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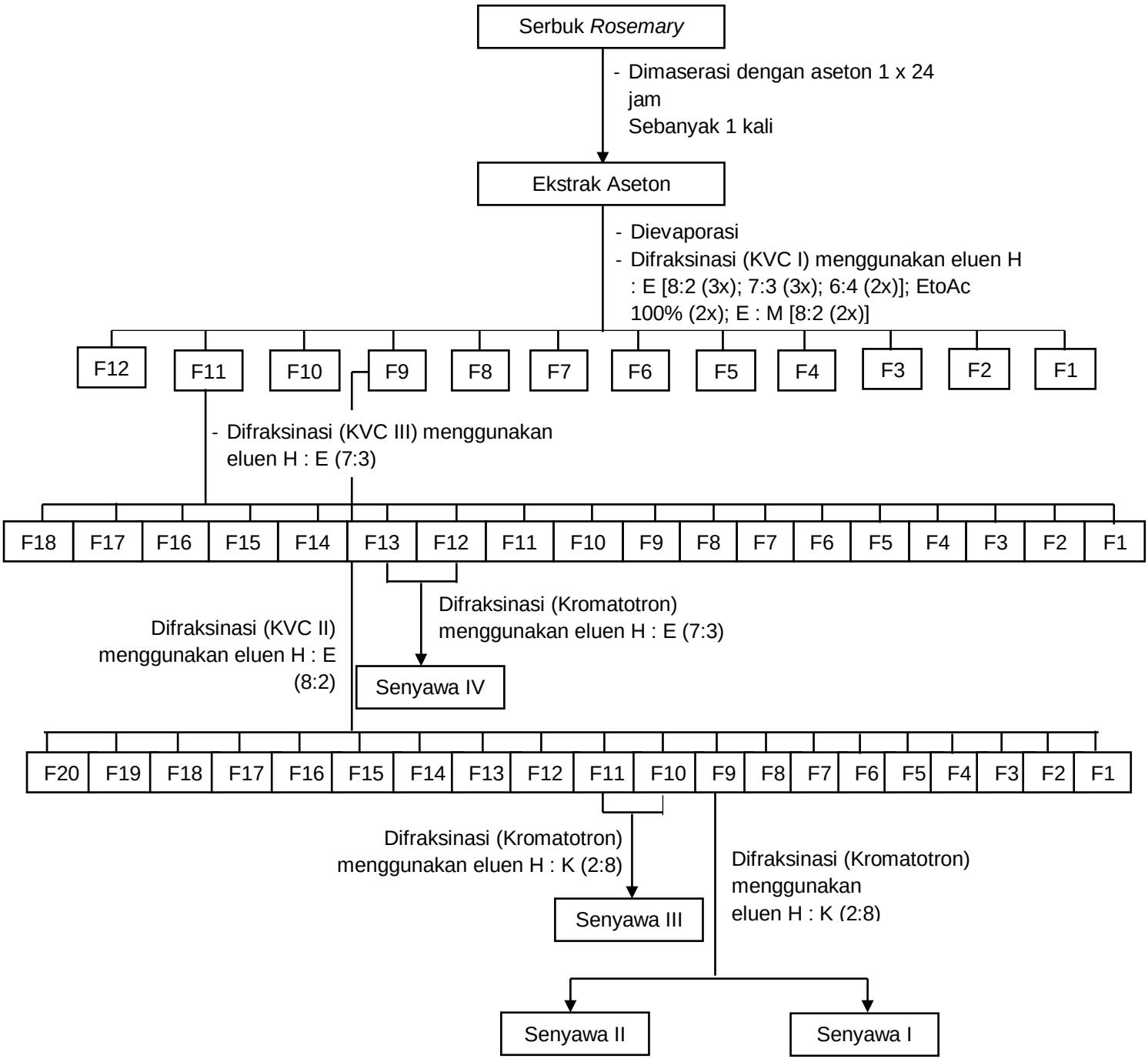
Zanella, C.A., Treichel, H., Cansian, R.L., Roman, S.S. 2012. The effects of acute administration of the hydroalcoholic extract of rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) in animal models of memory. Brazilian Journal of Pharmaceutical Sciences. 48(3): 389-397.

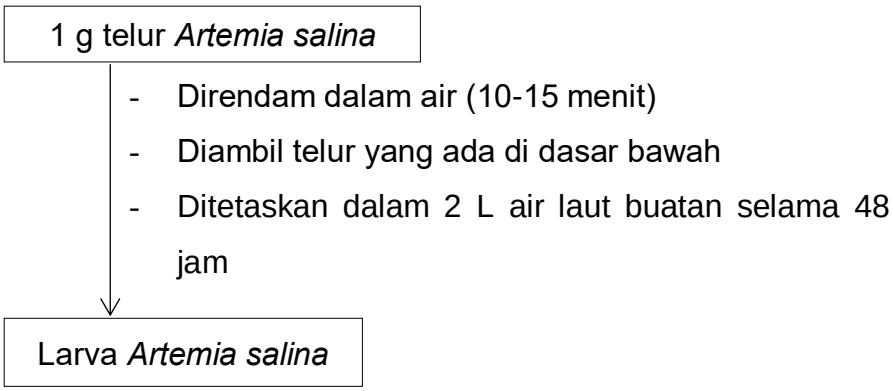
LAMPIRAN

Lampiran 1. Bagan isolasi senyawa metabolit sekunder dari ekstrak aseton *Rosmarinus officinalis* L. (rosemary)

