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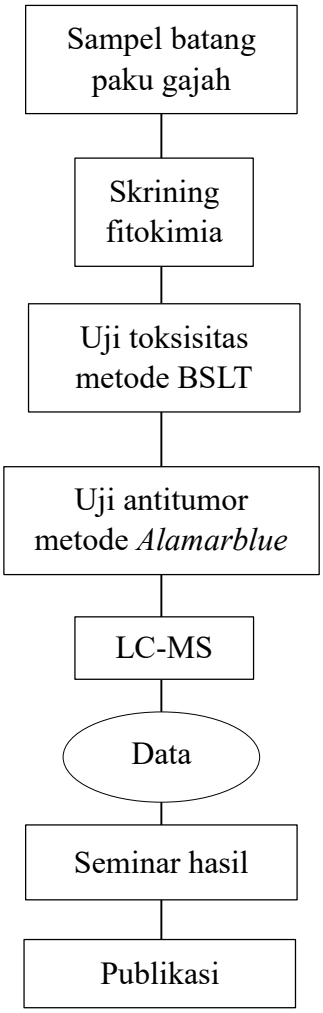
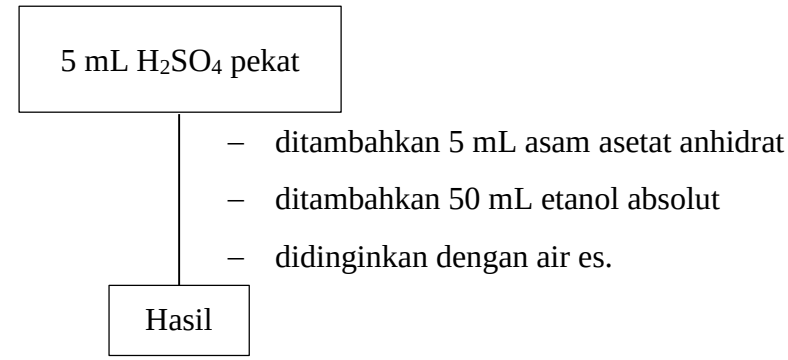
Lampiran 1. Diagam Alir Penelitian



