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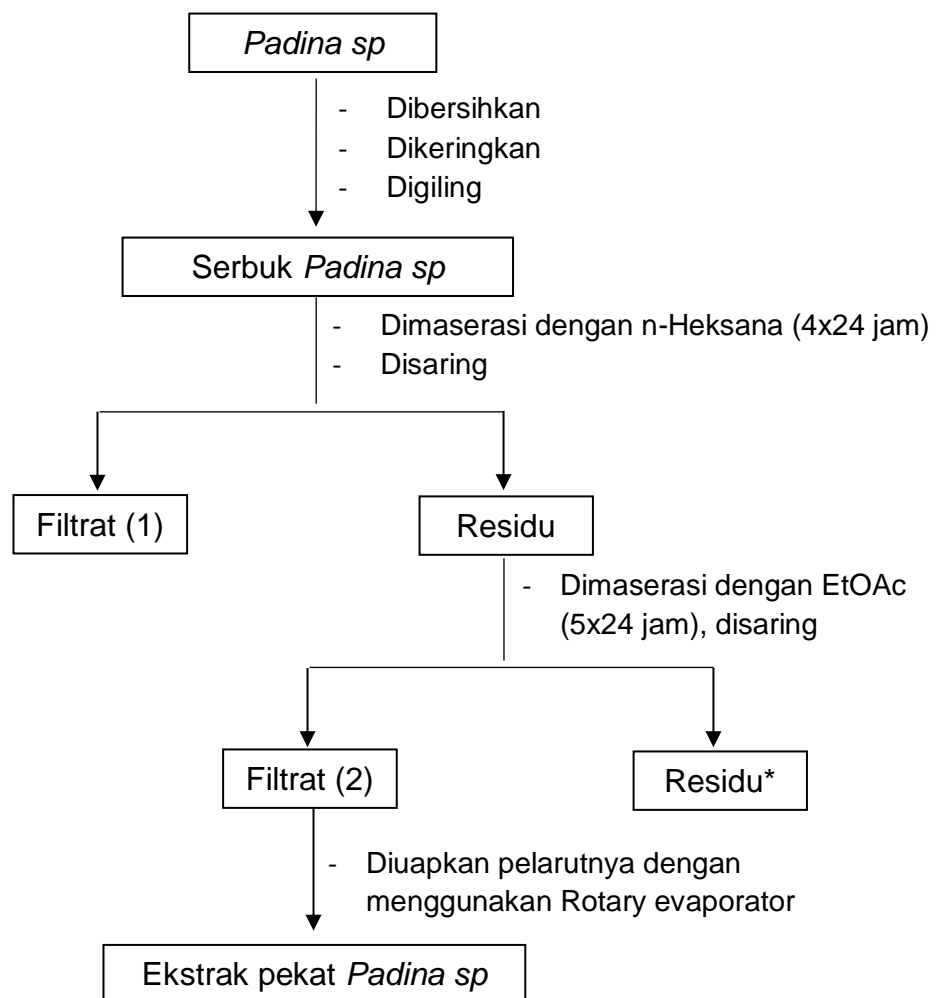
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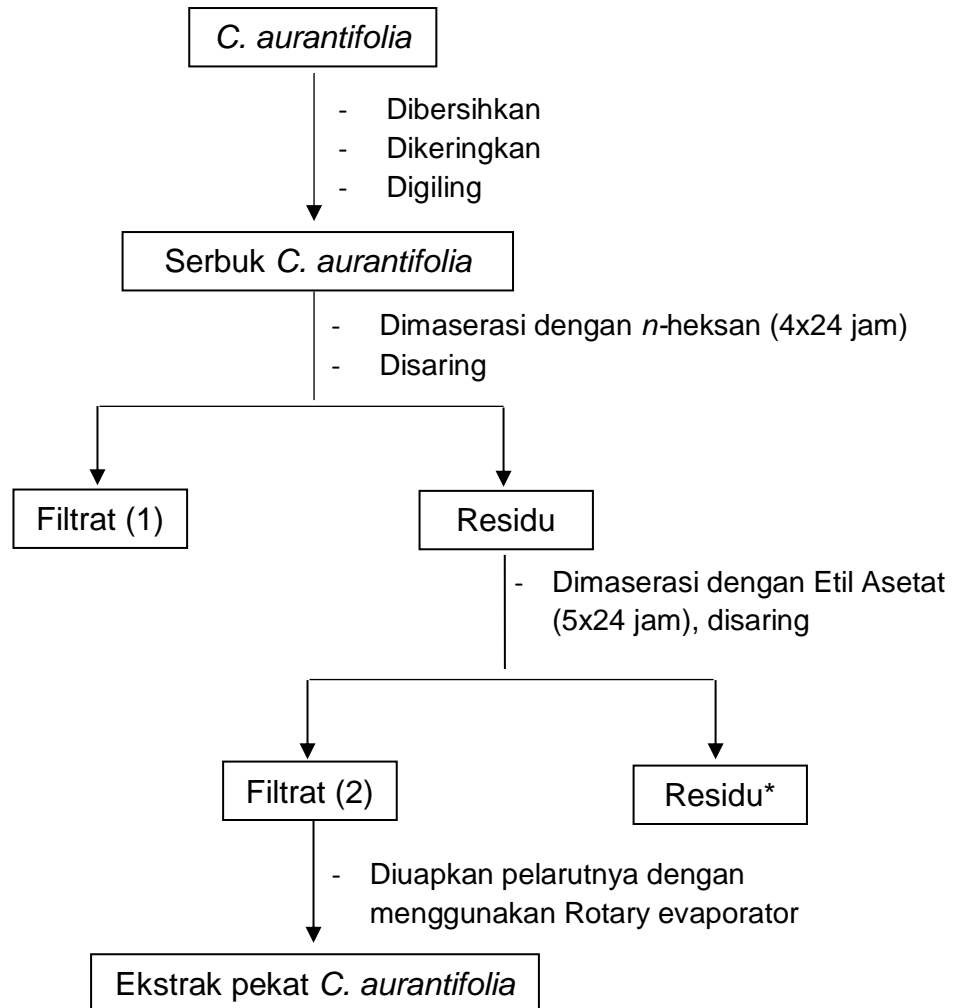
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## LAMPIRAN BAGAN KERJA

