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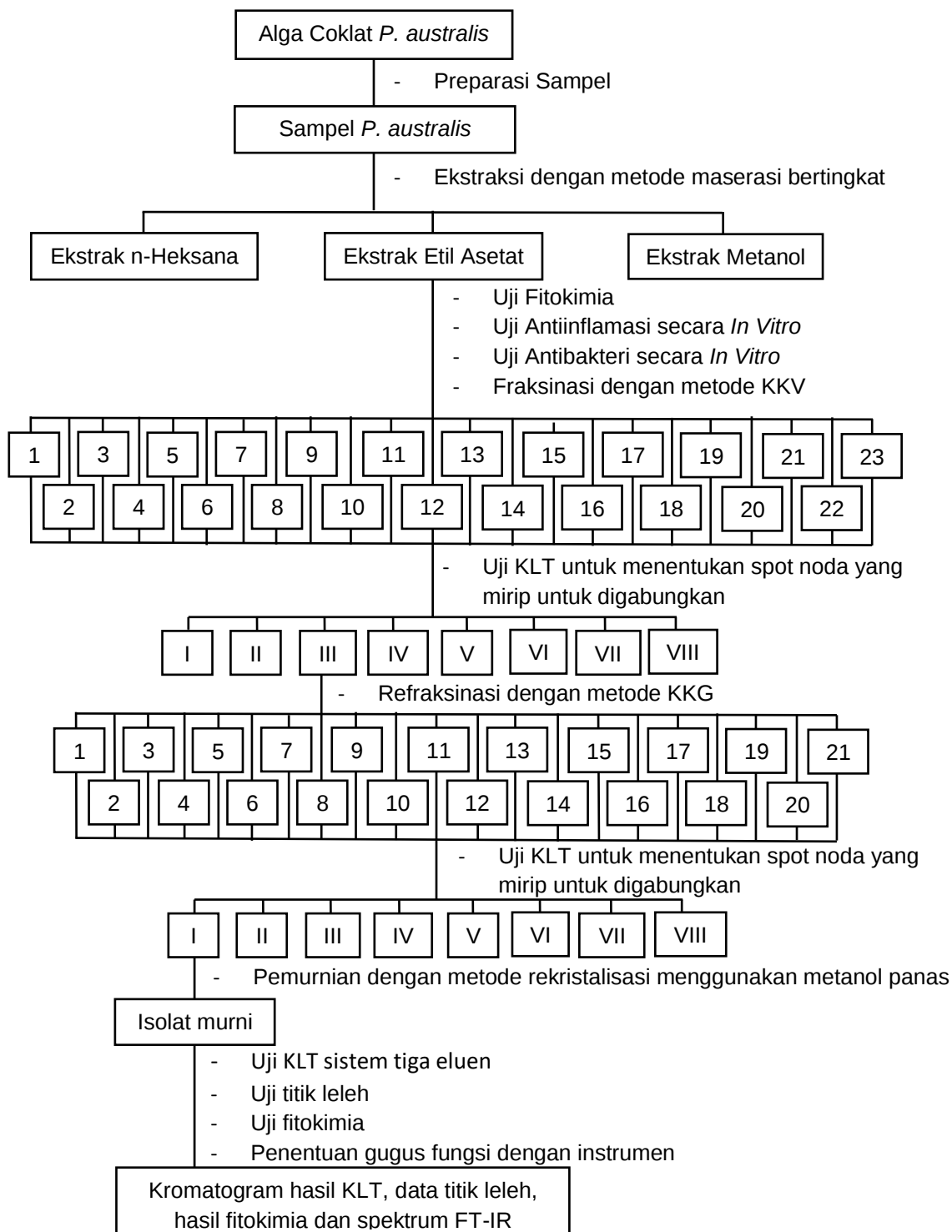
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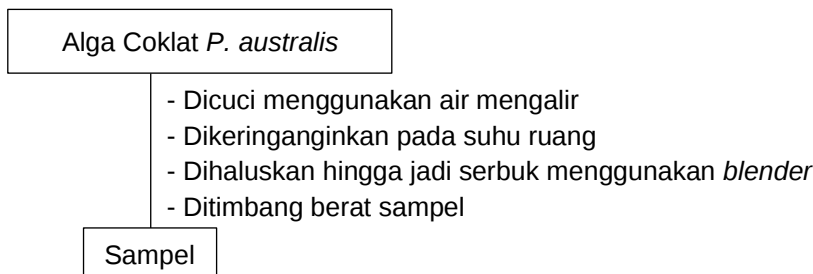
LAMPIRAN

