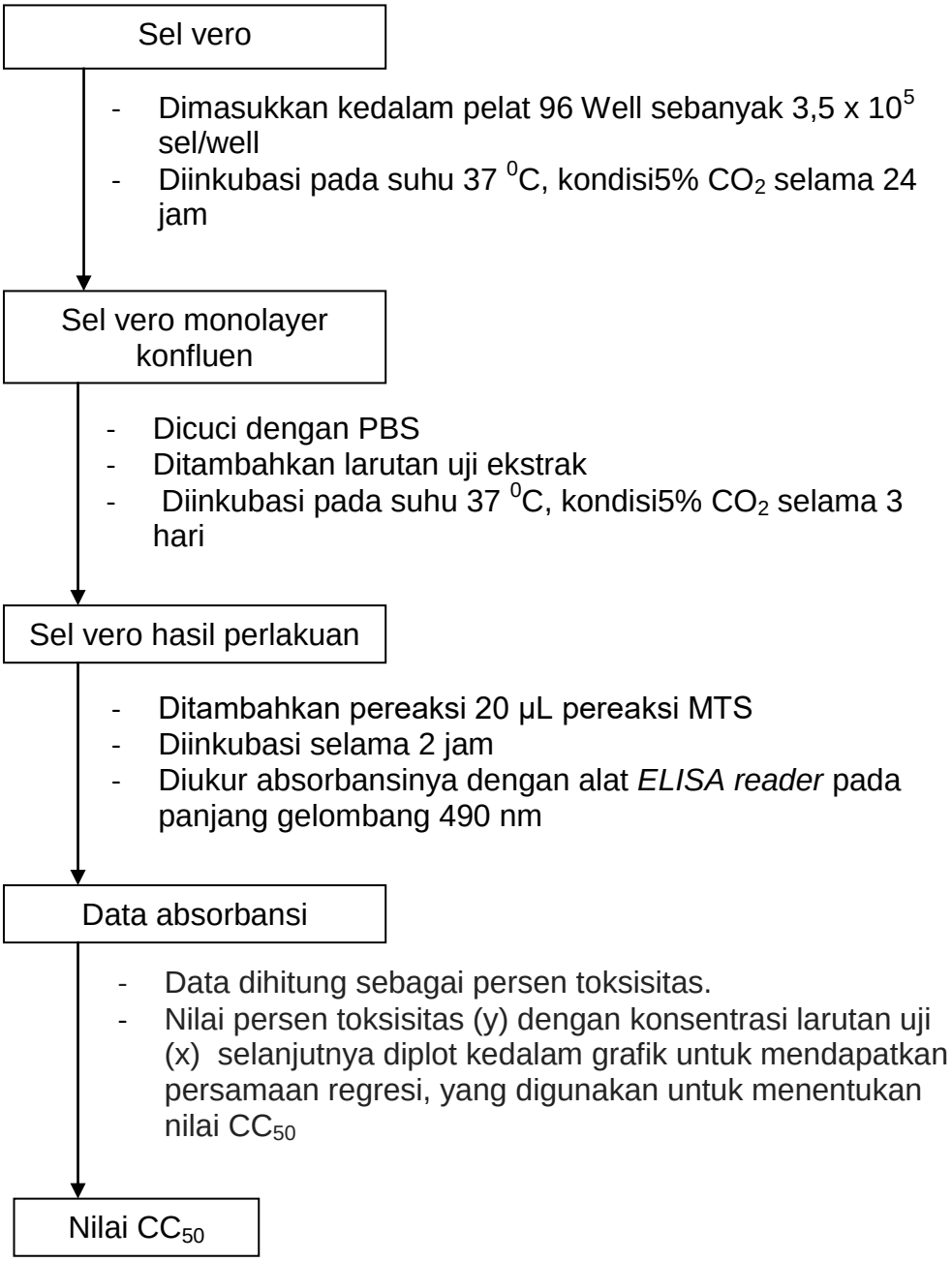
- Di infeksikan pada sel Vero dalam flask (tambahkan 50 µL virus yang telah diencerkan dengan 1 mL medium MEM)
- Di kocok
- Di inkubasi (37 ^{0}C, kondisi 5% CO_2)
- Setiap 15-20 menit dikocok perlahan, selama 1 jam
- Setelah 1 jam ditambahkan medium MEM (2% FBS) 5 mL
- Di inkubasi (37 ^{0}C, kondisi 5% CO2) sampai terlihat efek sitopatik