Lampiran 1. Bagan kerja isolasi metabolit sekunder dari kombinasi ekstrak etil asetat *Padina sp* dan *Citrus aurantifolia*1.1. Ekstraksi sampel *Padina sp*

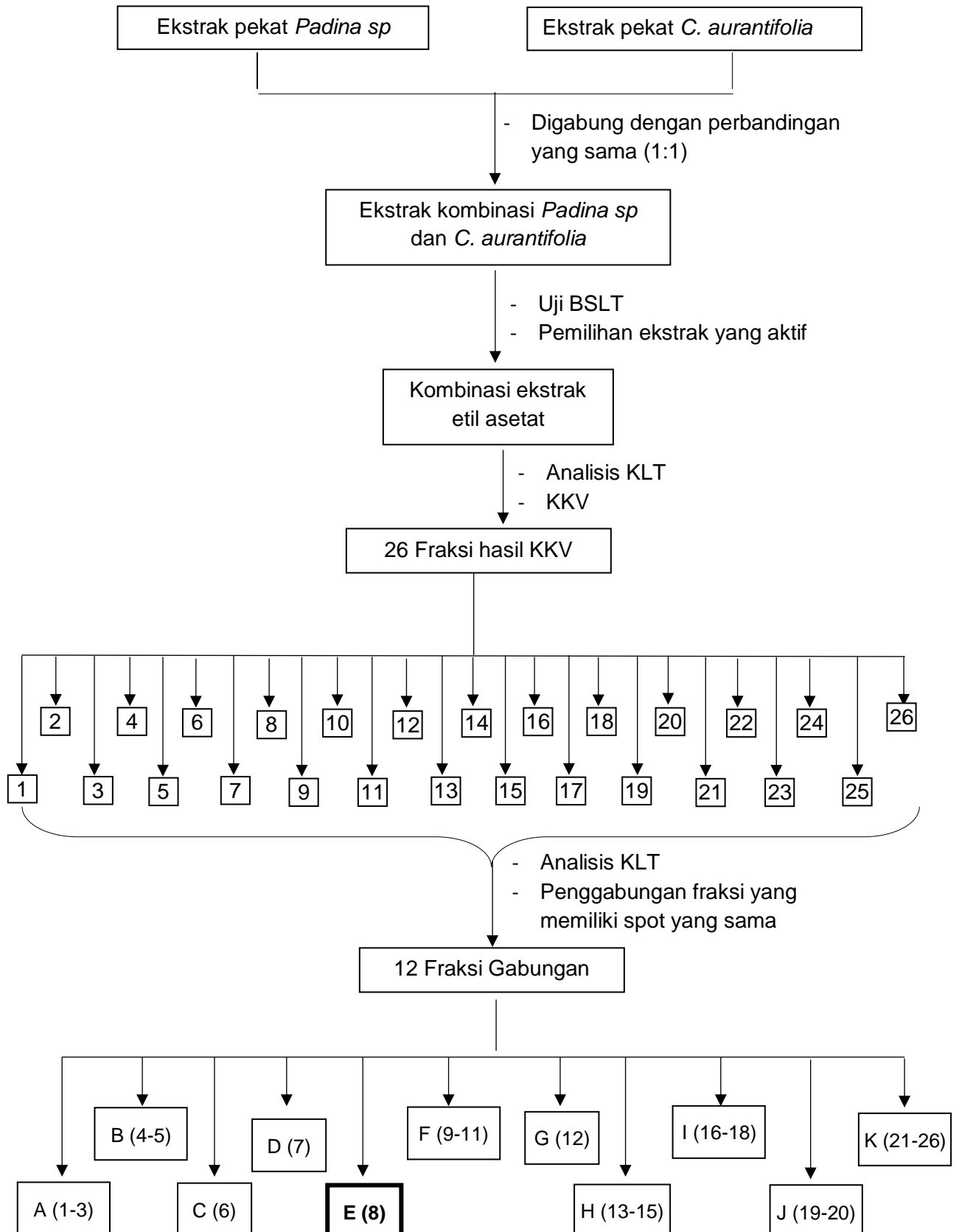
\*Catatan : dilanjutkan dengan maserasi menggunakan pelarut Aseton (4x24 jam) dan metanol (4x24 jam)