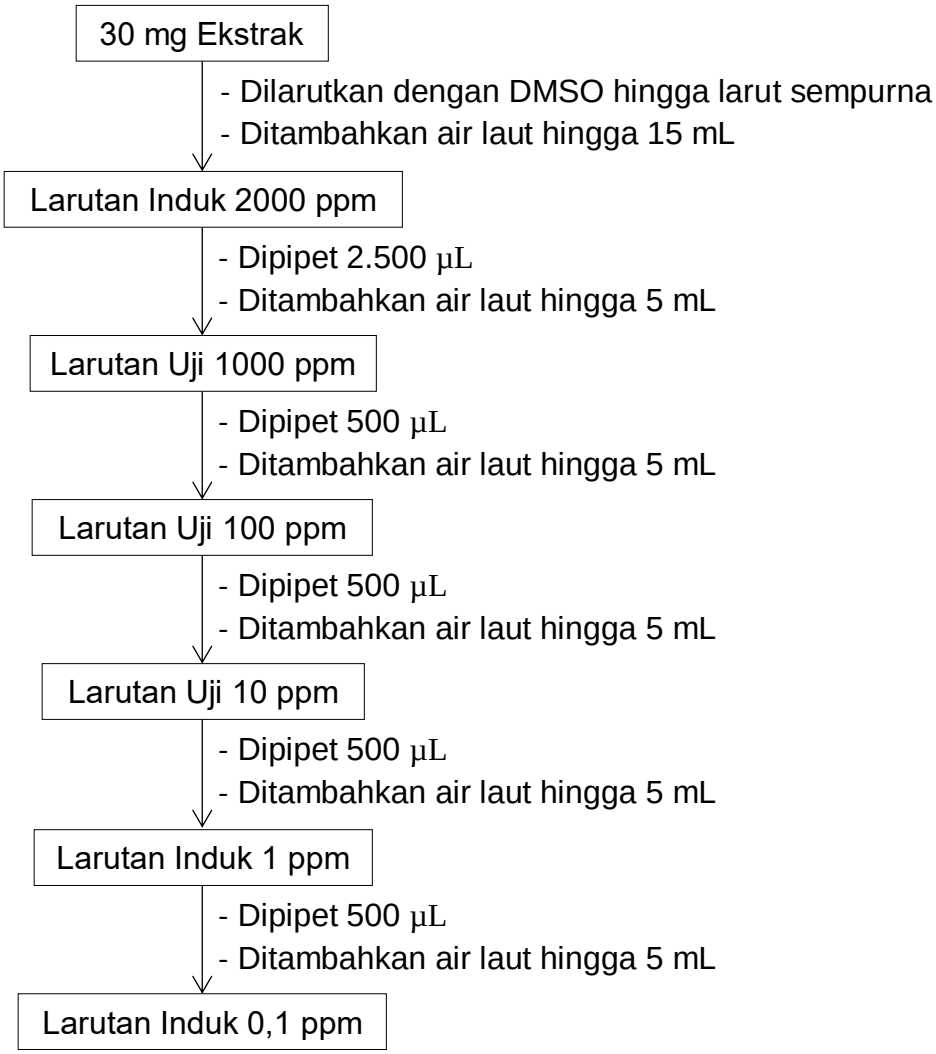


Lampiran 2. Skema kerja uji toksisitas (BSLT)

A. Penyiapan Larva



B. Penyiapan Sampel Uji

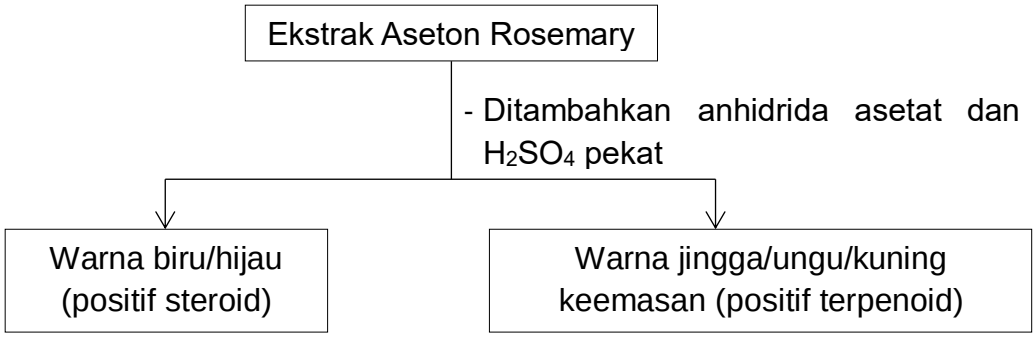


C. Pengujian Toksisitas

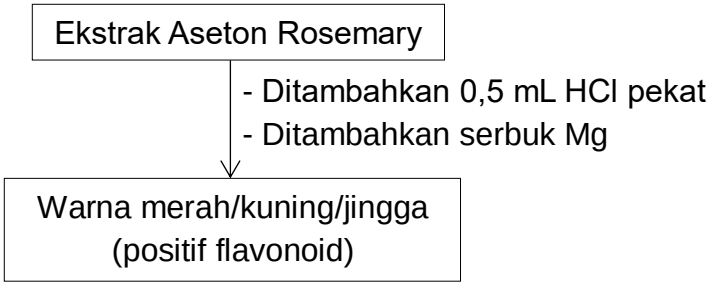
kontrol untuk setiap konsentrasi larutan uji disiapkan menggunakan air laut dan pelarut DMSO dengan perlakuan yang sama dengan sampel.

Lampiran 3. Bagan uji fitokimia

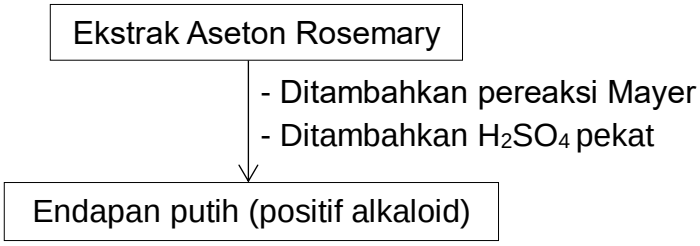
A. Uji Terpenoid dan Steroid (Pereaksi Libermann-Burchard)



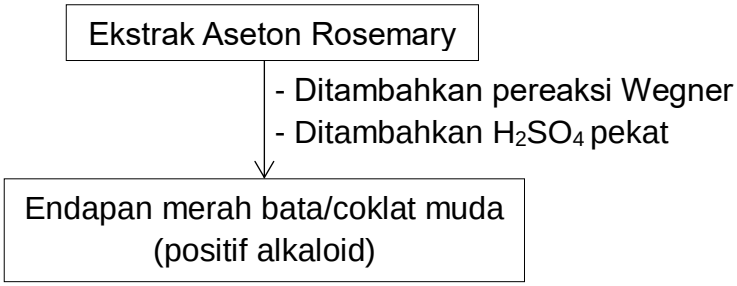
B. Uji Flavonoid (Pereaksi Shinoda)



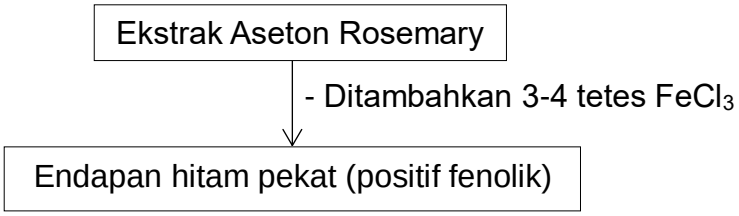
C. Uji Alkaloid (Pereaksi Mayer)