Lampiran 1. Diagram Alir Penelitian

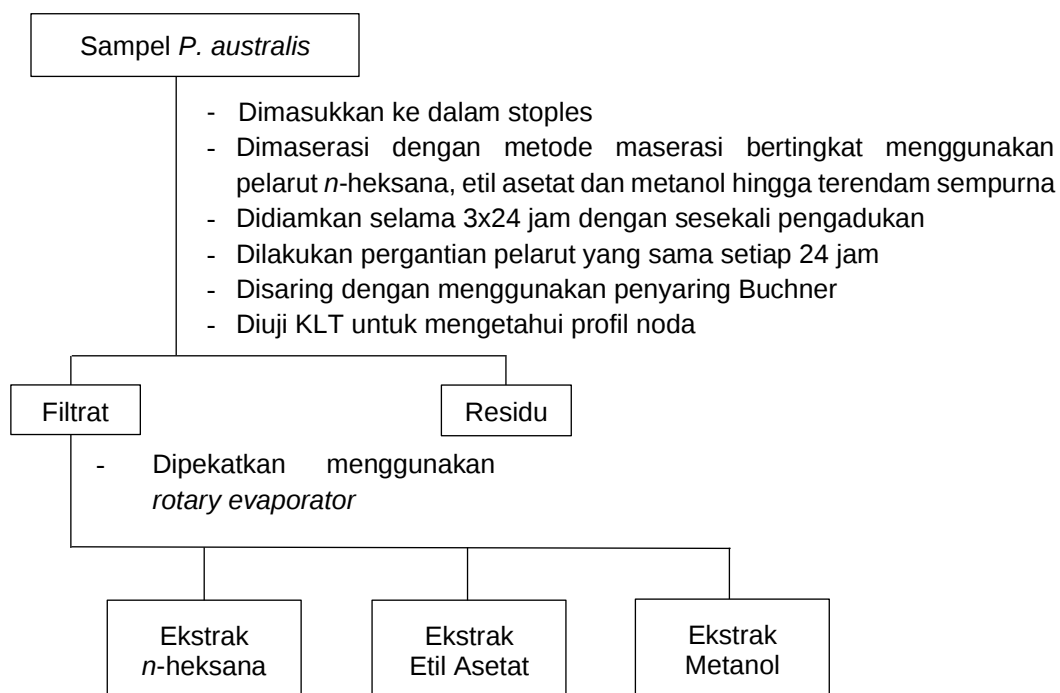


Lampiran 2. Prosedur Penelitian

1. Preparasi Sampel



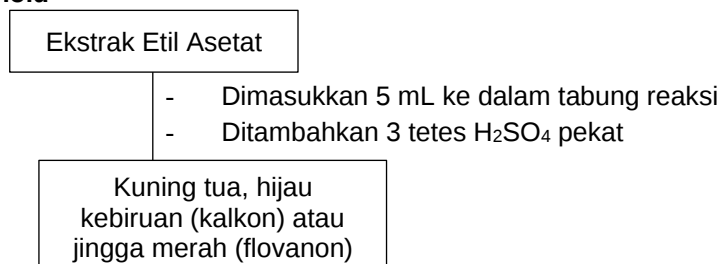
2. Ekstraksi Sampel

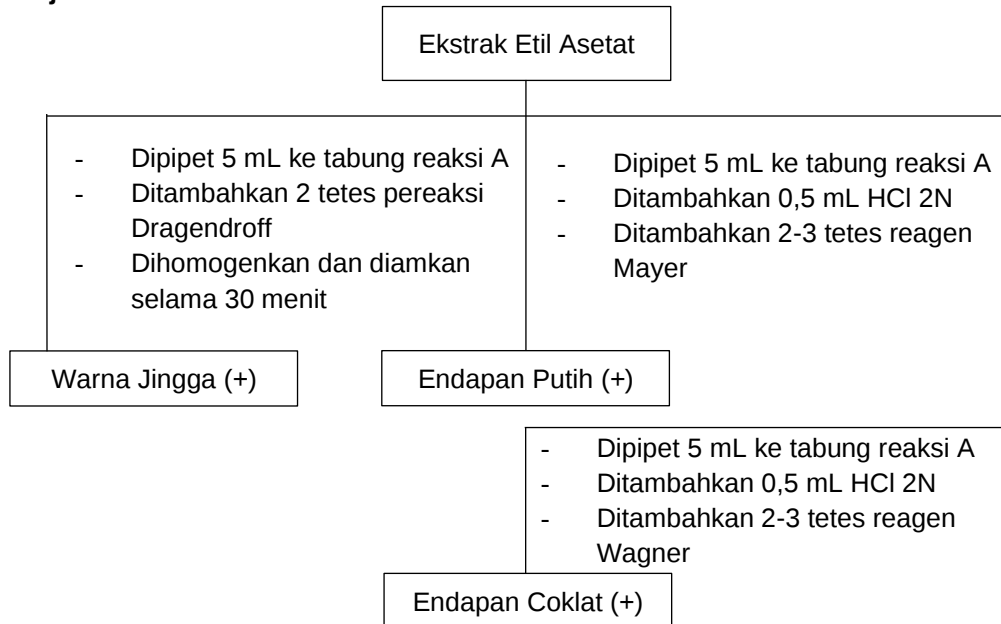
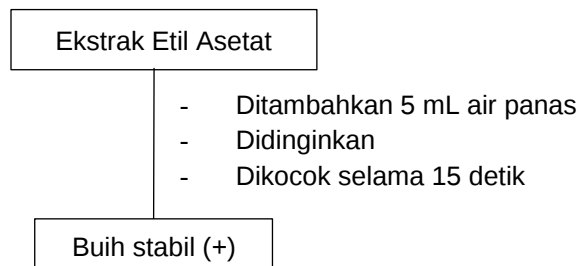
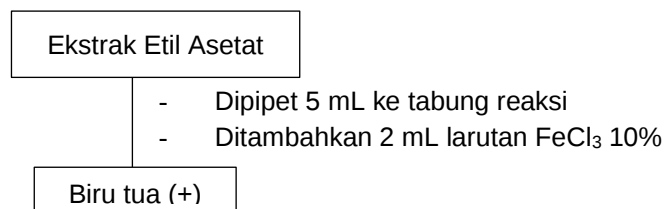
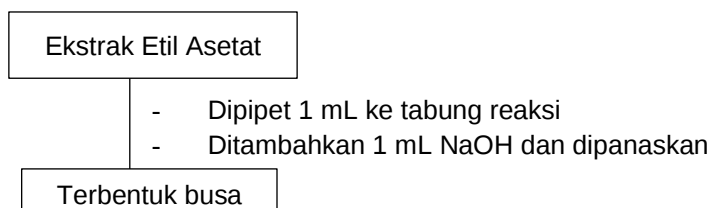