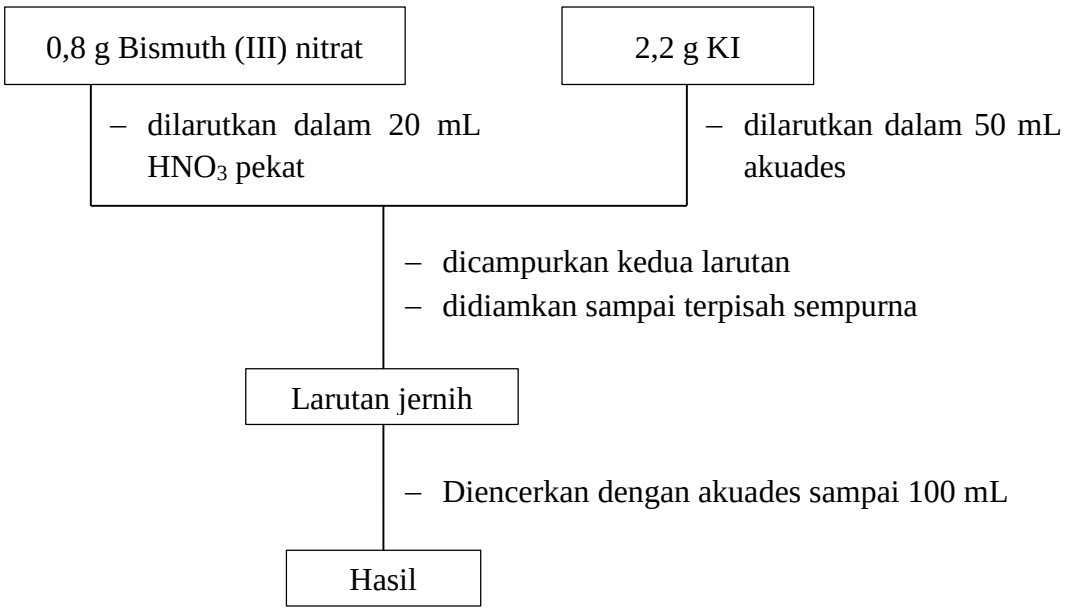
Lampiran 2. Bagan Prosedur Kerja

1. Pembuatan Reagen (Shaikh dan Patil, 2020)

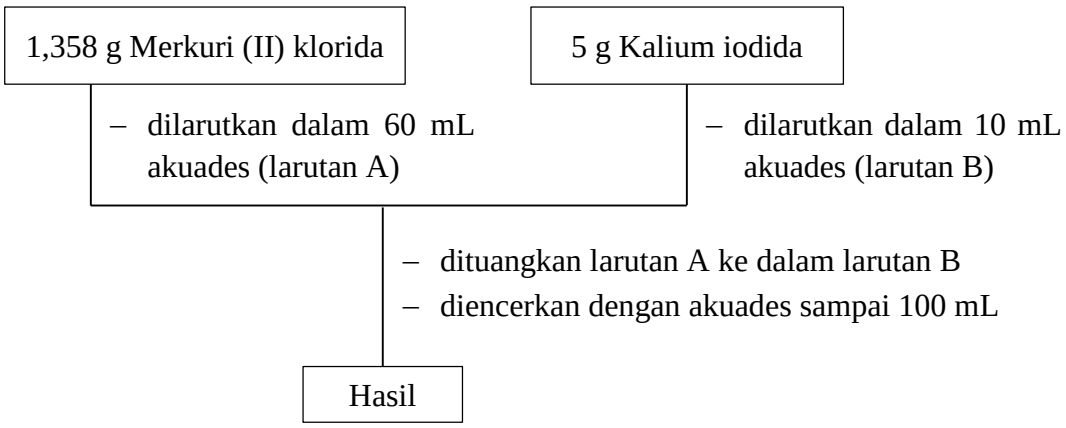
a. Pembuatan Reagen Libermann-Burchard



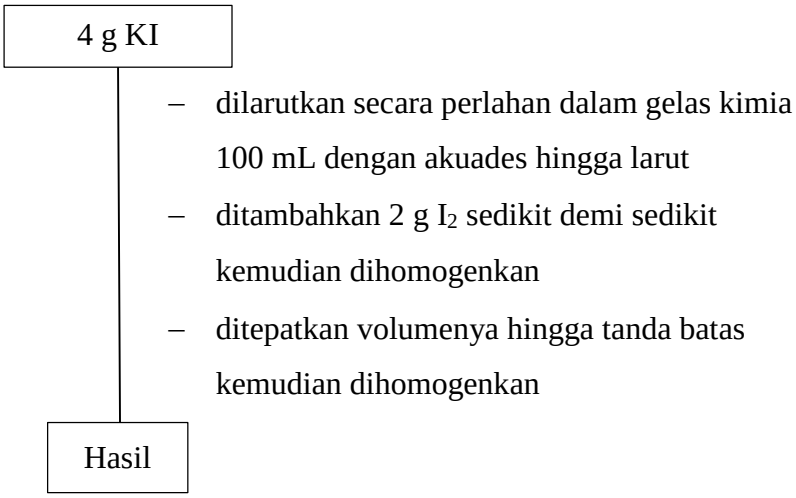
b. Pembuatan Reagen Dragendroff



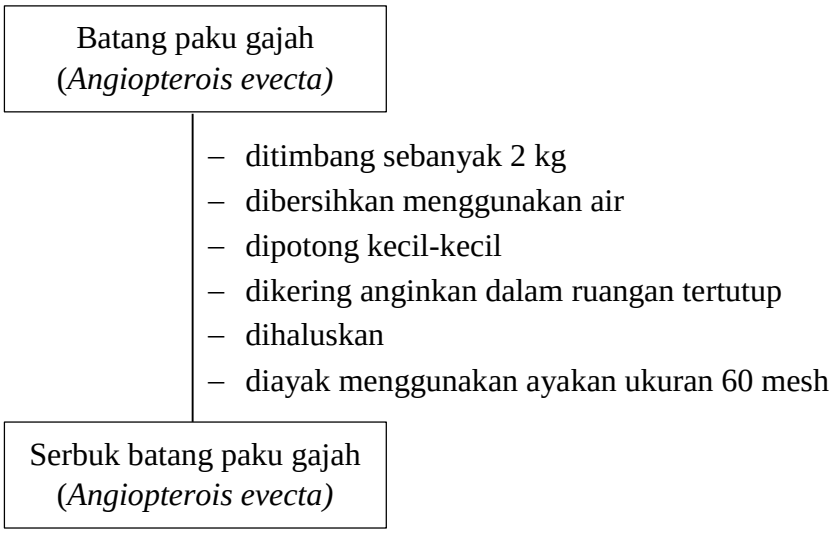
c. Pembuatan Reagen Mayer



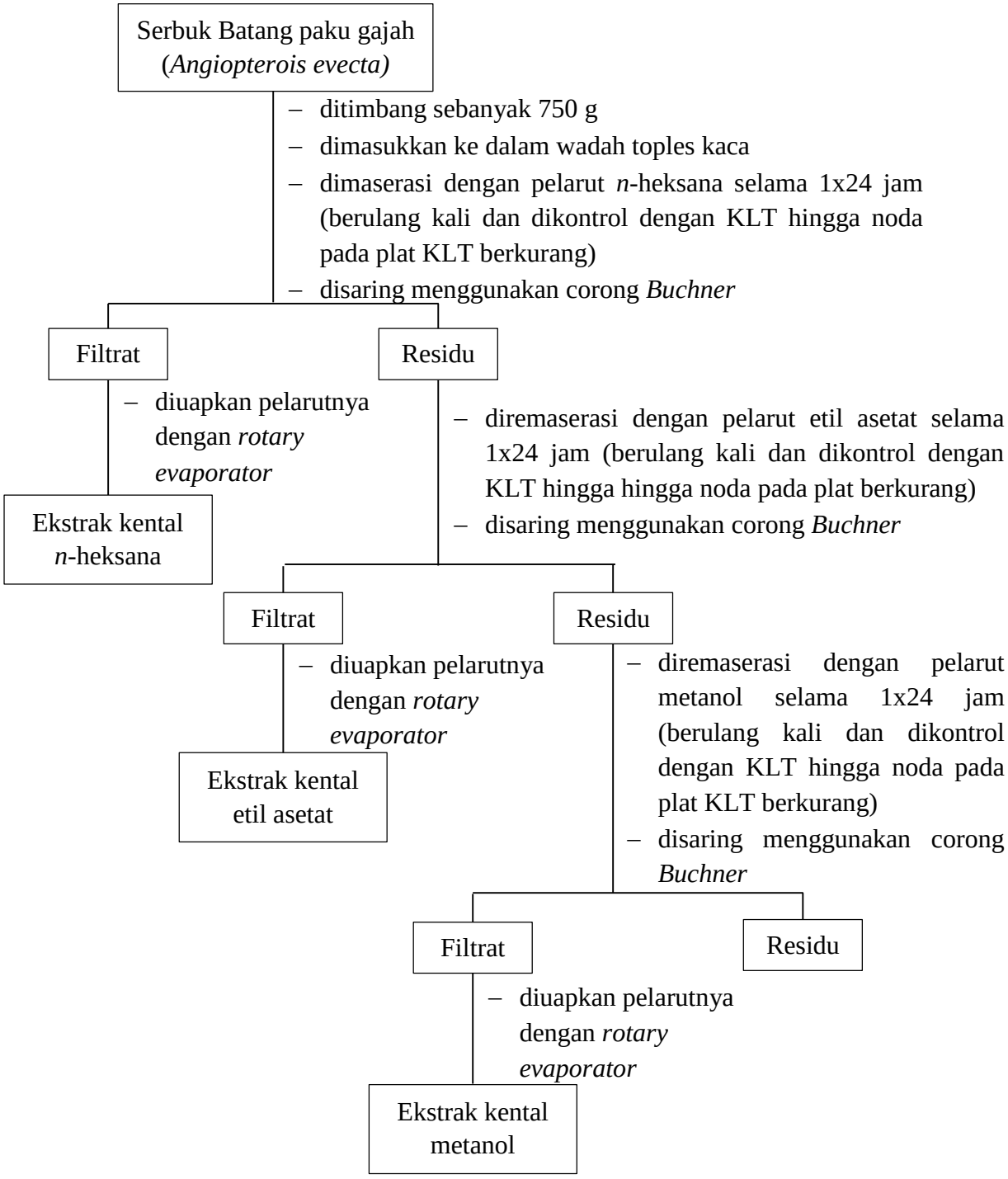
d. Pembuatan Reagen Wagner



2. Preparasi Sampel

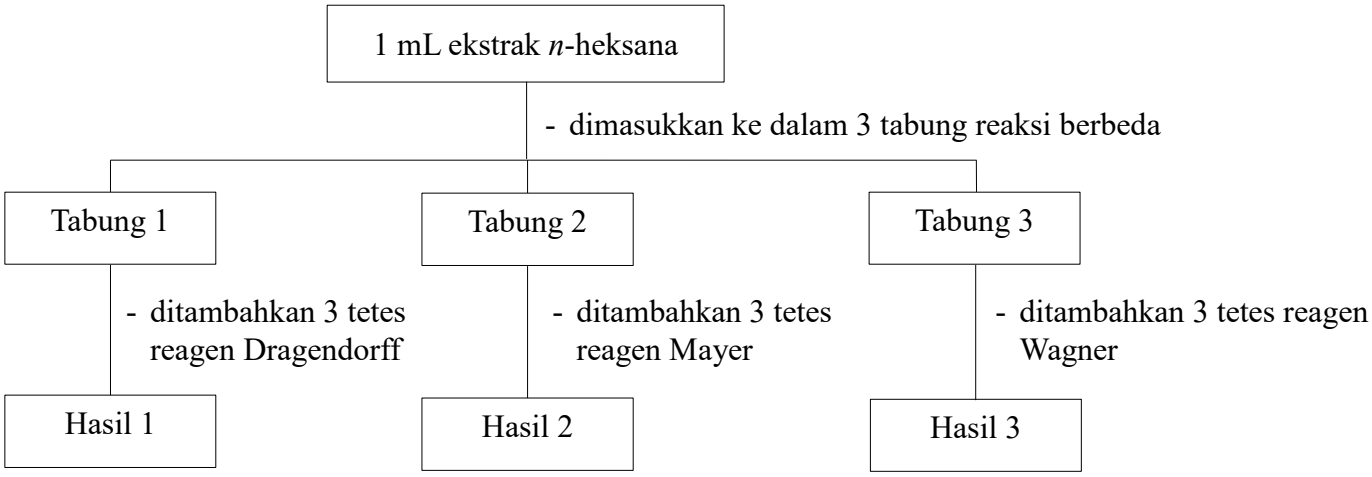