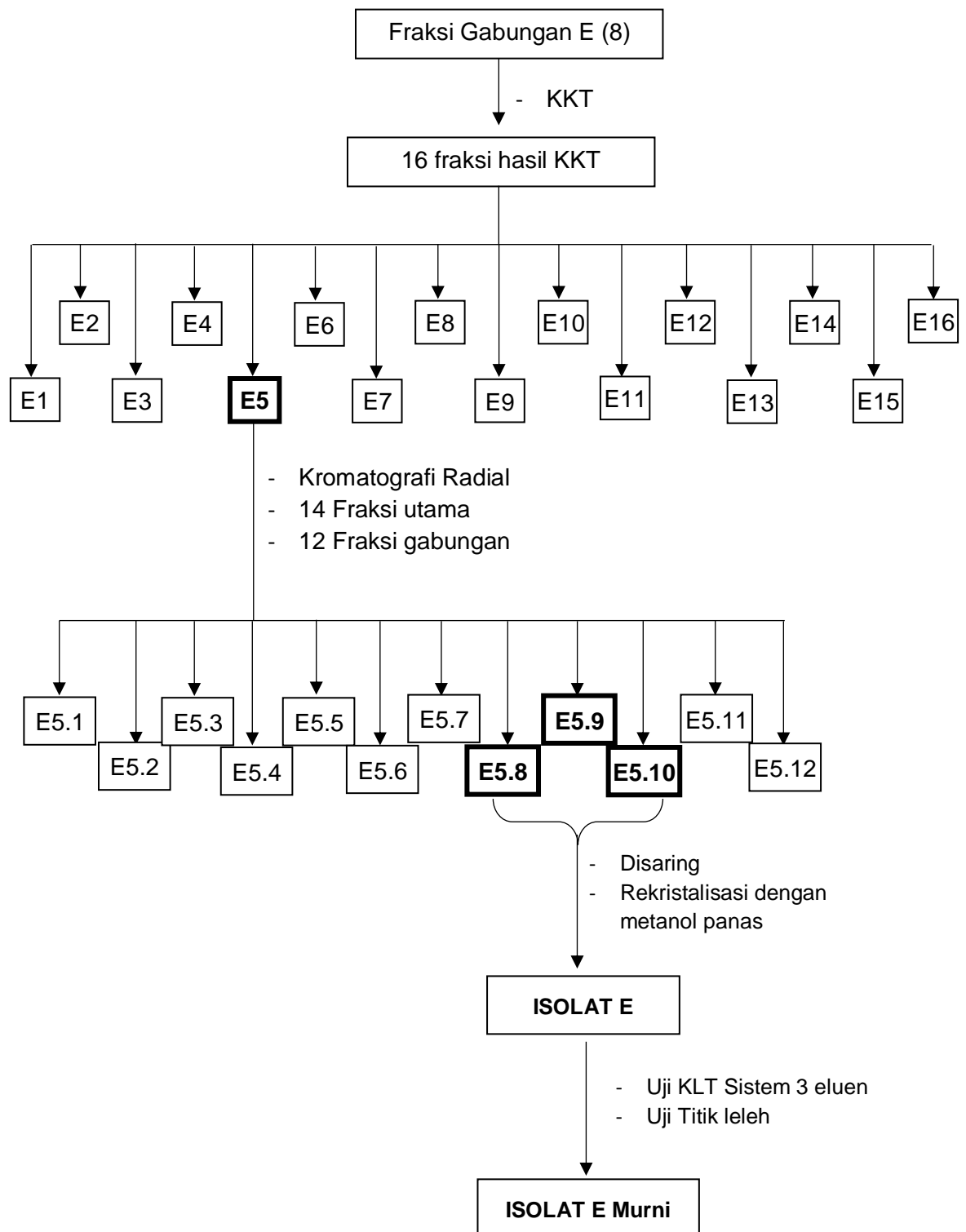
## 1.2. Ekstraksi sampel *C. aurantifolia*