Catatan: proses maserasi dihentikan apabila noda KLT memudar

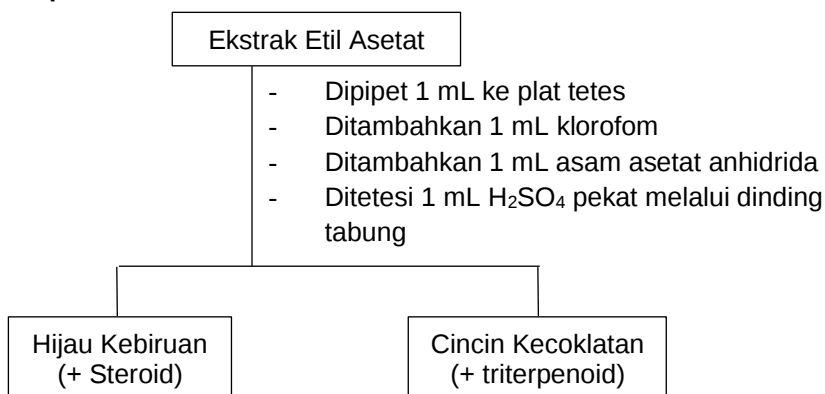
3. Uji Kandungan Ekstrak

a. Uji Flavonoid

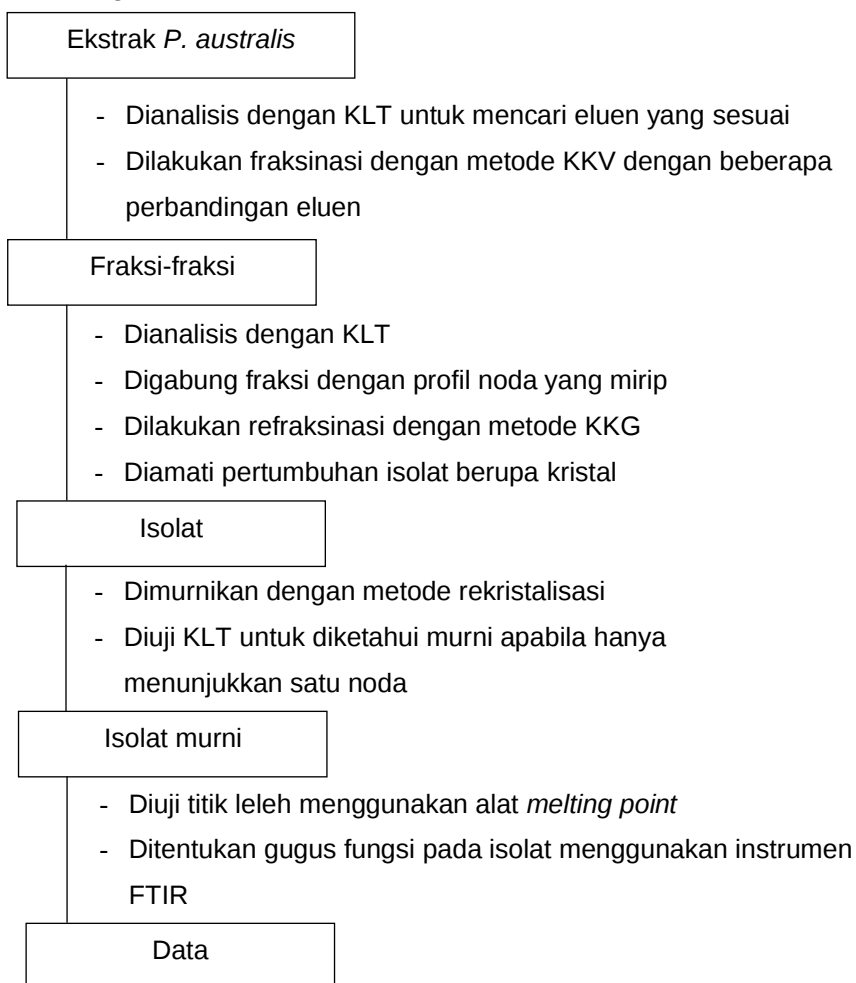


b. Uji Alkaloid**c. Uji Saponin****d. Uji Tanin****e. Uji Saponifikasi Asam Lemak**