Supernatan

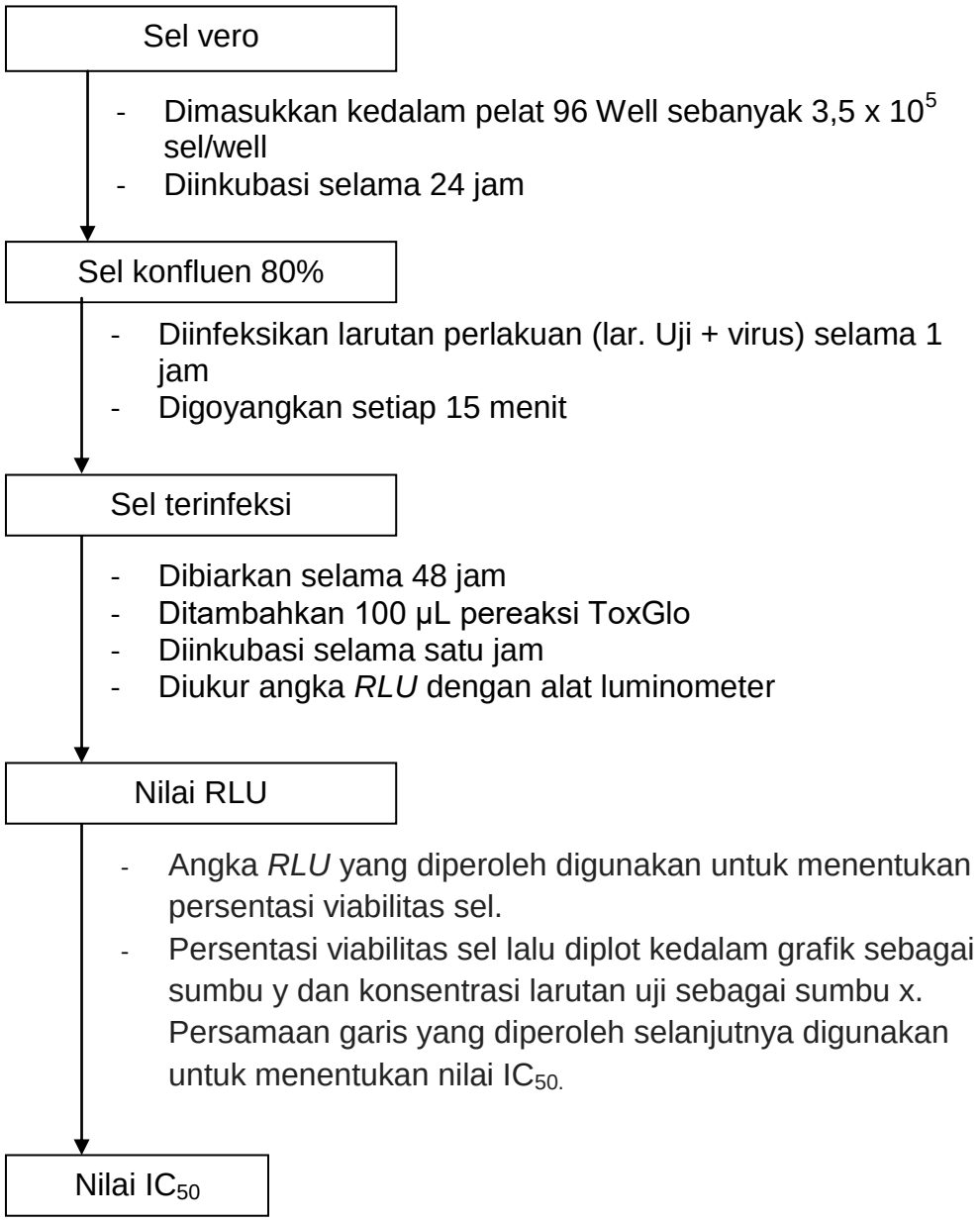
- Di panen (di pipet secara perlahan)
- Di sentrifugasi pada 4000 rpm, 4 ^{0}C selama 10 menit

Supernatan virus (stok virus disimpan pada suhu 80 ^{0}C)

e. Uji Sitotoksisitas



f. Uji Penghambatan terhadap Virus *Dengue*



Lampiran 5. Perhitungan pada pengujian BSLT

a. Persentase kematian larva pada larutan ekstrak

$$\%\ Kematian = \frac{\text{jumlah larva mati}}{\text{jumlah larva awal}} \text{ x } 100$$

- 0,1 ppm

$$\%\ Kematian = \frac{10}{30} \text{ x } 100$$
$$= 33$$

- 1 ppm

$$\%\ Kematian = \frac{16}{30} \text{ x } 100$$
$$= 53$$

- 10 ppm

$$\%\ Kematian = \frac{29}{30} \text{ x } 100$$
$$= 97$$

1) Persentase kematian larva pada kontrol

$$\%\ Kematian = \frac{\text{jumlah larva mati}}{\text{jumlah larva awal}} \text{ x } 100$$

- 0,1 ppm

$$\%\ Kematian = \frac{0}{30} \text{ x } 100$$
$$= 0$$

- 1 ppm

$$\%\ Kematian = \frac{1}{30} \text{ x } 100$$
$$= 3$$

- 10 ppm

$$\%\ Kematian = \frac{3}{30}\ x\ 100$$
$$= 10$$

b. Persentase kematian akhir

$$\%\ Kematian\ akhir = \%\ \text{kematian lar. ekstrak} - \%\ \text{kematian kontrol}$$