\*Catatan : dilanjutkan dengan maserasi menggunakan pelarut Aseton (4x24 jam) dan metanol (4x24 jam)

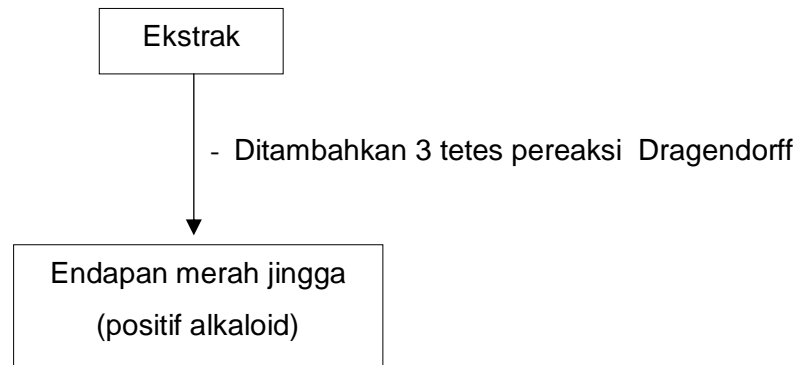
### 1.3 Fraksinasi Kombinasi Ekstrak *Padina sp* dan *C. aurantifolia*



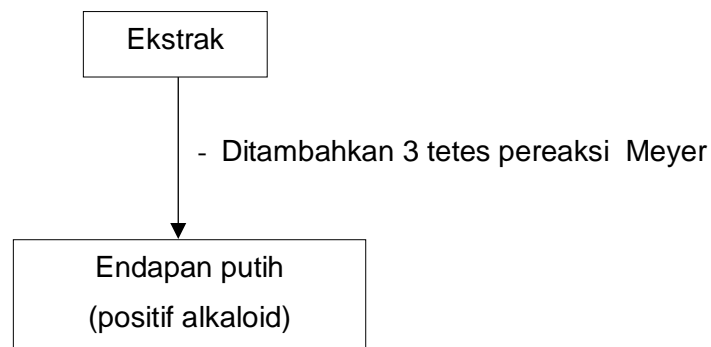


## Lampiran 2. Uji fitokimia

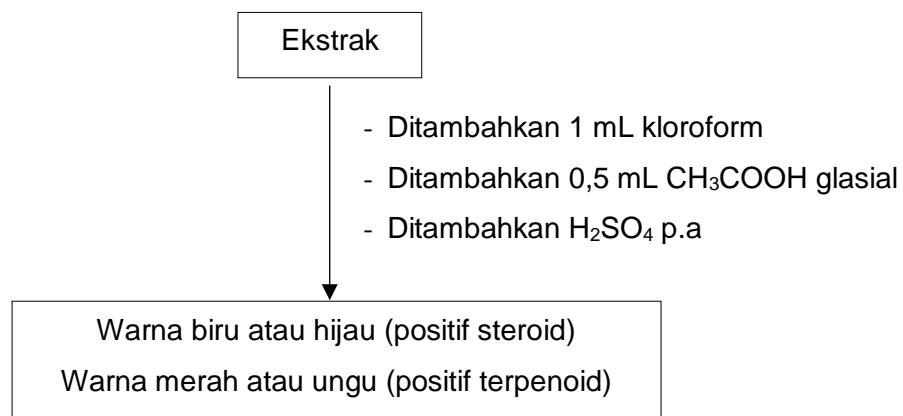
### 2.1 Uji Alkaloid (Pereaksi Dragendorff)