f. Uji Steroid/Triterpenoid

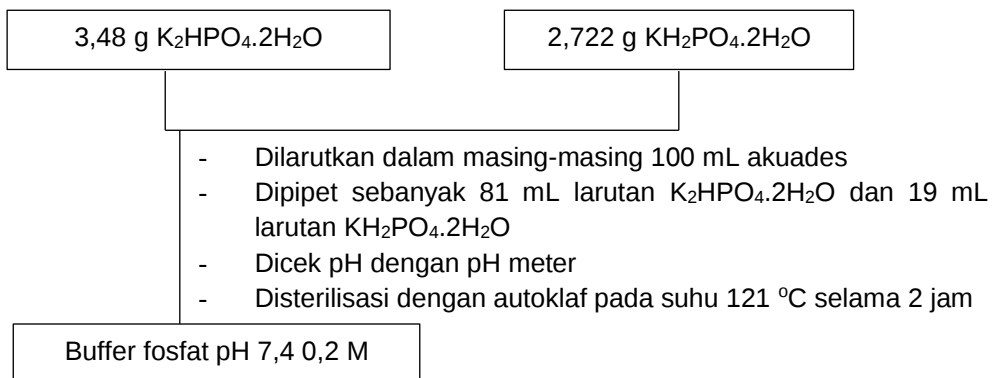


4. Fraksinasi dengan Metode KKV dan Pemurnian Isolat

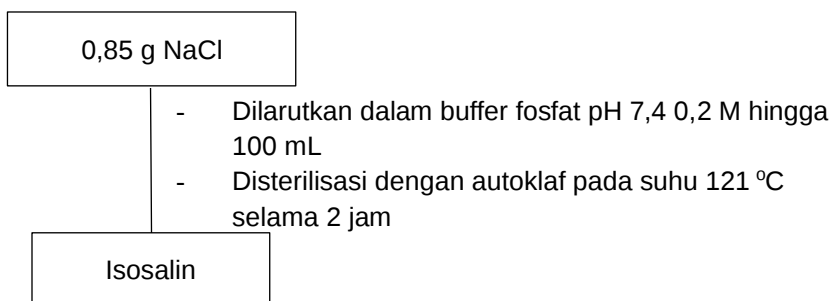


5. Uji Antiinflamasi Ekstrak Etil Asetat Alga Coklat *Padina australis* secara *In Vitro* dengan Metode HRBC

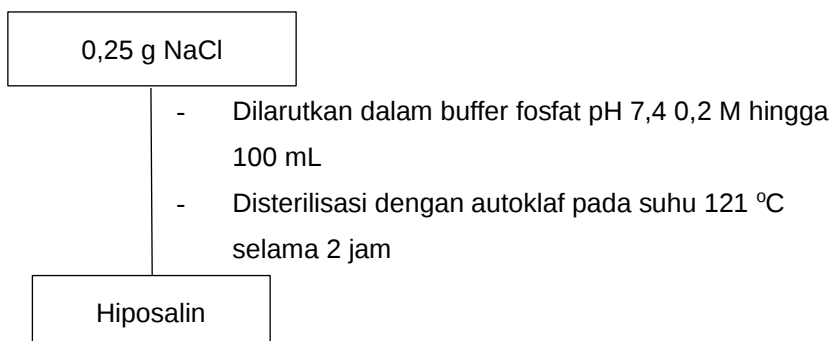
a. Pembuatan Buffer Fosfat pH 7,4 0,2 M

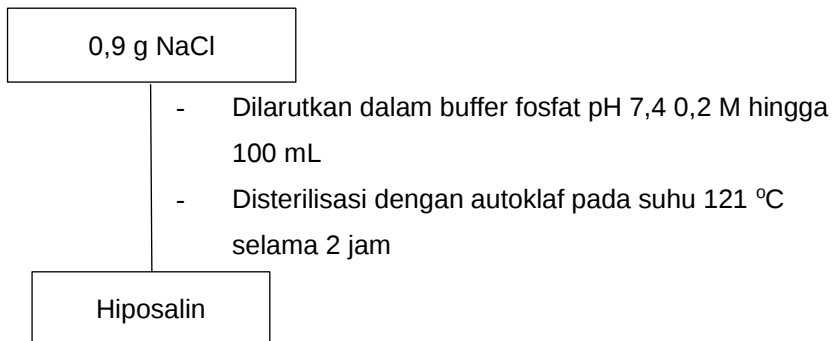
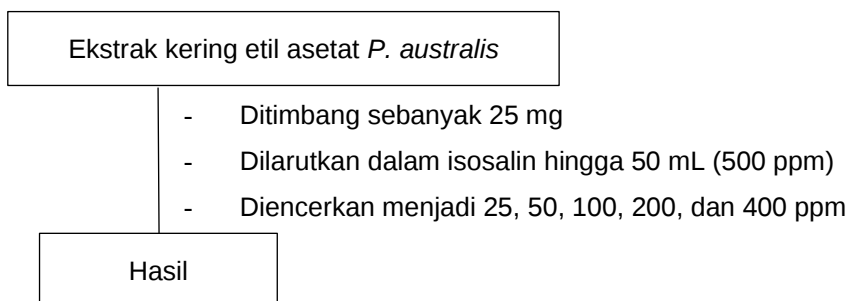
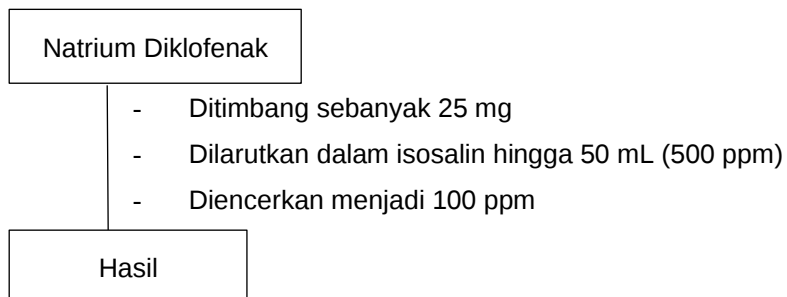