- 0,1 ppm

$$\%\ Kematian = 33 - 0$$
$$= 33$$

- 1 ppm

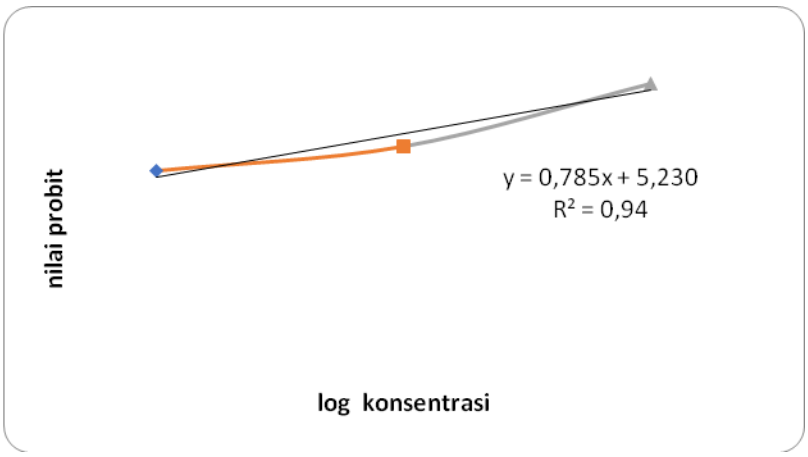
$$\%\ Kematian = 53 - 3$$
$$= 50$$

- 10 ppm

$$\%\ Kematian = 97 - 10$$
$$= 87$$

Tabel nilai probit untuk setiap % kematian akhir

Konsentrasi (ppm)	Log konsentrasi	% kematian akhir	Nilai probit
0,1	-1	33	4,56
1	0	50	5,00
10	1	87	6,13



Grafik hubungan log konsentrasi (x) dengan nilai probit % kematian

c. Perhitungan nilai LC_{50}

Dari grafik diperoleh persamaan y = 0,785x + 5,230, jika y = 50 dan nilai LC_{50} = x maka:

$$y = 0{,}785x + 5{,}230$$
$$5 - 5{,}230 = 0{,}785x + 5{,}230$$
$$-0{,}23 = 0{,}785x$$
$$x = \frac{-0{,}23}{0{,}785}$$
$$x = -0{,}293$$
$$x = \text{antilog}\ (-0{,}293)$$
$$x = 0{,}5093$$

Karena x = LC_{50}, maka;

$$LC50 = x$$
$$LC50 = 0{,}5093$$

Lampiran 6. Perhitungan pada pengujian antivirus

a. Uji Sitotoksik (Perhitungan nilai CC_{50})

1) Menghitu rata rata absorbansi perlakuan

- 6,25 µg/mL; $\frac{0,419 + 0,338 + 0,357}{3} = 0,371333333$
- 12,5 µg/mL; $\frac{0,545 + 0,37 + 0,559}{3} = 0,491333333$
- 25 µg/mL; $\frac{1,16 + 0,568 + 0,913}{3} = 0,880333333$
- 50 µg/mL; $\frac{1,64 + 1,267 + 1,375}{3} = 1,427333333$
- 100 µg/mL; $\frac{2,217 + 2,007 + 2,219}{3} = 2,147666667$
- 200 µg/mL; $\frac{2,787 + 2,476 + 2,207}{3} = 2,49$
- Kontrol sel; $\frac{0,963 + 1,05 + 2,624}{3} = 1,545666667$
- Kontrol Media; $\frac{0,125 + 0,117 + 0,12}{3} = 0,120666667$

2) Menghitung (Abs. Perlakuan) - (Abs. Media)

- 6,25 µg/mL; $(0,371333333) - (0,120666667 = 0,250666667$
- 12,5 µg/mL; $(0,491333333) - (0,120666667 = 0,370666667$
- 25 µg/mL; $(0,880333333) - (0,120666667 = 0,759666667$
- 50 µg/mL; $(1,427333333) - (0,120666667 = 1,306666667$
- 100 µg/mL; $(2,147333333) - (0,120666667 = 2,027$
- 200 µg/mL; $(2,49) - (0,120666667 = 2,369333333$

3) Menghitung (Abs. Sel) - (Abs. Media)

$$(1{,}545666667) - (0{,}120666667 = 1{,}425$$

4) Menghitung % Toksisitas

$$\%\,Toksisitas = \frac{(\text{(Abs. perlakuan)} - \text{(Abs. Media)})}{(\text{(Abs. Sel)} - \text{(Abs. Media)})} \times 100$$