3. Ekstraksi Sampel

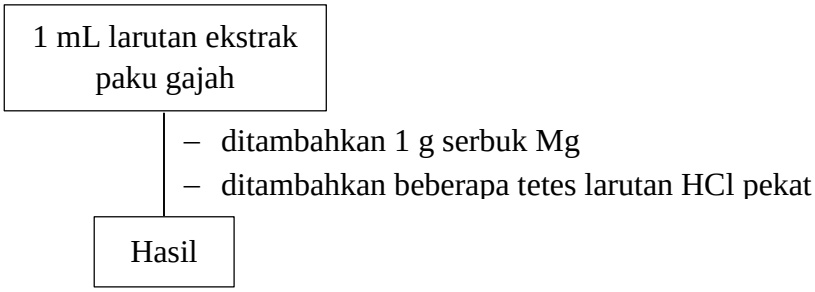


4. Skrining Fitokimia (Shaikh dan Patil, 2020)

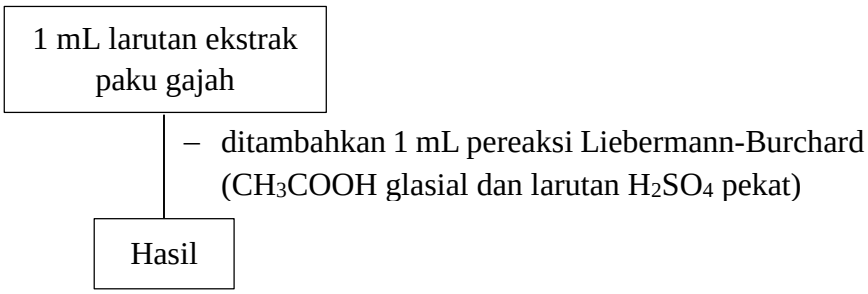
a. Uji Alkaloid



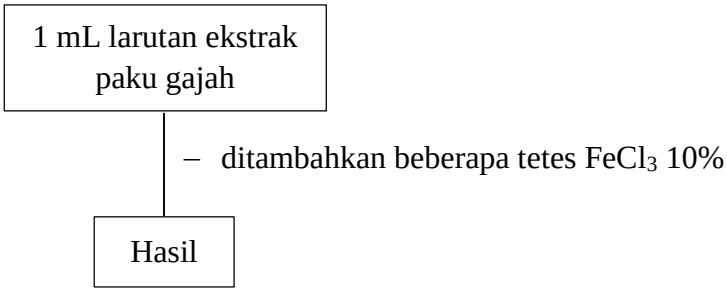
b. Uji Flavonoid



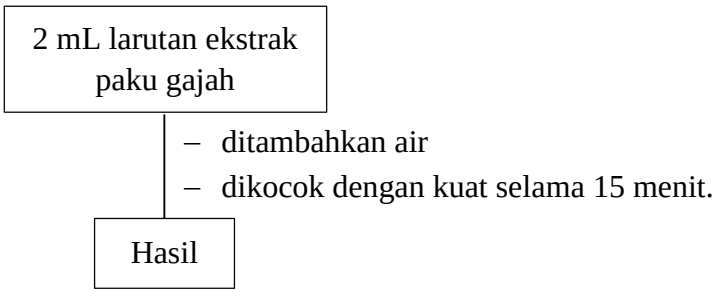
c. Uji Steroid dan Terpenoid



d. Uji Tanin



e. Uji Saponin



5. Uji Toksisitas metode *Brine Shrimp Lethality Test* (BSLT)

a. Penyiapan larva udang

2 g telur *Artemia salina* Leach

– ditempatkan pada wadah penetasan yang berisi 1 L air laut
– diaerasi selama ± 48 jam

Larva udang

b. Uji Toksisitas

Ekstrak paku gajah

– ditimbang 1 mg dalam tabung ependorf
– dilarutkan dalam 100 µL DMSO
– diencerkan dengan 150 µL akuades

Larutan uji konsentrasi 1000 ppm

– dipipet 100 µL ke dalam *microplate* baris A dan B
– ditambahkan 100 µL akuades pada *microplate* baris B hingga G
– dipipet 100 µL dari baris B lalu dimasukkan ke dalam baris C dan dari C dipipet 100 µL dimasukkan ke baris D, dst.