b. Pembuatan Larutan Isosalin

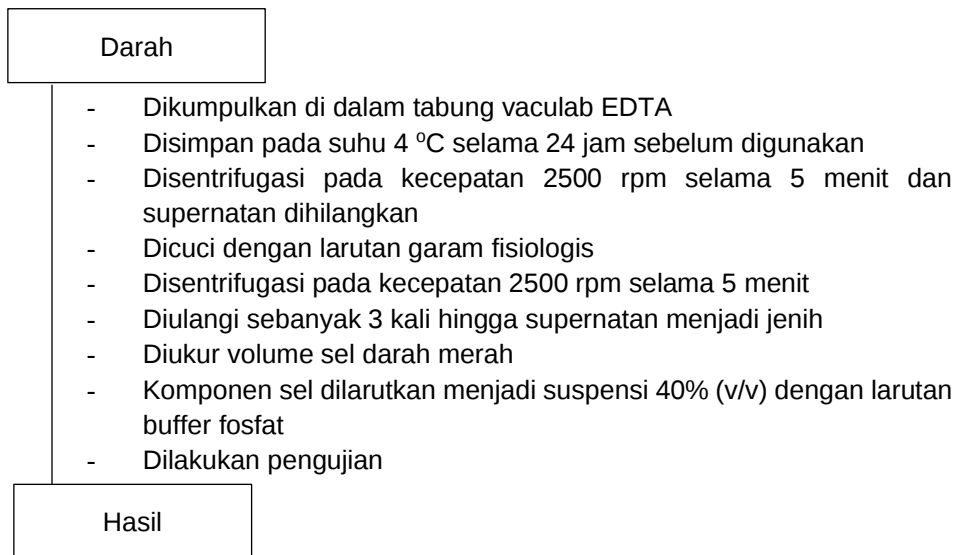


c. Pembuatan Larutan Hiposalin



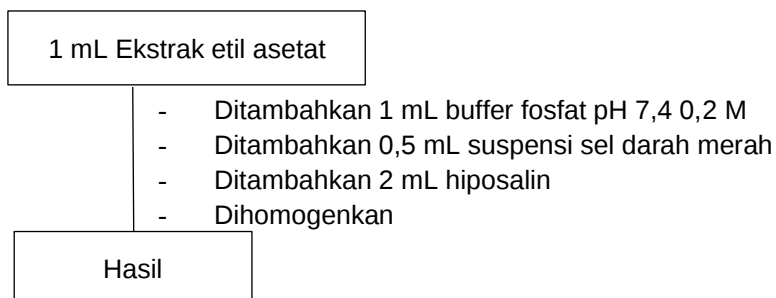
d. Pembuatan Larutan Garam Fisiologis**e. Pembuatan Konsentrasi Ekstrak****f. Pembuatan Konsentrasi Natrium Diklofenak**