- 6,25 µg/mL

$$\%\,Toksisitas = \frac{(0{,}250666667)}{(1{,}425)} \times 100$$

$$= 17{,}59064327$$

- 12,5 µg/mL

$$\%\,Toksisitas = \frac{(0{,}370666667)}{(1{,}425)} \times 100$$

$$= 26{,}01169591$$

- 25 µg/mL

$$\%\,Toksisitas = \frac{(0{,}759666667)}{(1{,}425)} \times 100$$

$$= 53{,}30994152$$

- 100 µg/mL

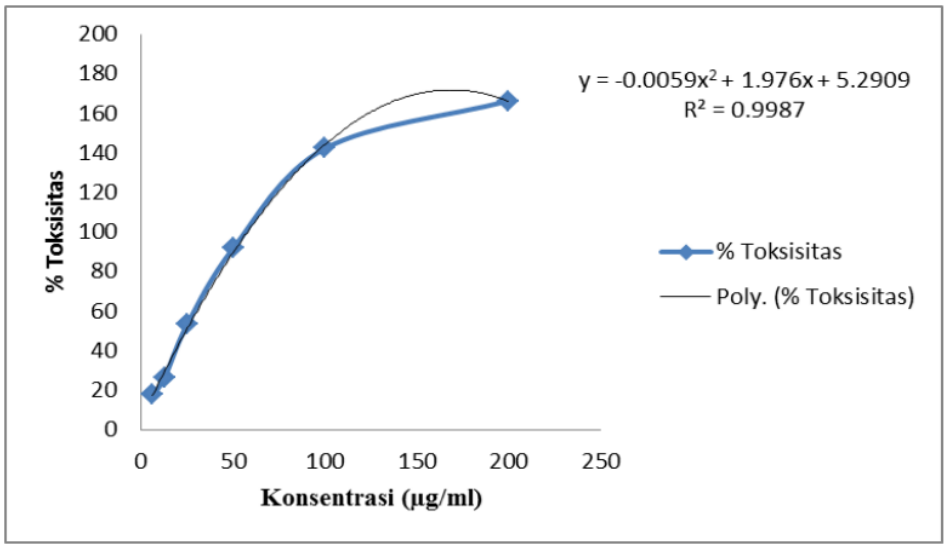
$$\%\,Toksisitas = \frac{(2{,}027)}{(1{,}425)} \times 100$$

$$= 142{,}245614$$

- 200 µg/mL

$$\%\,Toksisitas = \frac{(2{,}369333333)}{(1{,}425)} \times 100$$

$$= \quad 166{,}2690058$$



Grafik hubungan % Toksisitas (y) dan konsentrasi larutan ekstrak (x)

Dari persamaan pada grafik maka dapat dihitung nilai CC_{50} yang sama dengan nilai x (CC_{50} = x):

$$x1,x2 = \frac{-1{,}976 \pm \sqrt{(1{,}976)^2 - 4(-0{,}0059)(5{,}2909 - 50)}}{2\,(-0{,}0059)}$$

$$x1,x2 = \frac{-1{,}976 \pm \sqrt{(3{,}904576)\,(1{,}05513476)}}{-0{,}0118}$$

$$x1,x2 = \frac{-1{,}976 \pm \sqrt{2{,}84944124}}{-0{,}0118}$$

$$x1,x2 = \frac{-1{,}976 \pm 1{,}6880288031}{-0{,}0118}$$

- $x1 = \frac{-1{,}976 + 1{,}6880288031}{-0{,}0118}$

$$x1 = \frac{-0{,}287971197}{-0{,}0118}$$

$$x1 = 24{,}4$$

- $x2 = \frac{-1{,}976 - 1{,}6880288031}{-0{,}0118}$

$$x2 = \frac{-3{,}664028803}{-0{,}0118}$$

$$x2 = 310{,}5$$

b. Uji Antivirus (Perhitungan nilai IC_{50})

1) Menghitu rata rata Luminescence perlakuan

- 100 µg/mL; $\frac{(8{,}46E+04) + (7{,}99E+04) + (7{,}47E+04)}{3} = 7{,}97E+04$
- 50 µg/mL; $\frac{(1{,}00E+05) + (9{,}74E+04) + (8{,}83E+04)}{3} = 9{,}53E+04$
- 25 µg/mL; $\frac{(1{,}01E+05) + (8{,}19E+04) + (1{,}79E+05)}{3} = 1{,}21E+05$
- 12,5 µg/mL; $\frac{(1{,}55E+05) + (1{,}52E+05) + (1{,}73E+05)}{3} = 1{,}60E+05$
- 6,25 µg/mL; $\frac{(3{,}15E+05) + (2{,}59E+05) + (5{,}92E+05)}{3} = 3{,}89E+05$
- 3,13 µg/mL; $\frac{(6{,}90E+05) + (6{,}56E+05) + (9{,}55E+05)}{3} = 7{,}67E+05$
- 1,76 µg/mL; $\frac{(1{,}51E+06) + (1{,}36E+06) + (2{,}60E+06)}{3} = 1{,}82E+06$
- K. DENV + K. Sel;

$$\frac{(9{,}72E+06) + (8{,}71E+06) + (8{,}66E+06)}{3} = 9{,}03E+06$$

- K. Media; $\frac{(1{,}50E+03) + (7{,}00E+02) + (5{,}96E+02)}{3} = 9{,}33E+02$

2) Menghitung (L. Treatmen) - (L. K. Media)