Larutan uji konsentrasi 1000 ppm; 500 ppm; 250 ppm; 125 ppm; 62,5 ppm; 31,25 ppm; dan 15,6 ppm

– ditambahkan 100 µL media larva udang (berisi 10 ekor)

Larutan uji konsentrasi 500 ppm; 250 ppm; 125 ppm; 62,5 ppm; 31,25 ppm; 15,6 ppm; dan 7,8 ppm

– didiamkan selama 24 jam
– diamati kematian larva udang setelah 24 jam

Data

– dihitung nilai LC_{50} dengan analisis probit

Nilai LC_{50}

Catatan:
- dilakukan pengulangan sebanyak 3 kali (triplo) untuk setiap sampel
- dibuat kontrol negatif dengan menggunakan DMSO dan dilakukan pengerjaan yang sama tanpa menggunakan sampel

7. Uji Antitumor terhadap sel tumor payudara MCF-7 dengan Metode *Alamarblue*

a. Preparasi Sel

Sel yang telah konfluen 70%

- dibuang media pada dish
- dibilas sebanyak 2 kali dengan 1 mL PBS
- ditambahkan 1 mL tripsin-EDTA
- diinkubasi selama 5 menit
- dipindahkan sel kedalam tabung konikal berisi media
- disentrifugasi dengan kecepatan 3000 rpm selama 5 menit
- dibuang supernatan
- dilarutkan kedalam tabung konikal berisi media

Hasil

b. *Seeding* Sel ke dalam 96 *Well Plate*

Trypan blue

- disiapkan 10 µL *trypan blue* dalam tabung ependorf steril
- ditambahkan 10 µL suspensi sel dan dihomogenkan
- dipipet 10 µL ke dalam salah satu sisi bilik/chamber hemacytometer
- dihitung jumlah sel yang sehat dan ditentukan jumlah sel (viabel) per mL
- dikultur ke dalam 96 *well plate*
- diinkubasi selama 24 jam pada pada suhu 37°C dan 5% gas CO_2

Hasil

b. Perlakuan Sel dengan Sampel, Kontrol Positif, dan Kontrol Negatif

96 *well plate* yang telah berisi sel hasil inkubasi

- diberi label pada sepanjang margin kiri *plate* untuk baris mana yang akan diberi perlakuan dengan sampel dan kontrol
- dibuang media dari setiap *well*
- ditambahkan 100 µL sampel dengan 8 konsentrasi berbeda dan kontrol positif cisplatin dari tabung ependorf ke dalam masing-masing *well* yang sesuai dengan menggunakan mikropipet
- di inkubasi kembali selama 48 jam

Hasil

c. Pemberian Reagen Presto Blue dan Pengukuran Absorbansi

96 *well plate* yang berisi sel yang telah diberi perlakuan

- dibuang media pada setiap *well*
- dimasukkan 100 µL larutan campuran (9 mL media dan 1 mL reagen *presto blue cell viability*) ke dalam tiap *well microplate*
- diinkubasi selama 1-2 jam sampai terlihat perubahan warna
- diukur absorbansinya pada panjang gelombang 570 nm menggunakan *multimode reader*

Hasil

Lampiran 3. Perhitungan

1. Persen Rendemen Ekstrak

$$\text{Persen Rendemen} = \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$$

a. Ekstrak *n*-Heksana

Berat sampel serbuk awal = 750 g

Berat ekstrak yang dihasilkan = 1,875 g

Persen rendemen ekstrak *n*-heksana $= \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$

$= \frac{1,875}{750} \times 100$

= 0,250%

b. Ekstrak Etil Asetat

Berat sampel serbuk awal = 748,125 g

Berat ekstrak yang dihasilkan = 4,7 g

Persen rendemen ekstrak etil asetat $= \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$

$= \frac{4,7}{748,125} \times 100$

= 0,628%

c. Ekstrak Metanol

Berat sampel serbuk awal = 743,425 g

Berat ekstrak yang dihasilkan = 5,675 g

Persen rendemen ekstrak metanol $= \frac{\text{berat ekstrak}}{\text{berat sampel}} \times 100\%$

$= \frac{5,675}{743,425} \times 100$

= 0,763%

2. Perhitungan Konsentrasi untuk Uji Toksisitas

$$\text{Konsentrasi sampel} = \frac{\frac{200\ \mu L}{250\ \mu L} \text{ x } 1\ mg}{800\ \mu L}$$
$$= \frac{0{,}8\ mg}{800\ \mu L}$$
$$= 1\ \mu g/\mu L$$
$$= 1000\ ppm$$