D. Uji Alkaloid (Pereaksi Wegner)

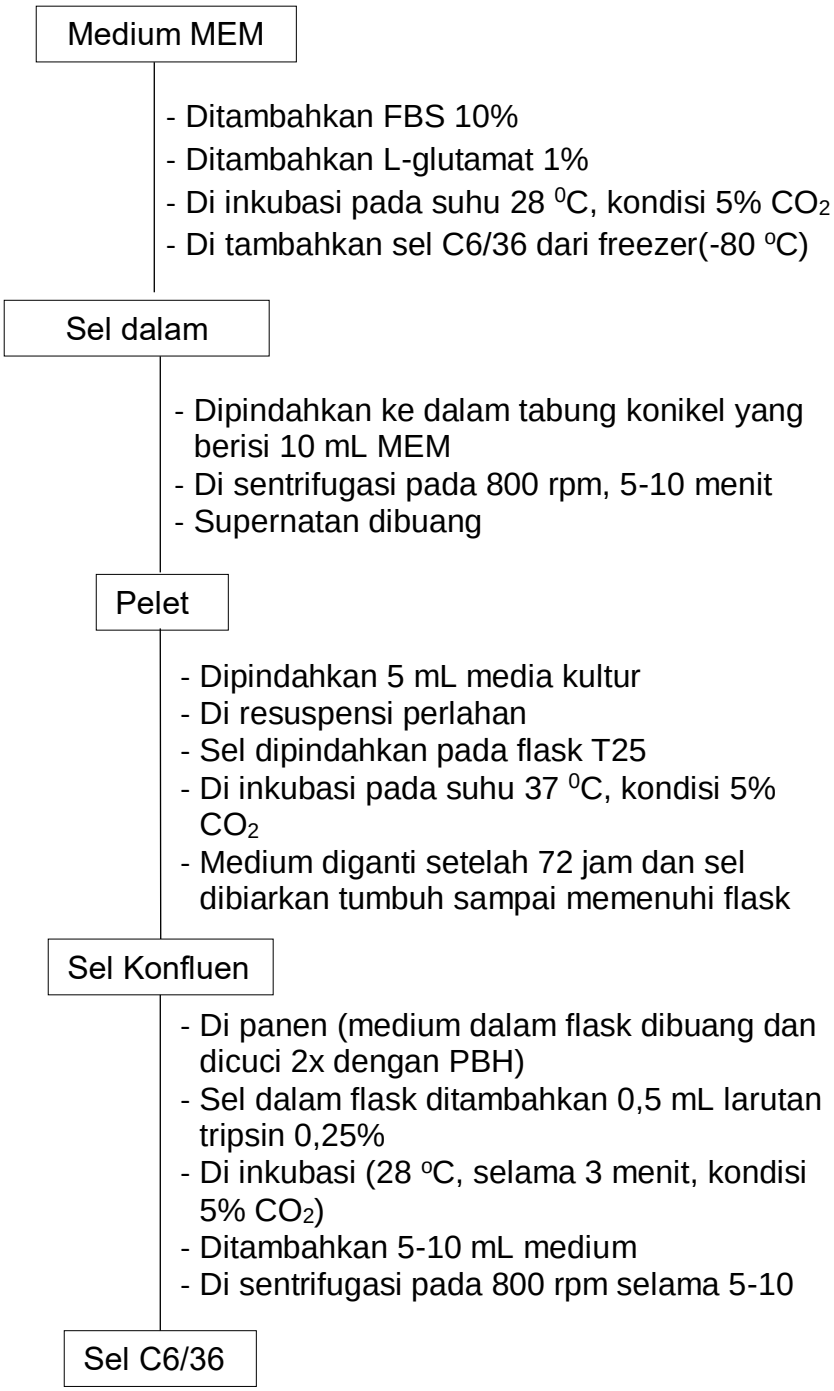


E. Uji Fenolik (Pereaksi $FeCl_3$)

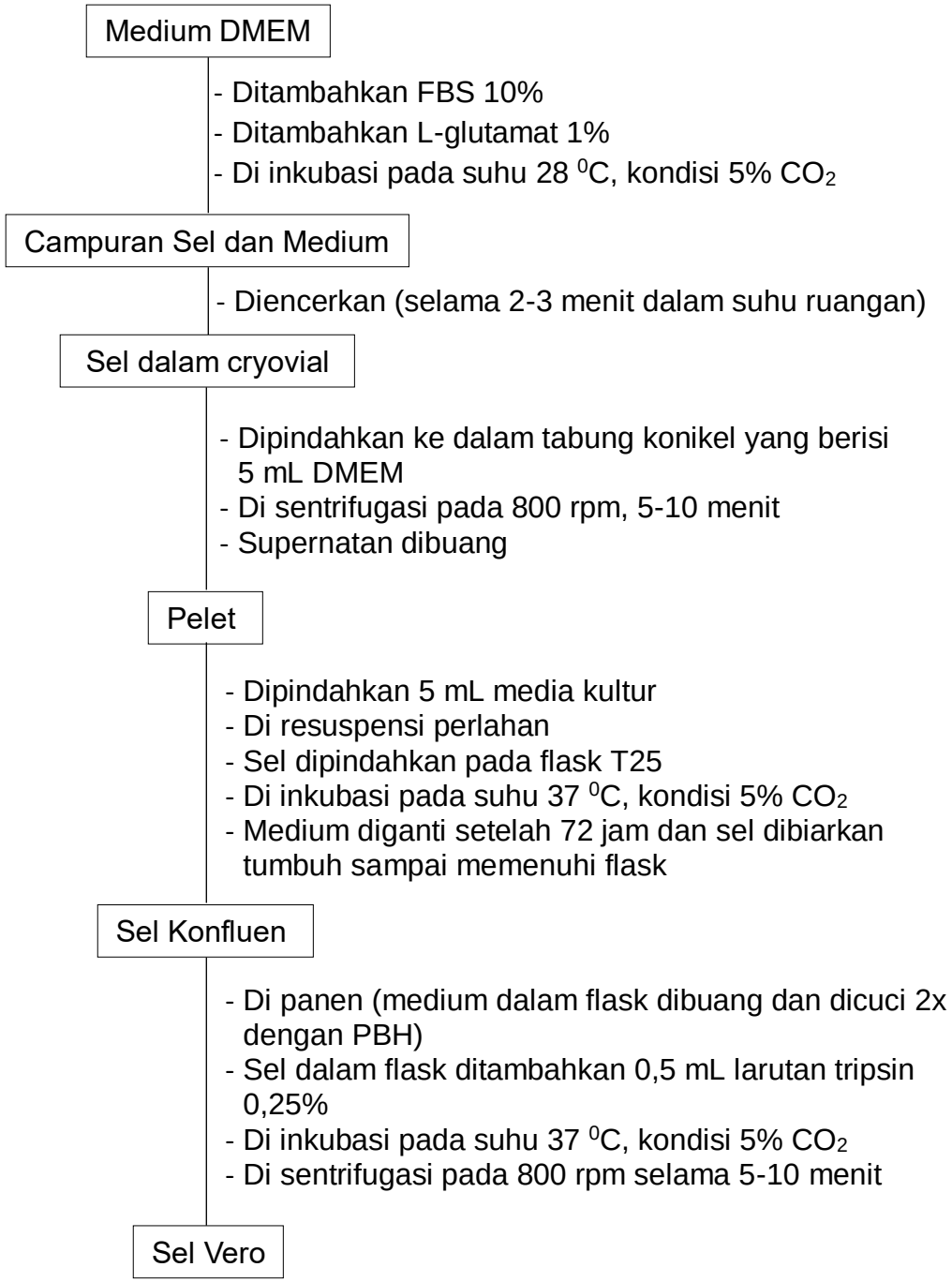


Lampiran 4. Bagan uji antivirus *dengue*

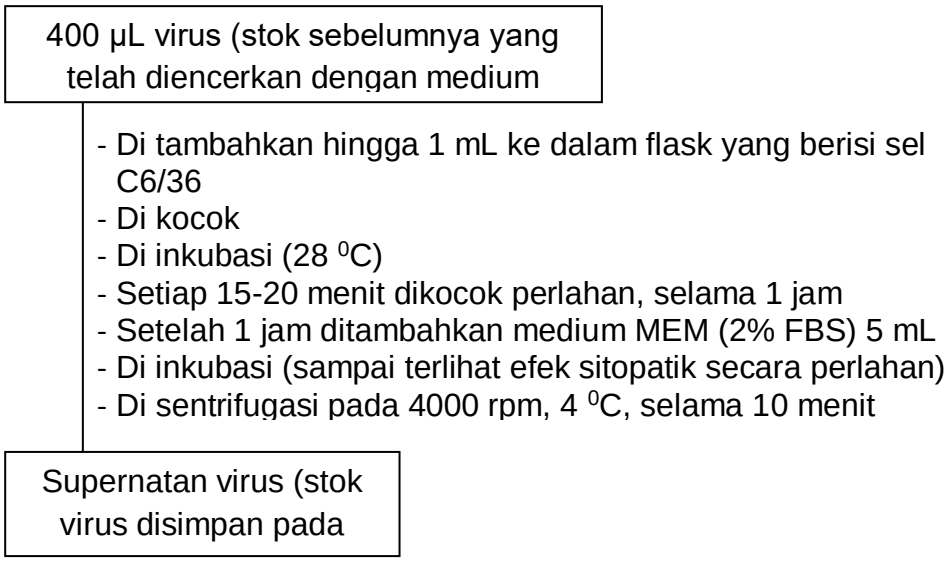
A. Kultur Sel C6/36



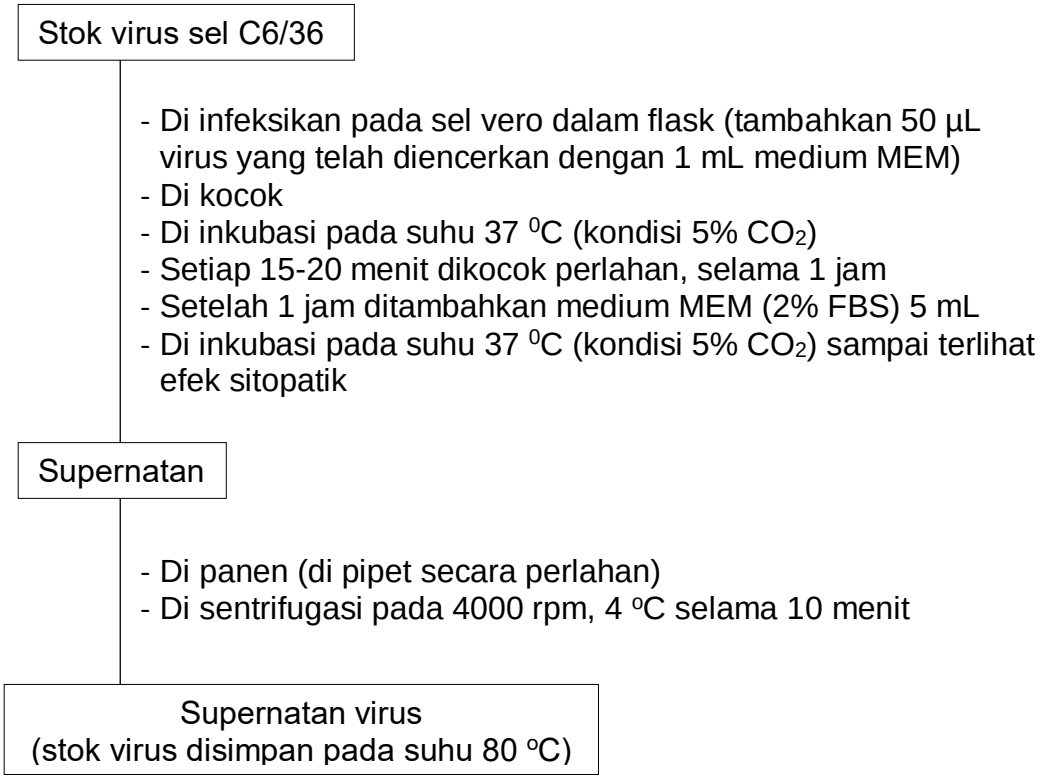
B. Kultur Sel Vero



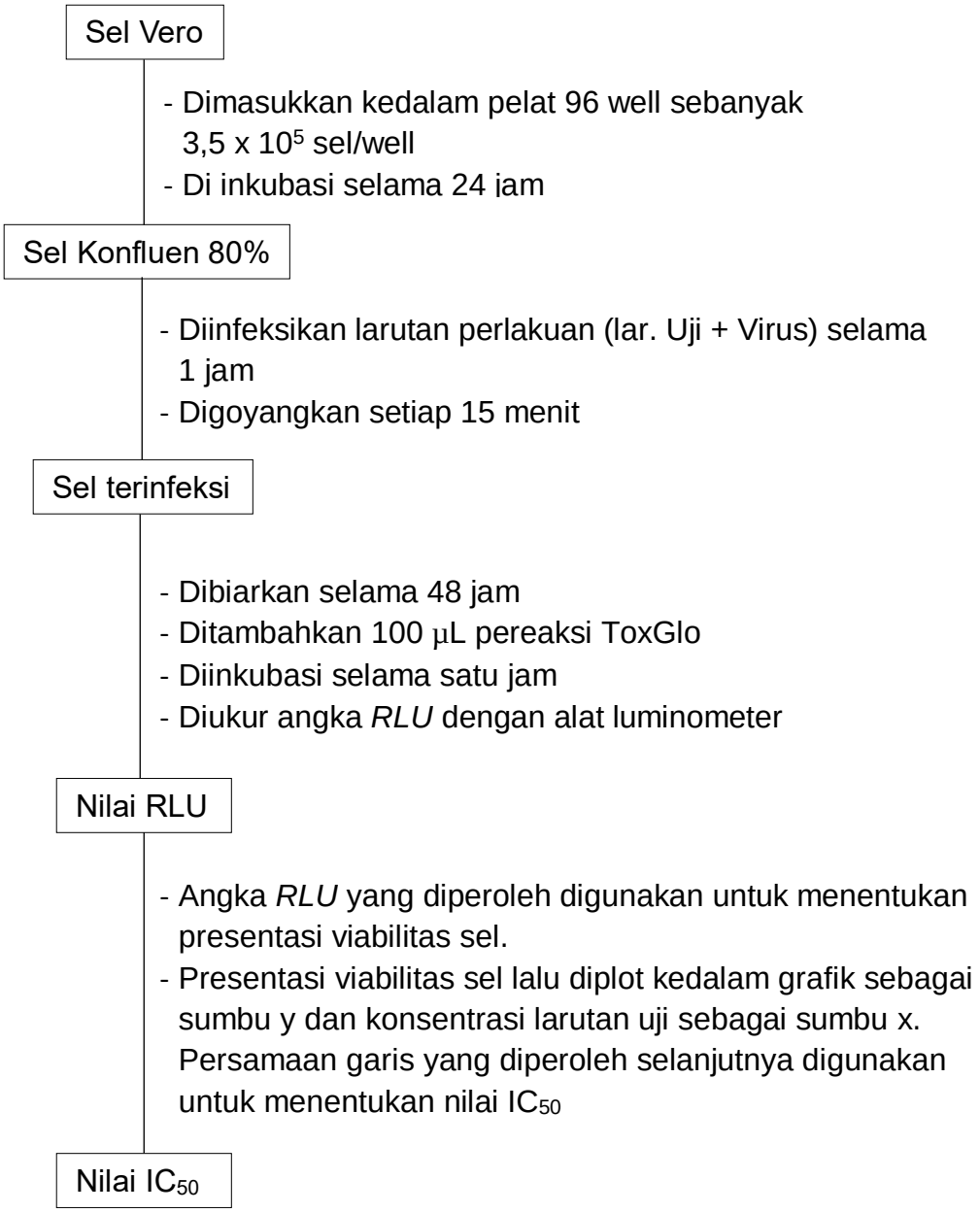
C. Pembuatan Stok Virus Pada Sel C6/36



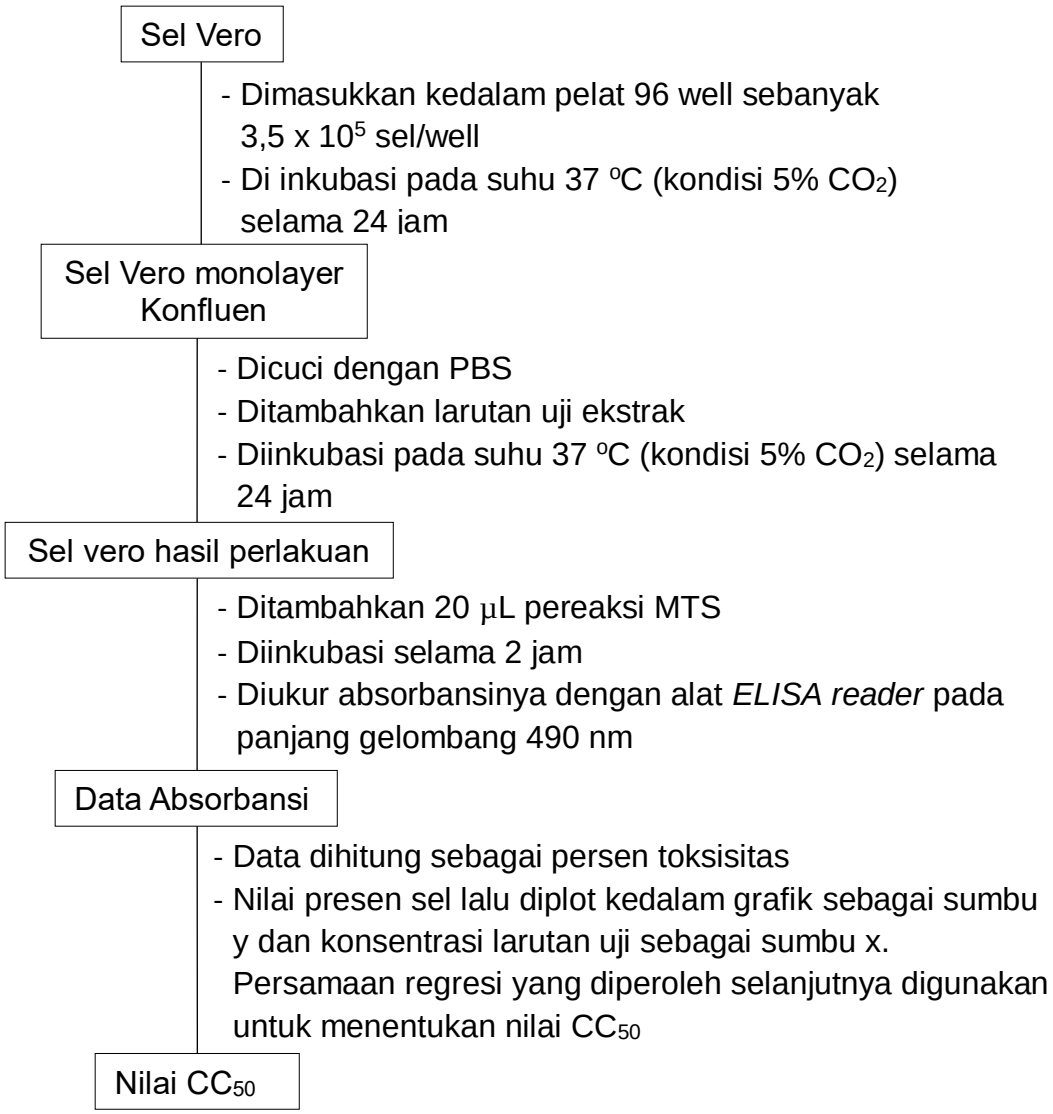
D. Pembuatan Stok Virus Pada Sel Vero



E. Uji Penghambatan Terhadap Virus *Dengue*



F. Uji Sitotoksitas



Lampiran 5. Perhitungan rendamen ekstrak aseton rosemary

Berat botol kosong = 177,2 g