g. Preparasi Suspensi Sel Darah Merah

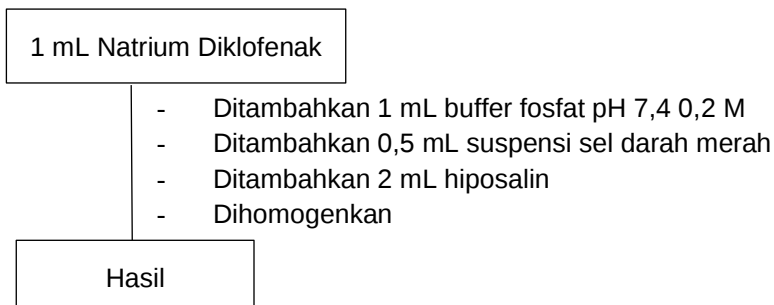


h. Persiapan Larutan untuk Pengujian Aktivitas Antiinflamasi

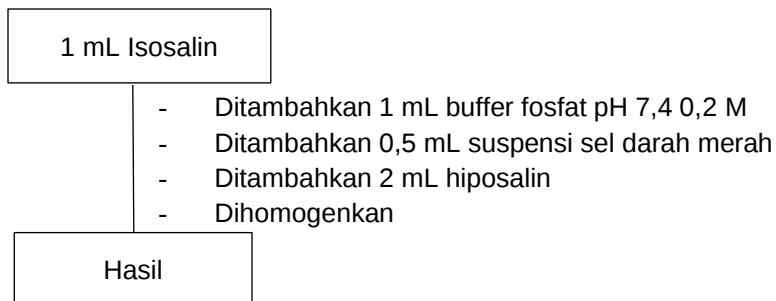
1. Pembuatan Larutan Uji



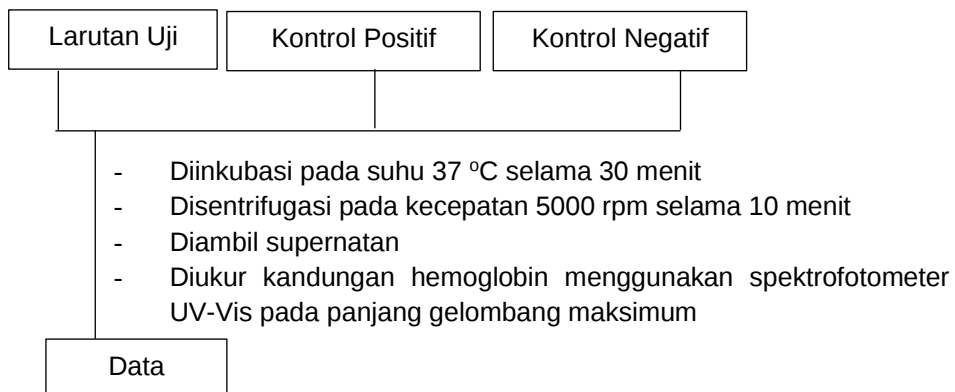
2. Pembuatan Larutan Kontrol Positif



3. Pembuatan Larutan Kontrol negatif

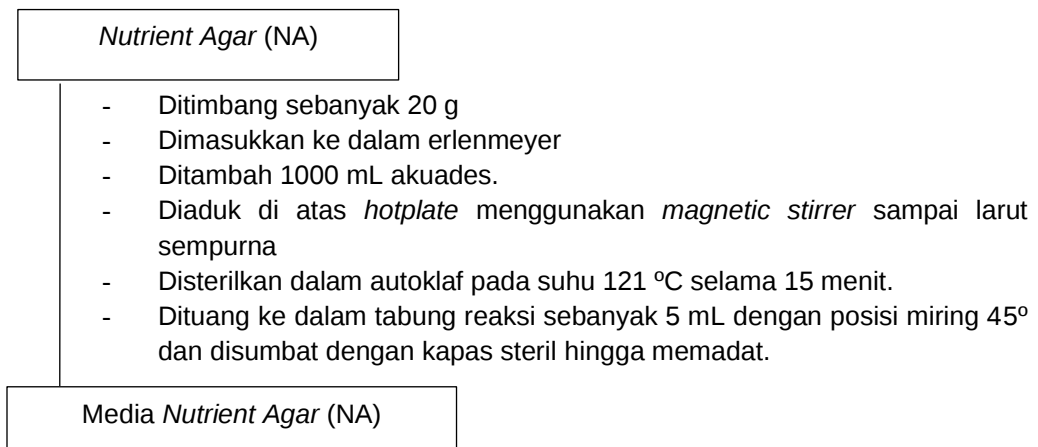


i. Uji Aktivitas Ekstrak Etil Asetat terhadap Stabilitas Membran Eritrosit

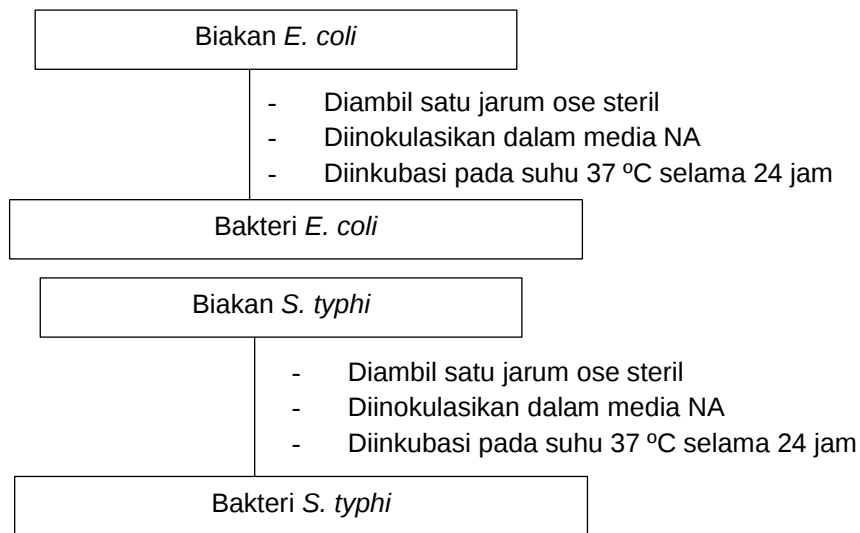


5. Uji Antibakteri Ekstrak Etil Asetat Alga Coklat *Padina australis* secara *In Vitro* dengan Metode Difusi Cakram

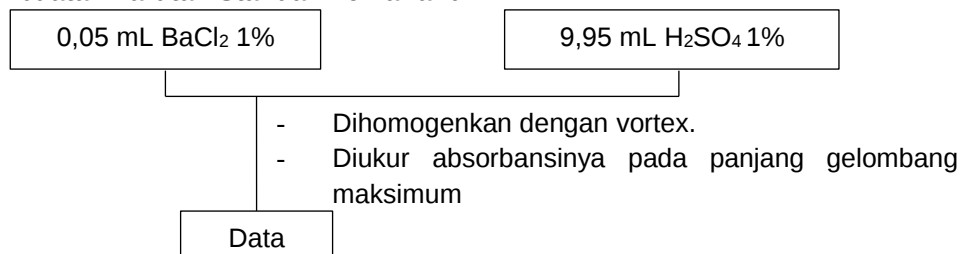
a. Pembuatan Media *Nutrient Agar* (NA)



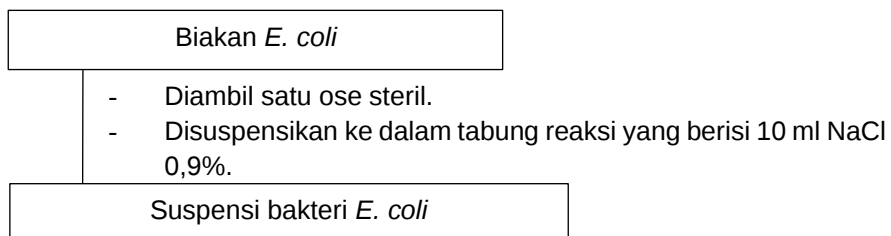
b. Peremajaan Bakteri



c. Pembuatan Larutan Standar Mc Farland

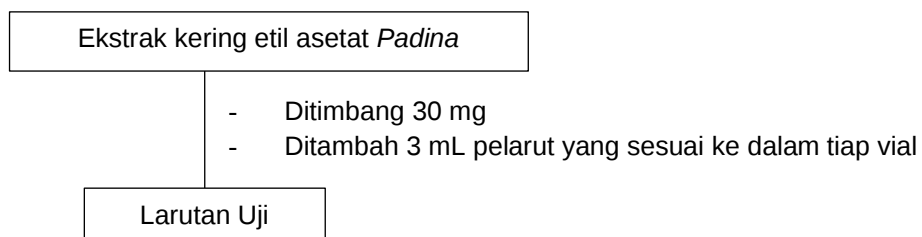


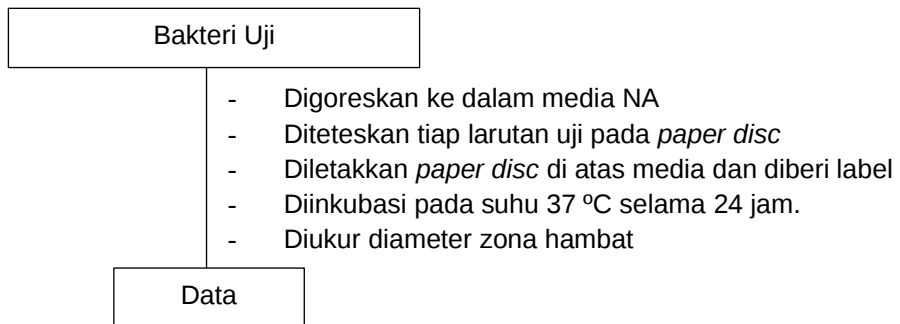
d. Pembuatan Suspensi Bakteri



Keterangan : prosedur serupa dilakukan untuk bakteri *S. typhi*

e. Pembuatan Larutan Uji



f. Pengukuran Diameter Zona Hambat

Lampiran 3. Perhitungan Data Penelitian

1. Perhitungan Redemen Ekstrak Alga Colat *Padina australis*

$$\% \text{ Rendemen} = \frac{\text{berat ekstrak (g)}}{\text{berat sampel (g)}} \times 100\%$$