Konsentrasi sampel setelah pengenceran pada *microplate*

$$\text{Baris A} = \frac{100\ \mu g}{100\ \mu L} = 1\ \mu g/\mu L = 1000\ ppm$$

$$B = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{100\ \mu g}{100\ \mu L} = \frac{50\ \mu g}{100\ \mu L} = 0{,}5\ \mu g/\mu L = 500\ ppm$$

$$C = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{50\ \mu g}{100\ \mu L} = \frac{25\ \mu g}{100\ \mu L} = 0{,}25\ \mu g/\mu L = 250\ ppm$$

$$D = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{25\ \mu g}{100\ \mu L} = \frac{12{,}5\ \mu g}{100\ \mu L} = 0{,}125\ \mu g/\mu L = 125\ ppm$$

$$E = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{12{,}5\ \mu g}{100\ \mu L} = \frac{6{,}25\ \mu g}{100\ \mu L} = 0{,}0625\ \mu g/\mu L = 62{,}5\ ppm$$

$$F = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{6{,}25\ \mu g}{100\ \mu L} = \frac{3{,}125\ \mu g}{100\ \mu L} = 0{,}03125\ \mu g/\mu L = 31{,}25\ ppm$$

$$G = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{3{,}125\ \mu g}{100\ \mu L} = \frac{1{,}5625\ \mu g}{100\ \mu L} = 0{,}015625\ \mu g/\mu L = 15{,}6\ ppm$$

Konsentrasi sampel setelah penambahan media udang 100 µL, maka masing-masing lubang berisi 200 µL (pengenceran 2x)

$$\text{Baris A} = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{100\ \mu g}{100\ \mu L} = 0{,}5\ \mu g/\mu L = 500\ ppm$$

$$B = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{50\ \mu g}{100\ \mu L} = \frac{25\ \mu g}{100\ \mu L} = 0{,}25\ mg/\mu L = 250\ ppm$$

$$C = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{25\ \mu g}{100\ \mu L} = \frac{12{,}5\ \mu g}{100\ \mu L} = 0{,}125\ mg/\mu L = 125\ ppm$$

$$D = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{12{,}5\ \mu g}{100\ \mu L} = \frac{6{,}25\ \mu g}{100\ \mu L} = 0{,}0625\ mg/\mu L = 62{,}5\ ppm$$

$$E = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{6{,}25\ \mu g}{100\ \mu L} = \frac{3{,}125\ \mu g}{100\ \mu L} = 0{,}03125\ mg/\mu L = 31{,}25\ ppm$$

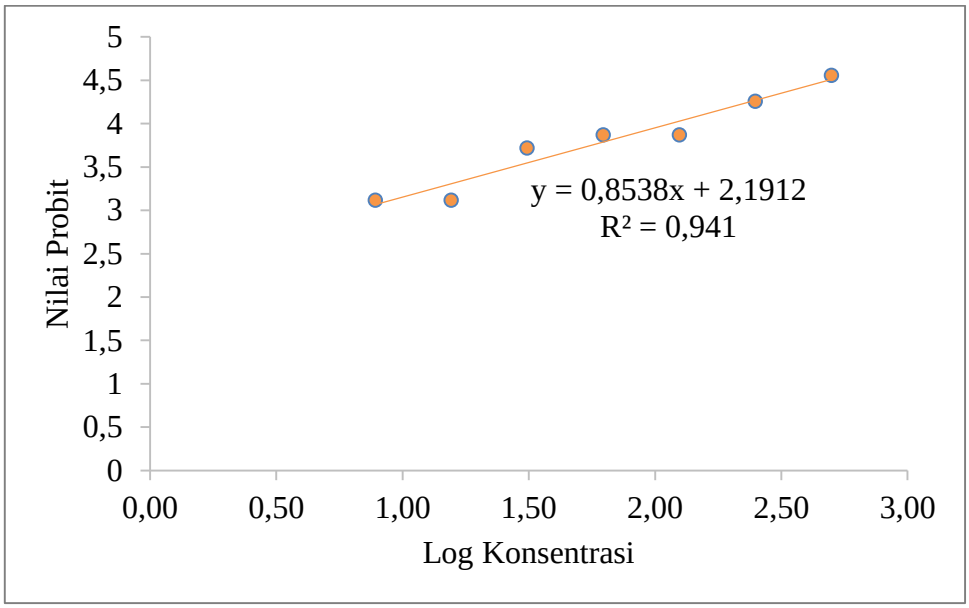
$$F = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{3{,}125\ \mu g}{100\ \mu L} = \frac{1{,}5625\ \mu g}{100\ \mu L} = 0{,}015625\ mg/\mu L = 15{,}6\ ppm$$

$$G = \frac{100\ \mu L}{200\ \mu L} \text{ x } \frac{1{,}5625\ \mu g}{100\ \mu L} = \frac{0{,}78125\ \mu g}{100\ \mu L} = 0{,}0078125\ mg/\mu L = 7{,}8\ ppm$$