Berat botol kosong + ekstrak pekat = 245,4 g

Berat ekstrak pekat = (berat botol kosong+ekstrak) – berat botol kosong

= 245,4 g – 177,2 g = 68,2 g

Rendamen $= \frac{\text{berat ekstrak pekat}}{\text{berat sampel awal}} \times 100\%$

$= \frac{68{,}2\ \text{g}}{500\ \text{g}} \times 100\% = 13{,}64\%$

Lampiran 6. Perhitungan pada pengujian BSLT

A. Perhitungan Konsentrasi Larutan Ekstrak Untuk Uji Toksisitas

a. Pembuatan Larutan Induk 2000 ppm Ekstrak Aseton Rosamary

$$\text{Konsentrasi Larutan} = \frac{\text{Bahan yang ditimbang}}{\text{Volume larutan}}$$

$$\frac{2000\ \text{mg}}{1000\ \text{mL}} = \frac{x}{15\ \text{mL}}$$

$$1000\ X = 30.000$$

$$X = \frac{30.000\ \text{mg/mL}}{10.000\ \text{mL}}$$

$$X = 30\ \text{mg} = 0{,}03\ \text{g}$$

Jadi, larutan induk 2000 ppm dibuat dengan melarutkan 30 mg ekstrak ke dalam 15 mL pelarut.

b. Pembuatan Larutan Ekstrak 1000 ppm

$$V_1 \times M_1 = V_2 \times M_2$$

$$V_1\ (2000\ \text{ppm}) = (5\ \text{mL})\ (1000\ \text{ppm})$$

$$V_1 = \frac{5.000\text{mL}}{2.000}$$

$$V_1 = 2{,}5\ \text{mL} = 2.500\ \mu\text{L}$$

Jadi, larutan uji 1000 ppm dibuat dengan mengambil 2.500 µL larutan induk menggunakan mikropipet kemudian dimasukkan ke dalam botol vial, selanjutnya dilarutkan dalam 5 mL air laut.