- 100 µg/mL; $(7{,}97E+04) - (9{,}33E+02) = 7{,}88E+04$
- 50 µg/mL; $(9{,}53E+04) - (9{,}33E+02) = 9{,}44E+04$
- 25 µg/mL; $(1{,}21E+05) - (9{,}33E+02) = 1{,}20E+05$
- 12,5 µg/mL; $(1{,}60E+05) - (9{,}33E+02) = 1{,}59E+05$
- 6,25 µg/mL; $(3{,}89E+05) - (9{,}33E+02) = 3{,}88E+05$
- 3,13 µg/mL; $(7{,}67E+05) - (9{,}33E+02) = 7{,}66E+05$
- 1,76 µg/mL; $(1{,}82E+06) - (9{,}33E+02) = 1{,}82E+06$

3) Menghitung L. (K. DENV + K. Sel) - L. (K. Media)

$$(9{,}03E+06) - (9{,}33E+02) = 9{,}03E+06$$

4) Menghitung % Viabilitas Sel

$$\%\ Viabilitas\ Sel = \frac{(\text{(L. Treatmen)} - \text{(L. K. Media)})}{(\text{L.(K. DENV + K. Sel)} - \text{(L. K. Media)})} \times 100$$

- 100 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(7{,}88E+04)}{(9{,}03E+06)} \times 100$$

$$= 0{,}8726762$$

- 50 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(9{,}44E+04)}{(9{,}03E+06)} \times 100$$

$$= 1{,}0455495$$

- 25 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(1{,}20E+05)}{(9{,}03E+06)} \times 100$$

$$= 1{,}3262887$$

- 12,5 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(1{,}59E+05)}{(9{,}03E+06)} \times 100$$

$$= 1{,}7649136$$

- 6,25 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(3{,}88E+05)}{(9{,}03E+06)} \times 100$$

$$= 4{,}2935966$$

- 3,13 µg/mL

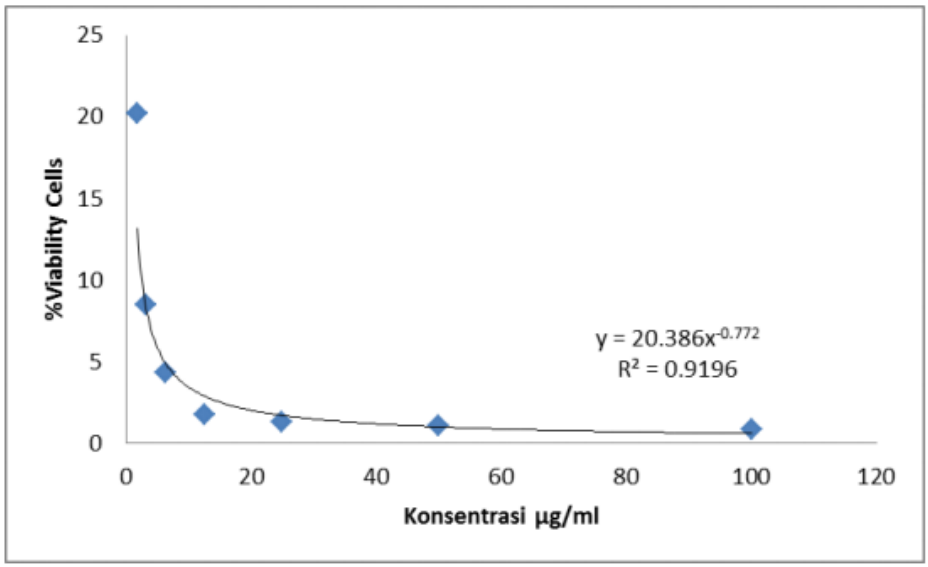
$$\%\ Viabilitas\ Sel = \frac{(7{,}66E+05)}{(9{,}03E+06)} \times 100$$

$$= 8{,}4793974$$

- 1,76 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(1{,}82E+06)}{(9{,}03E+06)} \times 100$$

$$= 2\ 0{,}171141$$



Grafik hubungan % Viabilitas sel (y) dengan konsentrasi larutan ekstrak (x)