3. Perhitungan LC_{50}

a. Ekstrak *n*-Heksana

Konsentrasi Uji (µg/mL)	Log Konsentrasi	Jumlah Larva Udang Uji (Ekor)	Jumlah Larva Udang yang Mati				Kontrol Negatif				Persen Kematian (%)	Nilai Probit
			1	2	3	Rata-rata	1	2	3	Rata-rata		
500	2,70	10	5	4	4	4,33	1	1	1	1	33,33	4,56
250	2,40	10	2	2	3	2,33	0	0	0	0	23,33	4,26
125	2,10	10	1	2	1	1,33	0	0	0	0	13,33	3,87
62,5	1,80	10	0	2	2	1,33	0	0	0	0	13,33	3,87
31,2	1,49	10	1	1	1	1,00	0	0	0	0	10,00	3,72
15,6	1,19	10	1	0	0	0,33	0	0	0	0	3,33	3,12
7,8	0.89	10	0	1	0	0,33	0	0	0	0	3,33	3,12



y = 0,8538x + 2,1912

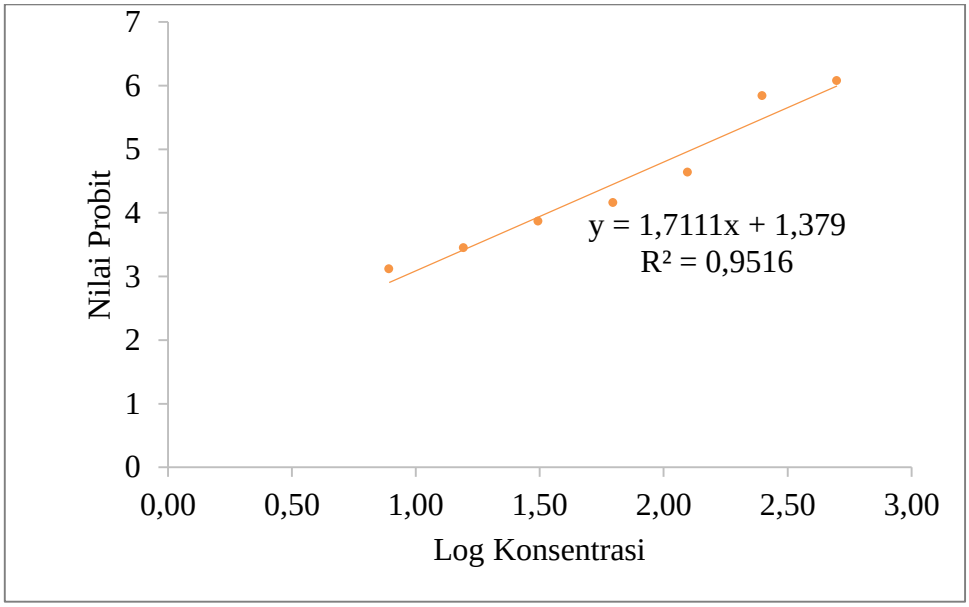
5 = 0,8538x + 2,1912

x = 3,2897634

LC_{50} $=10^{x}=10^{(3.2897634)}$ = 1948,78 µg/mL

b. Ekstrak Etil Asetat

Konsentrasi Uji (µg/mL)	Log Konsentrasi	Jumlah Larva Udang Uji (Ekor)	Jumlah Larva Udang yang Mati				Kontrol Negatif				Persen Kematian (%)	Nilai Probit
			1	2	3	Rata-rata	1	2	3	Rata-rata		
500	2,70	10	10	10	9	9,67	1	1	1	1	86,67	6,08
250	2,40	10	8	7	9	8,00	0	0	0	0	80,00	5,84
125	2,10	10	3	4	4	3,67	0	0	0	0	36,67	4,64
62,5	1,80	10	2	1	3	2,00	0	0	0	0	20,00	4,16
31,2	1,49	10	1	2	1	1,33	0	0	0	0	13,33	3,87
15,6	1,19	10	0	1	1	0,67	0	0	0	0	6,67	3,45
7,8	0,89	10	0	0	1	0,33	0	0	0	0	3,33	3,12