c. Pembuatan Larutan Ekstrak 100 ppm

$$V_1 \times M_1 = V_2 \times M_2$$

$$V_1\ (1000\ \text{ppm}) = (5\ \text{mL})\ (100\ \text{ppm})$$

$$V_1 = \frac{500\text{mL}}{1.000}$$

$$V_1 = 0{,}5\ \text{mL} = 500\ \mu\text{L}$$

Jadi, larutan uji 100 ppm dibuat dengan mengambil 500 µL larutan induk menggunakan mikropipet kemudian dimasukkan ke dalam botol vial, selanjutnya dilarutkan dalam 5 mL air laut.

d. Pembuatan Larutan Ekstrak 10 ppm

$$V_1 \times M_1 = V_2 \times M_2$$
$$V_1 \text{ (100 ppm)} = \text{(5 mL) (10 ppm)}$$
$$V_1 = \frac{50 \text{ mL}}{100}$$
$$V_1 = 0{,}5 \text{ mL} = 500 \ \mu\text{L}$$

Jadi, larutan uji 10 ppm dibuat dengan mengambil 500 µL larutan induk menggunakan mikropipet kemudian dimasukkan ke dalam botol vial, selanjutnya dilarutkan dalam 5 mL air laut.

e. Pembuatan Larutan Ekstrak 1 ppm

$$V_1 \times M_1 = V_2 \times M_2$$
$$V_1 \text{ (10 ppm)} = \text{(5 mL) (1 ppm)}$$
$$V_1 = \frac{5 \text{ mL}}{10}$$
$$V_1 = 0{,}5 \text{ mL} = 500 \ \mu\text{L}$$

Jadi, larutan uji 1 ppm dibuat dengan mengambil 500 µL larutan induk menggunakan mikropipet kemudian dimasukkan ke dalam botol vial, selanjutnya dilarutkan dalam 5 mL air laut.

f. Pembuatan Larutan Ekstrak 0,1 ppm

$$V_1 \times M_1 = V_2 \times M_2$$
$$V_1 \text{ (1 ppm)} = \text{(5 mL) (0,1 ppm)}$$
$$V_1 = \frac{0{,}5 \text{ mL}}{1}$$
$$V_1 = 0{,}5 \text{ mL} = 500 \ \mu\text{L}$$

Jadi, larutan uji 0,1 ppm dibuat dengan mengambil 500 µL larutan induk menggunakan mikropipet kemudian dimasukkan ke dalam botol vial, selanjutnya dilarutkan dalam 5 mL air laut.

B. Persentase Kematian Larva Pada Larutan Uji

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

a) 1000 ppm

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

b) 100 ppm

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

c) 10 ppm

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

d) 1 ppm

$$\%\ Kematian\ = \frac{27}{30}\ \text{x}\ 100$$
$$= 90$$

e) 0,1 ppm

$$\%\ Kematian\ = \frac{11}{30}\ \text{x}\ 100$$
$$= 36{,}67$$

C. Persentase Kematian Larva Pada Kontrol

a) 1000 ppm

$$\%\ Kematian\ = \frac{1}{30}\ \text{x}\ 100$$
$$= 3{,}33$$

b) 100 ppm

$$\%\ Kematian = \frac{1}{30}\ \text{x}\ 100$$
$$= 3{,}33$$

c) 10 ppm

$$\%\ Kematian = \frac{1}{30}\ \text{x}\ 100$$
$$= 3{,}33$$

d) 1 ppm

$$\%\ Kematian = \frac{10}{30}\ \text{x}\ 100$$
$$= 3{,}33$$

e) 0,1 ppm

$$\%\ Kematian = \frac{10}{30}\ \text{x}\ 100$$
$$= 3{,}33$$

D. Persentase Kematian Akhir

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

a) 1000 ppm

$$\%\ Kematian = 100 - 3{,}33$$
$$= 97$$

b) 100 ppm

$$\%\ Kematian = 100 - 3{,}33$$
$$= 97$$

c) 10 ppm

$$\%\ Kematian = 100 - 3{,}33$$
$$= 97$$

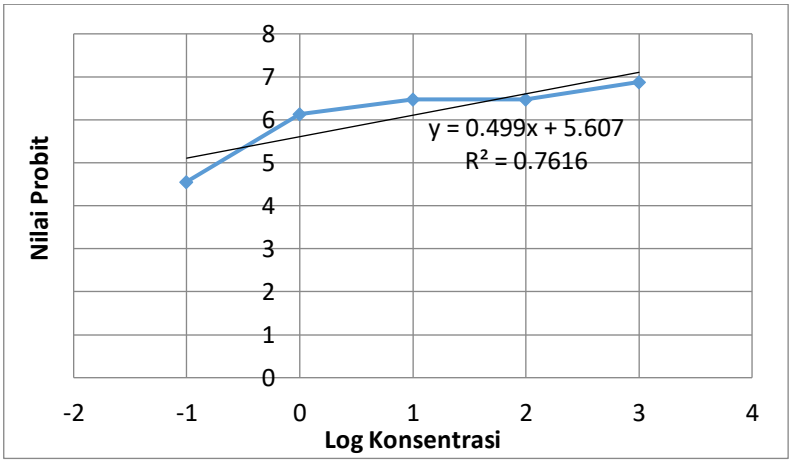
d) 1 ppm

$$\%\ Kematian = 90 - 3{,}33$$
$$= 87$$

e) 0,1 ppm

$$\% \, Kematian = 36{,}67 - 3{,}33$$
$$= 33$$

E. Grafik Hubungan Log Konsentrasi (x) dengan nilai probit % kematian (y)



F. Perhitungan nilai LC_{50}

Perhitungan nilai LC_{50}: Persamaan regresi: y = ax+b;

y = 0,499x + 5,607

LC_{50} nilai probit adalah 5

LC_{50} = 0,499x + 5,61

5 = 0,499x + 5,607

X = $\frac{5 - 5{,}607}{0{,}499}$ = -1,2164

Sehingga, log x = -1,2164

X = antilog (-1,2164) = 0,061

Keterangan : Toxic

Lampiran 7. Perhitungan pada pengujian antivirus

A. Perhitungan Nilai IC_{50}

1) Menghitung rata-rata Lumninescence perlakuan

- 100 µg/mL

$$\frac{(5{,}12\mathrm{E}+04)+(5{,}44\mathrm{E}+04)+(5{,}75\mathrm{E}+04)}{3} = 5{,}44\mathrm{E}+04$$

- 50 µg/mL

$$\frac{(9{,}72\mathrm{E}+04)+(8{,}41\mathrm{E}+04)+(9{,}12\mathrm{E}+04)}{3} = 9{,}08\mathrm{E}+04$$