Dari persamaan pada grafik maka dapat dihitung nilai IC_{50} yang sama dengan nilai x (IC_{50} = x):

$$y = (20{,}386)\ (x)^{-0{,}772}$$

$$50 = (20{,}386)\ (x)^{-0{,}772}$$

$$\frac{50}{20{,}386} = (x)^{-0{,}772}$$

$$2{,}4526635927 = (x)^{-0{,}772}$$

$$2{,}4526635927 = \frac{1}{(x) - 0{,}772}$$

$$(x)^{0{,}772} = \frac{1}{2{,}4526635927}$$

$$(x)^{0{,}772} = 0{,}40772$$

$$(x) = 0{,}31282$$

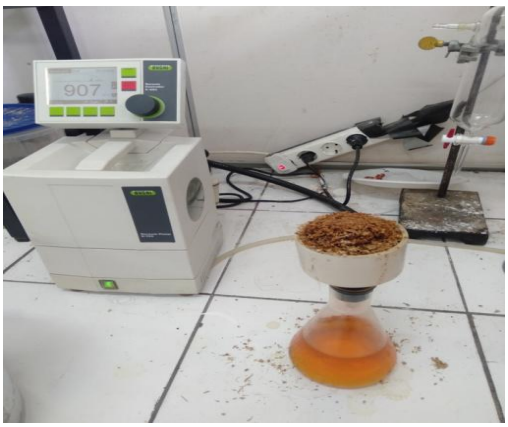
Lampiran 7. Dokumentasi Penelitian

a. Sampel rimpang lengkuas merah

b. Lengkuas merah kering

c. Serbuk lengkuas merah

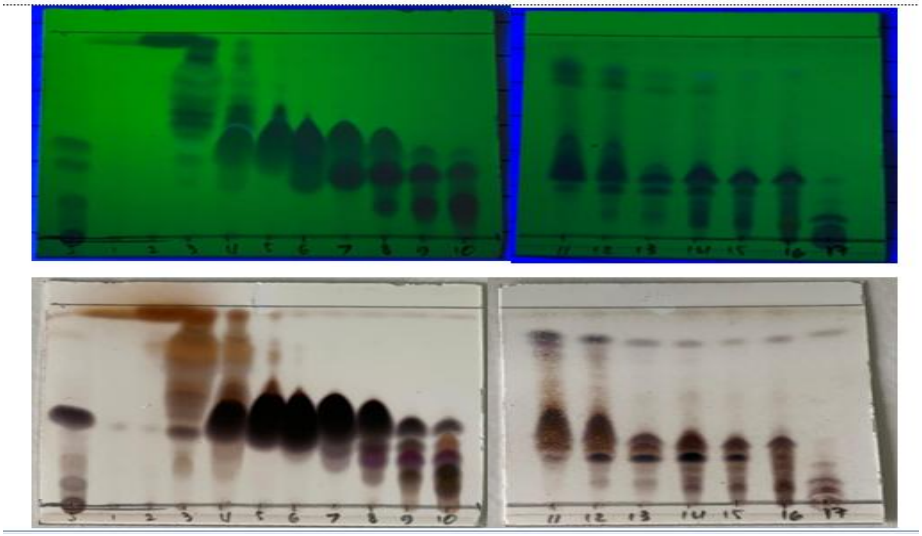
d. Proses maserasi



e. Proses penyaringan

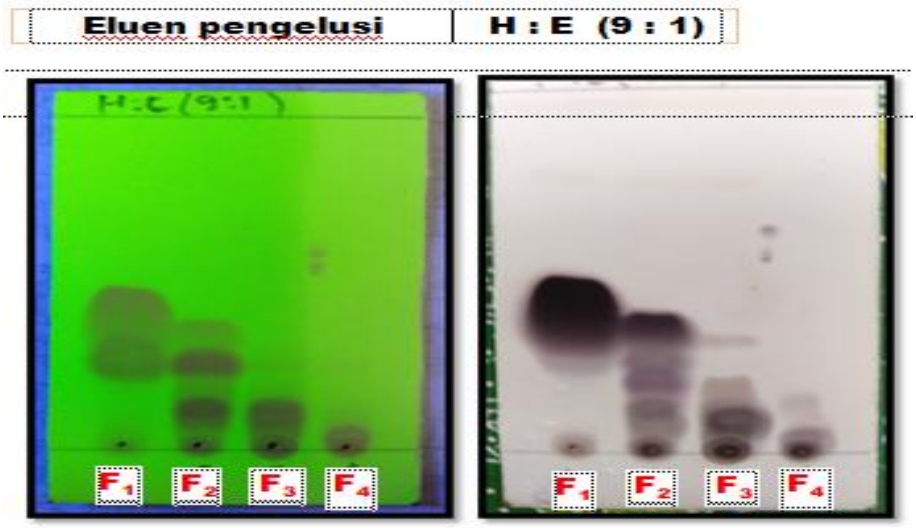
f. Proses evaporasi

g. Ekstrak rimpan lengkuas merah

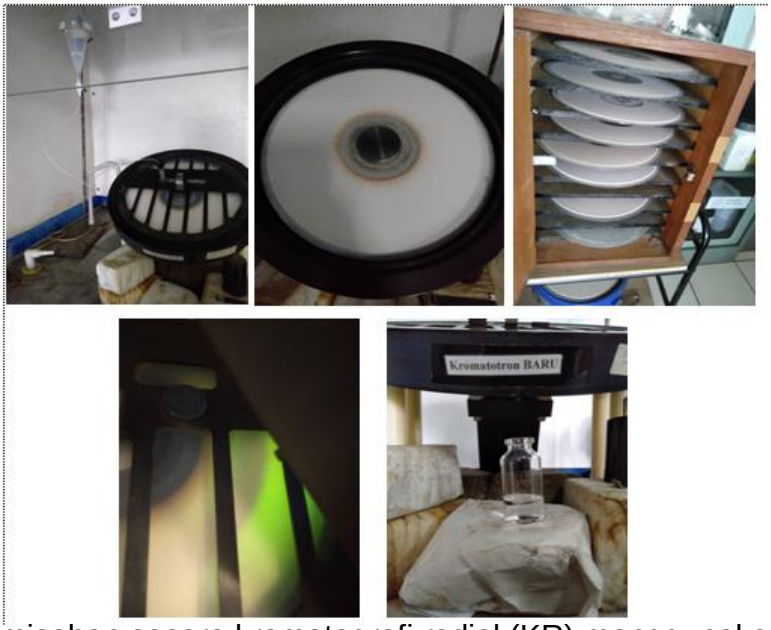


h. Hasil analisis KLT fraksi kromatografi kolom

i. Fraksi gabungan

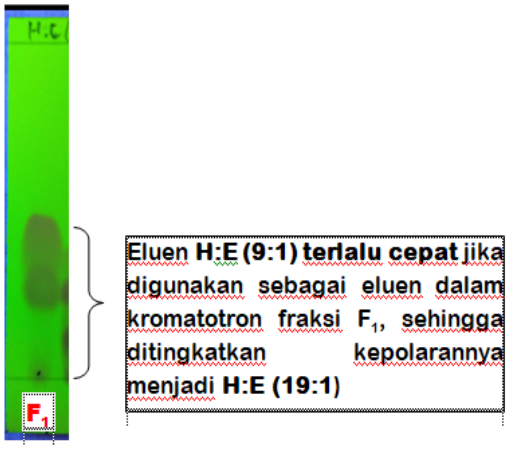