y = 1,7111x + 1,379

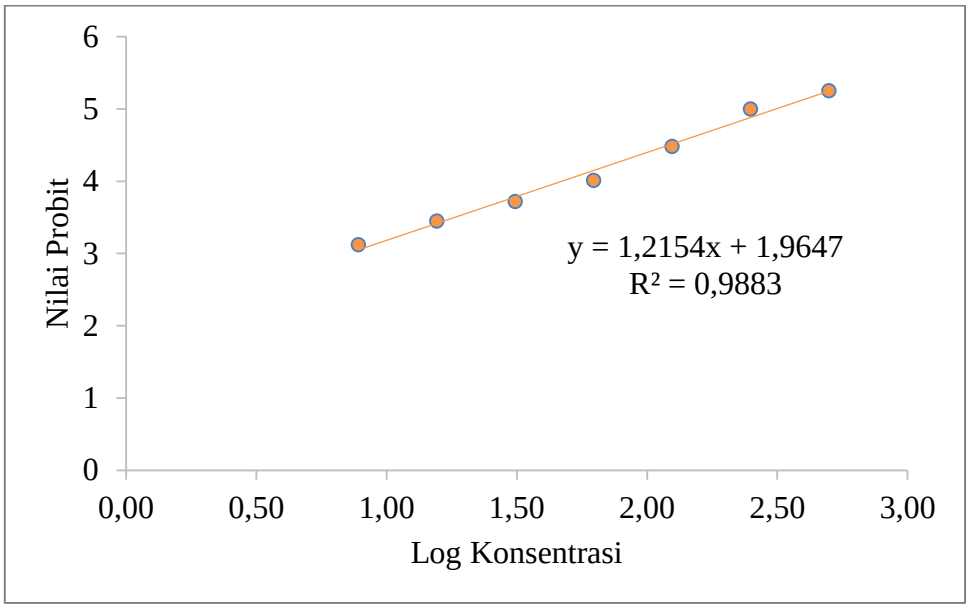
5 = 1,7111x + 1,379

x = 2,1161826

LC_{50} $=10^{x}=10^{(2,1161826)}$ = 130,67 µg/mL

c. Ekstrak Metanol

Konsentrasi Uji (µg/mL)	Log Konsentrasi	Jumlah Larva Udang Uji (Ekor)	Jumlah Larva Udang yang Mati				Kontrol Negatif				Persen Kematian (%)	Nilai Probit
			1	2	3	Rata-rata	1	2	3	Rata-rata		
500	2,70	10	8	7	6	7,00	1	1	1	1	60,00	5,25
250	2,40	10	5	5	5	5,00	0	0	0	0	50,00	5
125	2,10	10	4	2	3	3,00	0	0	0	0	30,00	4,48
62,5	1,80	10	2	1	2	1,67	0	0	0	0	16,67	4,01
31,2	1,49	10	1	1	1	1,00	0	0	0	0	10,00	3,72
15,6	1,19	10	1	1	0	0,67	0	0	0	0	6,67	3,45
7,8	0,89	10	0	0	1	0,33	0	0	0	0	3,33	3,12



y = 1,2154x + 1,9647

5 = 1,2154x + 1,9647

x = 2,49736712

LC_{50} $=10^{x}=10^{(2,49736712)}$ = 314,31 µg/mL

Lampiran 4. Dokumentasi

Pemotongan Sampel

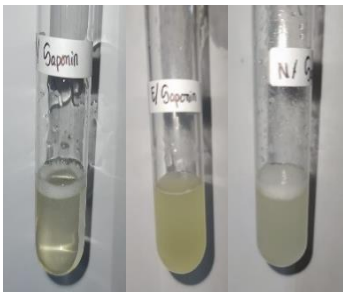
Pengeringan Sampel

Penghalusan Sampel

Maserasi Sampel

Penyaringan Hasil Maserasi

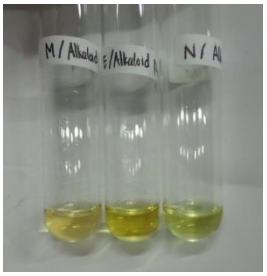
Pemekatan Maserat menjadi Ekstrak



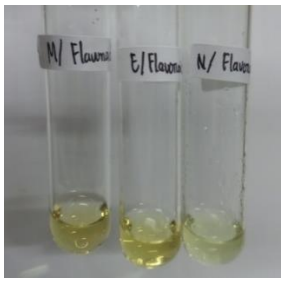
Uji Saponin

Uji Alkaloid Dragendorff

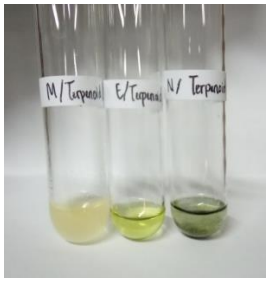
Uji Alkaloid Wagner



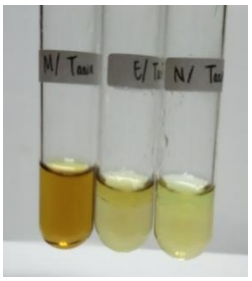
Uji Alkaloid Mayer



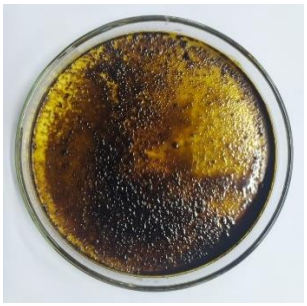
Uji Flavonoid



Uji Terpenoid/steroid



Uji Tanin

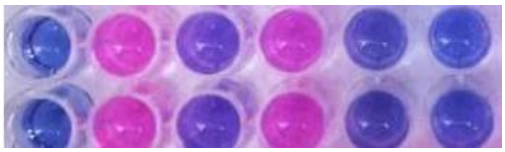


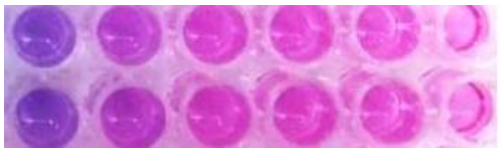
Ekstrak Etil Asetat

Ekstrak *n*-Heksana

Ekstrak Metanol

Uji Toksisitas Metode BSLT





Uji Aktivitas Antitumor Metode *Alamarblue*