- 25 µg/mL

$$\frac{(1{,}19\mathrm{E}+05)+(1{,}47\mathrm{E}+05)+(1{,}60\mathrm{E}+05)}{3} = 1{,}42\mathrm{E}+05$$

- 12,5 µg/mL

$$\frac{(3{,}57\mathrm{E}+05)+(2{,}90\mathrm{E}+05)+(3{,}58\mathrm{E}+05)}{3} = 3{,}35\mathrm{E}+05$$

- 6,25 µg/mL

$$\frac{(3{,}36\mathrm{E}+05)+(7{,}99\mathrm{E}+05)+(7{,}47\mathrm{E}+05)}{3} = 3{,}57\mathrm{E}+05$$

- 3,13 µg/mL

$$\frac{(9{,}29\mathrm{E}+05)+(6{,}21\mathrm{E}+05)+(6{,}29\mathrm{E}+05)}{3} = 7{,}26\mathrm{E}+05$$

- 1,76 µg/mL

$$\frac{(1{,}48\mathrm{E}+06)+(1{,}18\mathrm{E}+06)+(2{,}27\mathrm{E}+06)}{3} = 1{,}64\mathrm{E}+06$$

- K. DENV + K. Sel

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

- K. Media

$$\frac{(1{,}52\mathrm{E}+03)+(7{,}00\mathrm{E}+02)+(5{,}27\mathrm{E}+02)}{3} = 9{,}33\mathrm{E}+02$$

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

- 100 µg/m; $(5{,}44\mathrm{E}+04)-(9{,}33\mathrm{E}+02)=5{,}34\mathrm{E}+04$
- 50 µg/mL; $(9{,}08\mathrm{E}+04)-(9{,}33\mathrm{E}+02)=8{,}99\mathrm{E}+04$
- 25 µg/mL; $(1{,}42\mathrm{E}+05)-(9{,}33\mathrm{E}+02)=1{,}41\mathrm{E}+05$
- 12,5 µg/mL; $(3{,}35\mathrm{E}+05)-(9{,}33\mathrm{E}+02)=3{,}34\mathrm{E}+05$
- 6,25 µg/mL; $(3{,}57\mathrm{E}+05)-(9{,}33\mathrm{E}+02)=3{,}56\mathrm{E}+05$
- 3,13 µg/mL; $(7{,}26\mathrm{E}+05)-(9{,}33\mathrm{E}+02)=7{,}25\mathrm{E}+05$
- 1,76 µg/mL; $(1{,}64\mathrm{E}+06)-(9{,}33\mathrm{E}+02)=1{,}64\mathrm{E}+06$

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

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

4) Menghitung % Viabilitas Sel

$$\%\,Viabilitas\,Sel\;=\;\frac{((\mathrm{L.\,Treatmen})-(\mathrm{L.\,K.\,Media}))}{(\mathrm{L.\,(K.\,DENV+K.\,Sel)}-(\mathrm{L.\,K.\,Media}))}\;\mathrm{x}\;100$$

- 100 µg/mL

$$\%\,Viabilitas\,Sel\;=\;\frac{(5{,}34\mathrm{E}+04)}{(9{,}03\mathrm{E}+06)}\;\mathrm{x}\;100$$
$$=\;0{,}591605$$

- 50 µg/mL

$$\%\,Viabilitas\,Sel\;=\;\frac{(8{,}99\mathrm{E}+04)}{(9{,}03\mathrm{E}+06)}\;\mathrm{x}\;100$$
$$=\;0{,}995382$$

- 25 µg/mL

$$\%\,Viabilitas\,Sel\;=\;\frac{(1{,}41\mathrm{E}+05)}{(9{,}03\mathrm{E}+06)}\;\mathrm{x}\;100$$
$$=\;1{,}560773$$

- 12,5 µg/mL

$$\%\,Viabilitas\,Sel\;=\;\frac{(3{,}34\mathrm{E}+05)}{(9{,}03\mathrm{E}+06)}\;\mathrm{x}\;100$$
$$=3{,}700002$$

- 6,25 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(3{,}56\mathrm{E}+05)}{(9{,}03\mathrm{E}+06)}\ \mathrm{x}\ 100$$

$$= 3{,}944749$$

- 3,13 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(7{,}25\mathrm{E}+05)}{(9{,}03\mathrm{E}+06)}\ \mathrm{x}\ 100$$

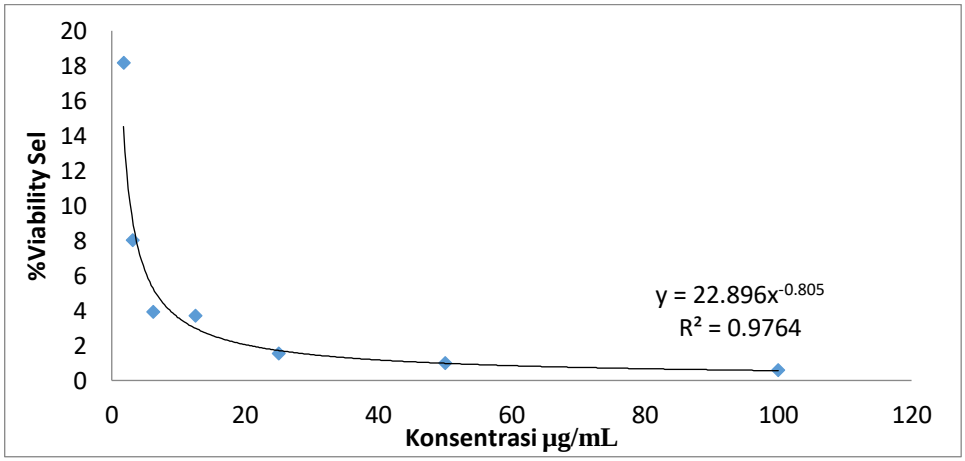
$$= 8{,}029772$$

- 1,76 µg/mL

$$\%\ Viabilitas\ Sel = \frac{(1{,}64\mathrm{E}+06)}{(9{,}03\mathrm{E}+06)}\ \mathrm{x}\ 100$$

$$= 18{,}18511$$

5) Grafik Hubungan konsentrasi larutan uji (x) dengan % Viabilitas sel (y)



Dari persamaan pada grafik maka dapat dihitung nilai IC_{50} Ekstrak Aseton Rosemary Terhadap DENV-2

y = $22{,}896x^{-0{,}805}$

50 = $22{,}896x^{-0{,}805}$

$\frac{50}{22{,}896} = x^{-0{,}805}$

$2{,}1838 = x^{-0{,}805}$

$2{,}1838 = \frac{1}{x^{0{,}805}}$

$x^{0{,}805} = \frac{1}{2{,}1838}$

$x^{0{,}805} = 0{,}45792$

*misalkan x = < 0,5

$x^{0,805}$ = 0,45792

$(0,37899)^{0,805}$ = 0,45792

Jadi nilai IC_{50} = 0,37899 μg/mL (< 0,5)