j. Hasil analisis KLT fraksi gabungan



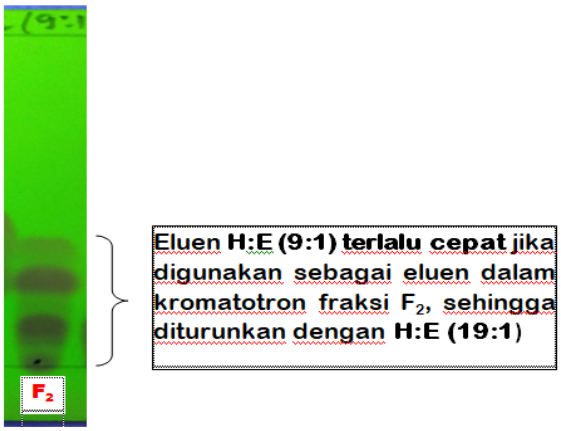
k. Proses pemisahan secara kromatografi radial (KR) menggunakan kromatotron

- Analisis Klt fraksi F_1 **(Eluen H:E-9:1)**



l. pemilihan eluen yang sesuai untuk proses kromagrafi radial untuk fraksi gab. F_{6-7}

- Uji Klt fraksi F_2 **(Eluen H:E-9:1)**



m. pemilihan eluen yang sesuai untuk proses kromagrafi radial untuk fraksi gab. F_{8-10}

- Analisis Klt fraksi F_3 **(Eluen H:E-9:1)**

Eluen H:E (9:1) agak lambat jika digunakan sebagai eluen dalam kromatotron fraksi F_3, sehingga dinaikkan menjadi H:E (8,5:1,5)

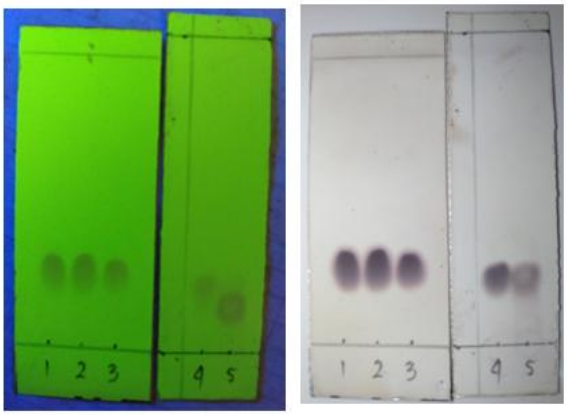
n. pemilihan eluen yang sesuai untuk proses kromagrafi radial untuk fraksi gab. F_{11-12}

- Analisi Klt fraksi F_4 **(Eluen H:E-9:1)**

Eluen H:E (9:1) terlalu rendah jika digunakan sebagai eluen dalam kromatotron fraksi F_4, sehingga dinaikkan menjadi H:E (8:2)