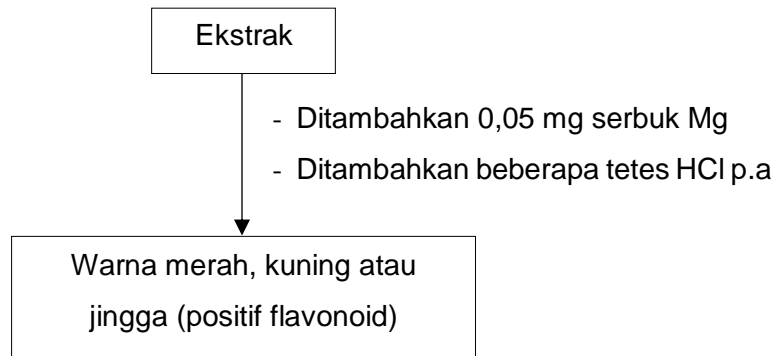
### 2.2 Uji Alkaloid (Pereaksi Meyer)



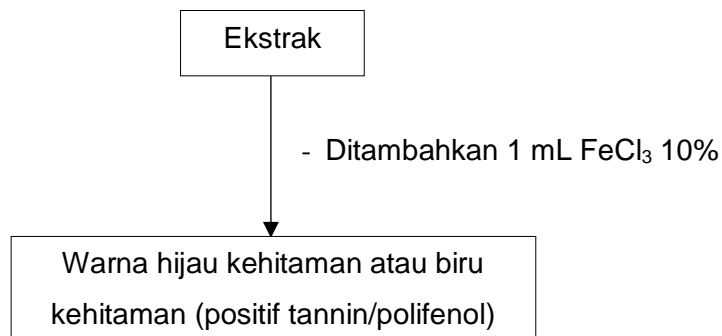
### 2.3 Uji Steroid/Terpenoid



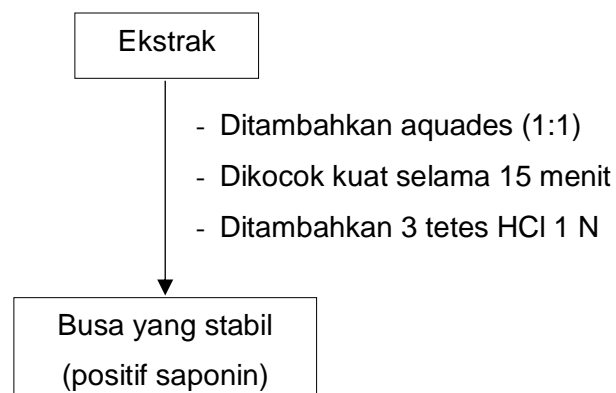
## 2.4 Uji Flavonoid



## 2.5 Uji Tanin



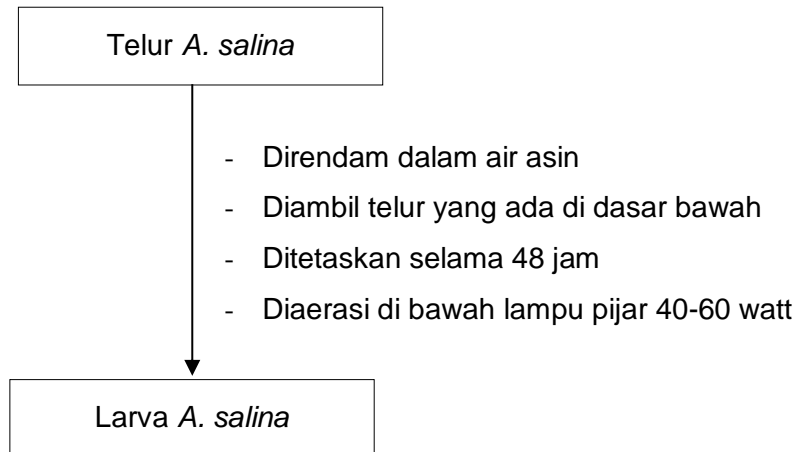
## 2.6 Uji Saponin



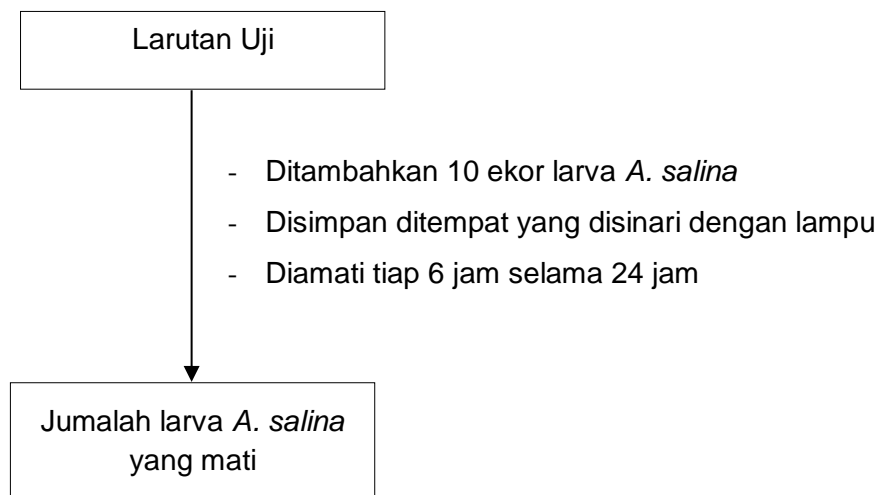