a. Ekstrak n-Heksana

$$\begin{aligned} \% \text{ Rendemen} &= \frac{8,41 \text{ g}}{3200 \text{ g}} \times 100\% \\ &= 0,2628\% \end{aligned}$$

b. Ekstrak Etil Asetat

$$\begin{aligned} \% \text{ Rendemen} &= \frac{28,6 \text{ g}}{3200 \text{ g}} \times 100\% \\ &= 0,8937\% \end{aligned}$$

c. Ekstrak Metanol

$$\begin{aligned} \% \text{ Rendemen} &= \frac{13,7 \text{ g}}{3200 \text{ g}} \times 100\% \\ &= 0,4281\% \end{aligned}$$

2. Pembuatan Larutan Uji Aktivitas Antiinflamasi

a. Larutan Induk 500 ppm dalam 50 mL

$$\begin{aligned} \text{Larutan Induk} &= \frac{\text{berat ekstrak (mg)}}{\text{volume pelarut (L)}} \\ &= \frac{25 \text{ mg}}{0,05 \text{ L}} \\ &= 500 \text{ ppm} \end{aligned}$$

b. Larutan Uji 400 ppm

$$\begin{aligned} V_1 \cdot C_1 &= V_2 \cdot C_2 \\ V_1 \cdot 500 \text{ ppm} &= 25 \text{ mL} \cdot 400 \text{ ppm} \\ V_1 &= 20 \text{ mL} \end{aligned}$$

c. Larutan Uji 200 ppm

$$\begin{aligned} V_1 \cdot C_1 &= V_2 \cdot C_2 \\ V_1 \cdot 500 \text{ ppm} &= 25 \text{ mL} \cdot 200 \text{ ppm} \\ V_1 &= 10 \text{ mL} \end{aligned}$$

d. Larutan Uji 100 ppm

$$\begin{aligned} V_1 \cdot C_1 &= V_2 \cdot C_2 \\ V_1 \cdot 500 \text{ ppm} &= 25 \text{ mL} \cdot 100 \text{ ppm} \\ V_1 &= 5 \text{ mL} \end{aligned}$$

e. Larutan Uji 50 ppm
 $V_1 \cdot C_1 = V_2 \cdot C_2$
 $V_1 \cdot 500 \text{ ppm} = 25 \text{ mL} \cdot 50 \text{ ppm}$
 $V_1 = 2,5 \text{ mL}$

f. Larutan Uji 25 ppm
 $V_1 \cdot C_1 = V_2 \cdot C_2$
 $V_1 \cdot 500 \text{ ppm} = 25 \text{ mL} \cdot 25 \text{ ppm}$
 $V_1 = 1,25 \text{ mL}$

3. Pembuatan Larutan Uji Aktivitas Antibakteri

- a. Larutan Induk 10000 ppm dalam 3 mL

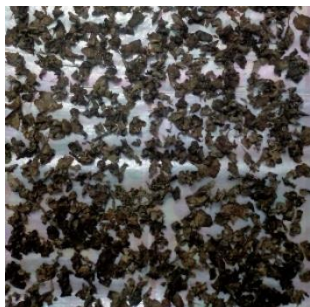
$$\begin{aligned} \text{Larutan Induk} &= \frac{\text{berat ekstrak (mg)}}{\text{volume pelarut (L)}} \\ &= \frac{30 \text{ mg}}{0,003 \text{ L}} \\ &= 10000 \text{ ppm} \end{aligned}$$

b. Larutan Uji 100 ppm
 $V_1 \cdot C_1 = V_2 \cdot C_2$
 $V_1 \cdot 10000 \text{ ppm} = 3 \text{ mL} \cdot 100 \text{ ppm}$
 $V_1 = 0,03 \text{ mL}$

c. Larutan Uji 500 ppm
 $V_1 \cdot C_1 = V_2 \cdot C_2$
 $V_1 \cdot 10000 \text{ ppm} = 3 \text{ mL} \cdot 500 \text{ ppm}$
 $V_1 = 0,15 \text{ mL}$

d. Larutan Uji 1000 ppm
 $V_1 \cdot C_1 = V_2 \cdot C_2$
 $V_1 \cdot 10000 \text{ ppm} = 3 \text{ mL} \cdot 1000 \text{ ppm}$
 $V_1 = 0,3 \text{ mL}$

Lampiran 4. Dokumentasi Penelitian



Pengeringan sampel *Padina australis*



Proses maserasi sampel



Pengentalan ekstrak dengan rotary evaporator



Ekstrak kental



Uji fitokimia ekstrak etil asetat *Padina australis*



Fraksinasi metode KKV



Fraksinasi metode KKG



Hasil fraksinasi fraksi EA III



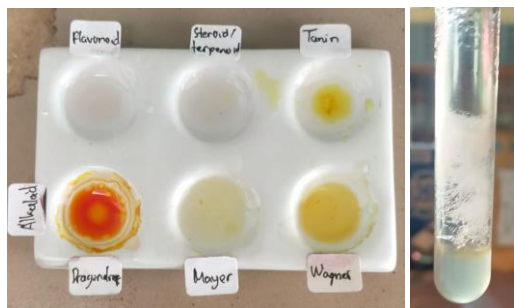
Hasil fraksinasi ekstrak etil asetat *Padina australis*