B. Perhitungan Nilai CC_{50}

1) Menghitung rata-rata absorbansi perlakuan

- 200 μg/mL

$$\frac{2,214 + 2,246 + 1,358}{3} = 0,120666667$$

- 100 μg/mL

$$\frac{1,177 + 1,186 + 1,138}{3} = 1,167$$

- 50 μg/mL

$$\frac{0,624 + 0,599 + 0,582}{3} = 0,601666667$$

- 25 μg/mL

$$\frac{0,337 + 0,356 + 0,367}{3} = 0,353333333$$

- 12,5 μg/mL

$$\frac{0,248 + 0,253 + 0,221}{3} = 0,240666667$$

- 6,25 μg/mL

$$\frac{0,301 + 0,469 + 0,25}{3} = 0,34$$

- 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)

- 200 μg/m; $(1,939333333) - (0,120666667) = 1,818666667$
- 100 μg/mL; $(1,167) - (0,120666667) = 1,046333333$
- 50 μg/mL; $(0,601666667) - (0,120666667) = 0,481$
- 25 μg/mL; $(0,353333333) - (0,120666667) = 0,232666667$

- 12,5 µg/mL; $(0{,}240666667) - (0{,}120666667) = 0{,}12$
- 6,25 µg/mL; $(0{,}34) - (0{,}120666667) = 0{,}129333333$

3) Menghitung L. (Abs. Sel) – L. (Abs. Media)

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

4) Menghitung % Viabilitas Sel

$$\%\ Toksisitas = \frac{((\text{Abs. Perlakuan}) - (\text{Abs. Media}))}{((\text{Abs. Sel}) - (\text{Abs. Media}))}\ \text{x}\ 100$$

- 200 µg/mL

$$\%\ Viabilitas\ Sel = \frac{1{,}818666667}{1{,}425}\ \text{x}\ 100$$
$$= 127{,}625731$$

- 100 µg/mL

$$\%\ Viabilitas\ Sel = \frac{1{,}046333333}{1{,}425}\ \text{x}\ 100$$
$$= 73{,}42690058$$

- 50 µg/mL

$$\%\ Viabilitas\ Sel = \frac{0{,}481}{1{,}425}\ \text{x}\ 100$$
$$= 33{,}75438596$$

- 25 µg/mL

$$\%\ Viabilitas\ Sel = \frac{0{,}232666667}{1{,}425}\ \text{x}\ 100$$
$$= 16{,}32748538$$

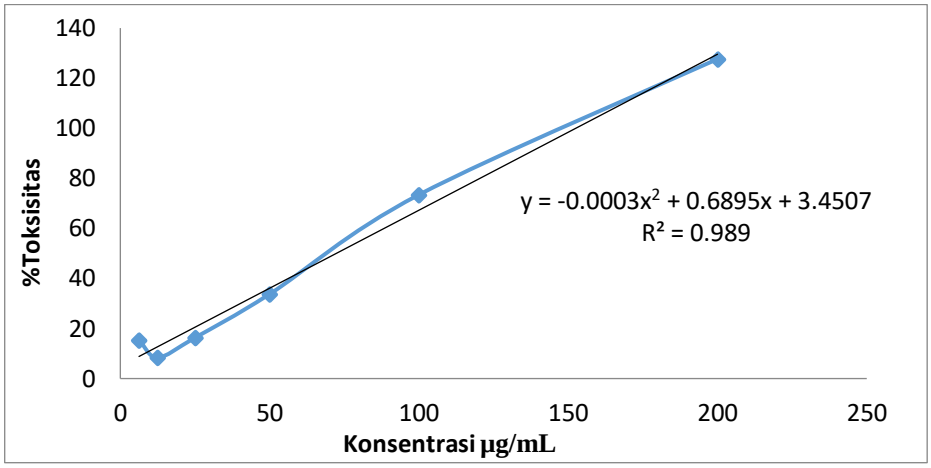
- 12,5 µg/mL

$$\%\ Viabilitas\ Sel = \frac{0{,}12}{1{,}425}\ \text{x}\ 100$$
$$= 8{,}421052632$$

- 6,25 µg/mL

$$\%\ Viabilitas\ Sel = \frac{0{,}219333333}{1{,}425}\ \text{x}\ 100$$
$$= 15{,}39181287$$

5) Grafik Hubungan konsentrasi larutan uji (x) dengan % Toksisitas (y)



Dari persamaan pada grafik maka dapat dihitung nilai CC_{50} ekstrak aseton rosemary terhadap sel vero:

y = -0,0003 x^2 + 0,6895 x + 3,4507

50 = -0,0003 x^2 + 0,6895 x + 3,4507

0 = -0,0003 x^2 + 0,6895 x + 3,4507 - 50

0 = -0,0003 x^2 + 0,6895 x – 46,5493

$$x_{1,2} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$= \frac{-0{,}6895 \pm \sqrt{(0{,}6895)^2 - 4(-0{,}0003)(-46{,}5493)}}{2(-0{,}0003)}$$

$$= \frac{-0{,}6895 \pm \sqrt{0{,}47541025 - 0{,}05585916}}{-0{,}0006}$$

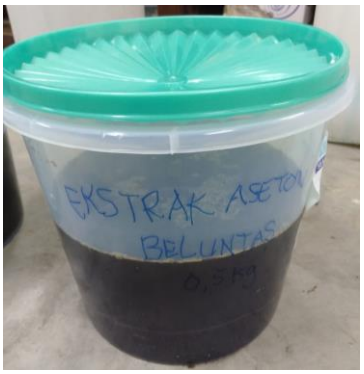
$$= \frac{-0{,}6895 \pm \sqrt{0{,}41955109}}{-0{,}0006}$$

$$= \frac{-0{,}6895 \pm 0{,}6477}{-0{,}0006}$$

$$x_1 = \frac{-0{,}6895 + 0{,}6477}{-0{,}0006} = \frac{-0{,}0418}{-0{,}0006} = 69{,}65$$

Jadi nilai CC_{50} = 69,65 µg/mL

Lampiran 8. Dokumentasi Penelitan



Proses Maserasi Sampel

Proses Evaporasi Ekstrak

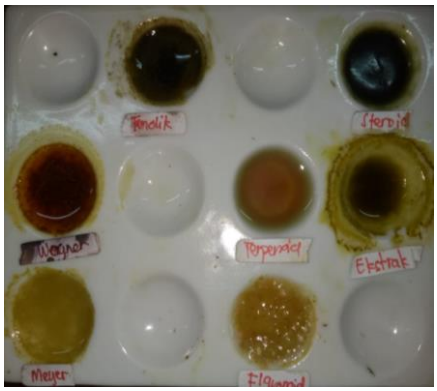
Ekstrak Aseton

Proses KKV

Fraksi-fraksi hasil KKV

Fraksi 9

Proses Kromatotron



Uji Fitokimia Ekstrak

Senyawa Isolat

Uji Fitokimia Senyawa 1

Uji Fitokimia Senyawa 2,3 & 4

Perubahan Warna Pada Uji Fitokimia