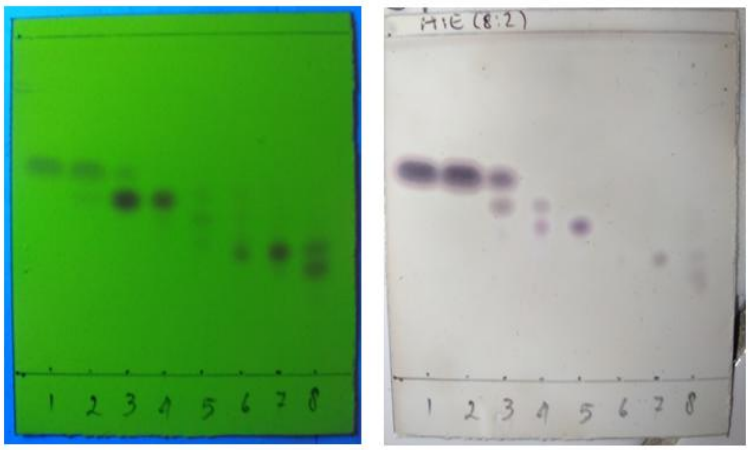
o. Pemilihan eluen yang sesuai untuk proses kromagrafi radial untuk raksi gab. F_{13-16}

- Hasil Analisis KLT Fraksi Fraksi Hasil Kromatotron. eluen yang digunakan dalam kromatotron yakni **H:E (19:1)**



p. Analisis KLT fraksi fraksi hasil kromatografi radial fraksi gab. F_{6-7}

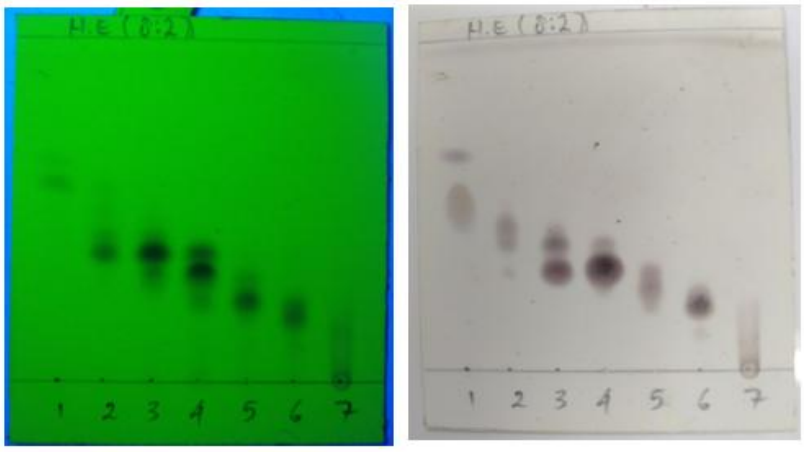
- Hasil Analisis KLT Fraksi Hasil Kromatotron, eluen yang digunakan dalam kromatotron yakni **H:E (19:1)**



Larutan pengelusi KLT H:E (8:2)

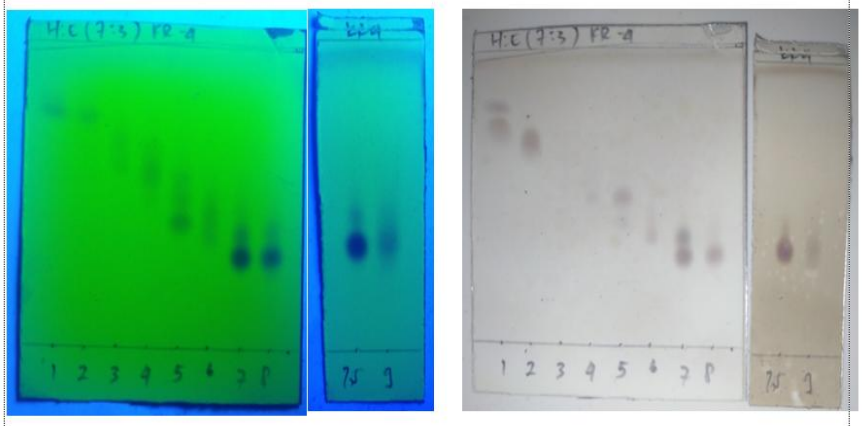
q. Analisis KLT fraksi fraksi hasil kromatografi radial fraksi gab. $F_{8\text{-}10}$

- Hasil Analisis KLT Fraksi Fraksi dari Kromatotron, eluen yang digunakan dalam kromatotron yakni **H:E (8,5:1,5)**



r. Analisis KLT fraksi fraksi hasil kromatografi radial fraksi gab. $F_{11\text{-}12}$

- Hasil Analisis KLT Fraksi Fraksi dari Kromatotron, eluen yang digunakan dalam kromatotron yakni **H:E (8:2)**
  (Bobot fraksi yg di kromatotron = 118 mg)



s. Analisis KLT fraksi fraksi hasil kromatografi radial fraksi gab. $F_{13\text{-}16}$