Kristal isolat 1



Uji titik leleh isolat 1



Uji fitokimia isolat 1



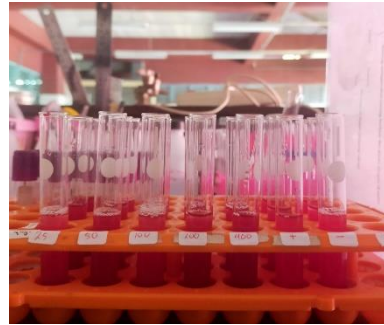
Hasil uji antibakteri ekstrak etil asetat terhadap *E. coli*



Hasil uji antibakteri ekstrak etil asetat terhadap *S. typhi*



Persiapan pengambilan darah tikus



Preparasi sampel ekstrak etil asetat
untuk pengujian antiinflamasi

Lampiran 5. Data Analisis Uji Antiinflamasi Metode *Human Red Blood Cell*

1. Tabel Hasil Pengujian UV-Vis Terhadap Larutan Uji

Konsentrasi (ppm)	Absorbansi (A) = 577 nm			Rata-rata
	Simplo	Duplo	Triplo	
25	0,368	0,256	0,149	0,25766667
50	0,376	0,176	0,187	0,24633333
100	0,164	0,304	0,253	0,24033333
200	0,204	0,286	0,101	0,197
400	0,205	0,098	0,128	0,14366667
Kontrol +	0,017	0,023	0,038	0,026
Kontrol -	0,337	0,366	0,373	0,35867

2. Hasil % Stabilitas Membran Sel Darah Merah

Konsentrasi (ppm)	Rata-rata	Stabilitas (%)
25	0,257667	28,16
50	0,246333	31,32
100	0,240333	32,99
200	0,197	45,07
400	0,143667	59,94
Kontrol +	0,026	92,75
Kontrol -	0,3586	-

a. Rumus % Stabilitas

$$\% \text{ Stabilitas} = 100 - \left(\frac{\text{Abs. Larutan Uji}}{\text{Abs. Kontrol Negatif}} \right) \times 100\%$$

b. Larutan Uji 25 ppm

$$\begin{aligned} \% \text{ Stabilitas} &= 100 - \left(\frac{0,257667}{0,3586} \right) \times 100\% \\ &= 28,16\% \end{aligned}$$

c. Larutan Uji 50 ppm

$$\begin{aligned} \% \text{ Stabilitas} &= 100 - \left(\frac{0,246333}{0,3586} \right) \times 100\% \\ &= 31,32\% \end{aligned}$$

d. Larutan Uji 100 ppm

$$\begin{aligned} \% \text{ Stabilitas} &= 100 - \left(\frac{0,240333}{0,3586} \right) \times 100\% \\ &= 32,99\% \end{aligned}$$

e. Larutan Uji 200 ppm

$$\begin{aligned} \% \text{ Stabilitas} &= 100 - \left(\frac{0,197}{0,3586} \right) \times 100\% \\ &= 45,07\% \end{aligned}$$

f. Larutan Uji 400 ppm

$$\% \text{ Stabilitas} = 100 - \left(\frac{0,143667}{0,3586} \right) \times 100\%$$

$$= 59,94\%$$

g. Larutan Kontrol Positif

$$\% \text{ Stabilitas} = 100 - \left(\frac{0,026}{0,3586} \right) \times 100\%$$

$$= 92,75\%$$

3. Hasil % Inhibisi Hemolisis Membran Sel Darah Merah

Konsentrasi (ppm)	Rata-rata	Inhibisi Hemolisis (%)
25	0,257667	71,8535044
50	0,246333	68,6930656
100	0,240333	67,0198922
200	0,197	54,9358617
400	0,143667	40,0632088
Kontrol -	0,3586	-

a. Rumus % Inhibisi Hemolisis

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{\text{Abs. Larutan Uji}}{\text{Abs. Kontrol Negatif}} \right) \times 100\%$$

b. Larutan Uji 25 ppm

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{0,257667}{0,3586} \right) \times 100\%$$

$$= 71,85\%$$

c. Larutan Uji 50 ppm

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{0,246333}{0,3586} \right) \times 100\%$$

$$= 68,69\%$$

d. Larutan Uji 100 ppm

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{0,240333}{0,3586} \right) \times 100\%$$

$$= 67,01\%$$

e. Larutan Uji 200 ppm

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{0,197}{0,3586} \right) \times 100\%$$

$$= 54,93\%$$

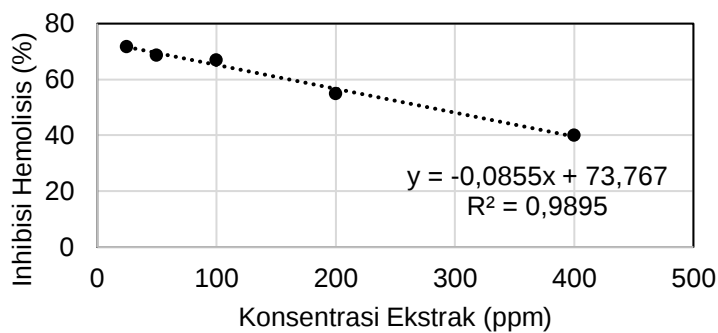
f. Larutan Uji 400 ppm

$$\% \text{ Inhibisi Hemolisis} = \left(\frac{0,143667}{0,3586} \right) \times 100\%$$

$$= 40,06\%$$

g. Penentuan Nilai IC_{50}

Nilai IC_{50} Uji Antiinflamasi Ekstrak Etil Asetat
Padina australis



$$y = ax + b$$

$$y = -0,0855x + 73,767$$

$$IC_{50} = \frac{50 - 73,767}{-0,0855}$$

$$IC_{50} = 277,977 \text{ ppm}$$