### Lampiran 3. Pengujian toksisitas dengan metode BSLT

#### 3.1 Penetasan telur *Artemia salina* (*A. salina*)

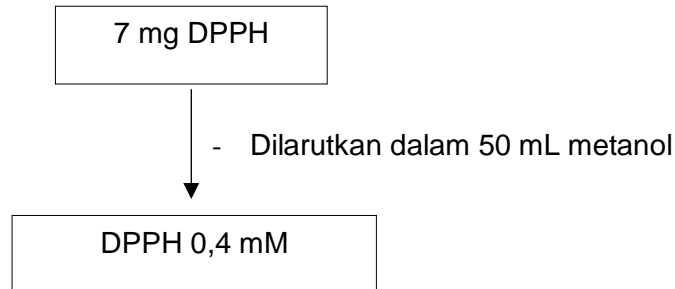


#### 3.2 Pengujian Toksisitas

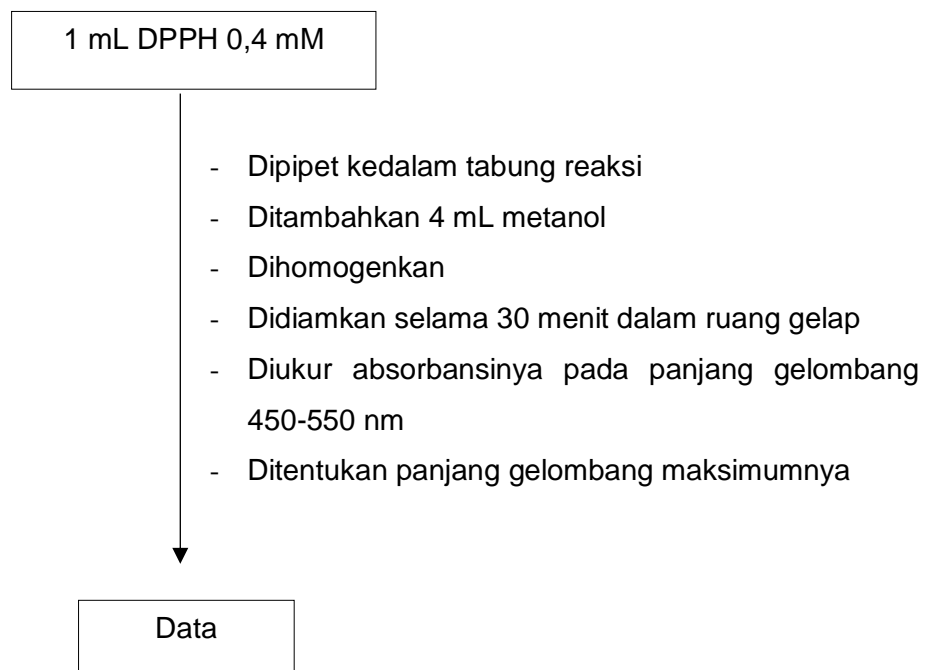


## Lampiran 4. Pengujian aktivitas antioksidan dengan metode DPPH

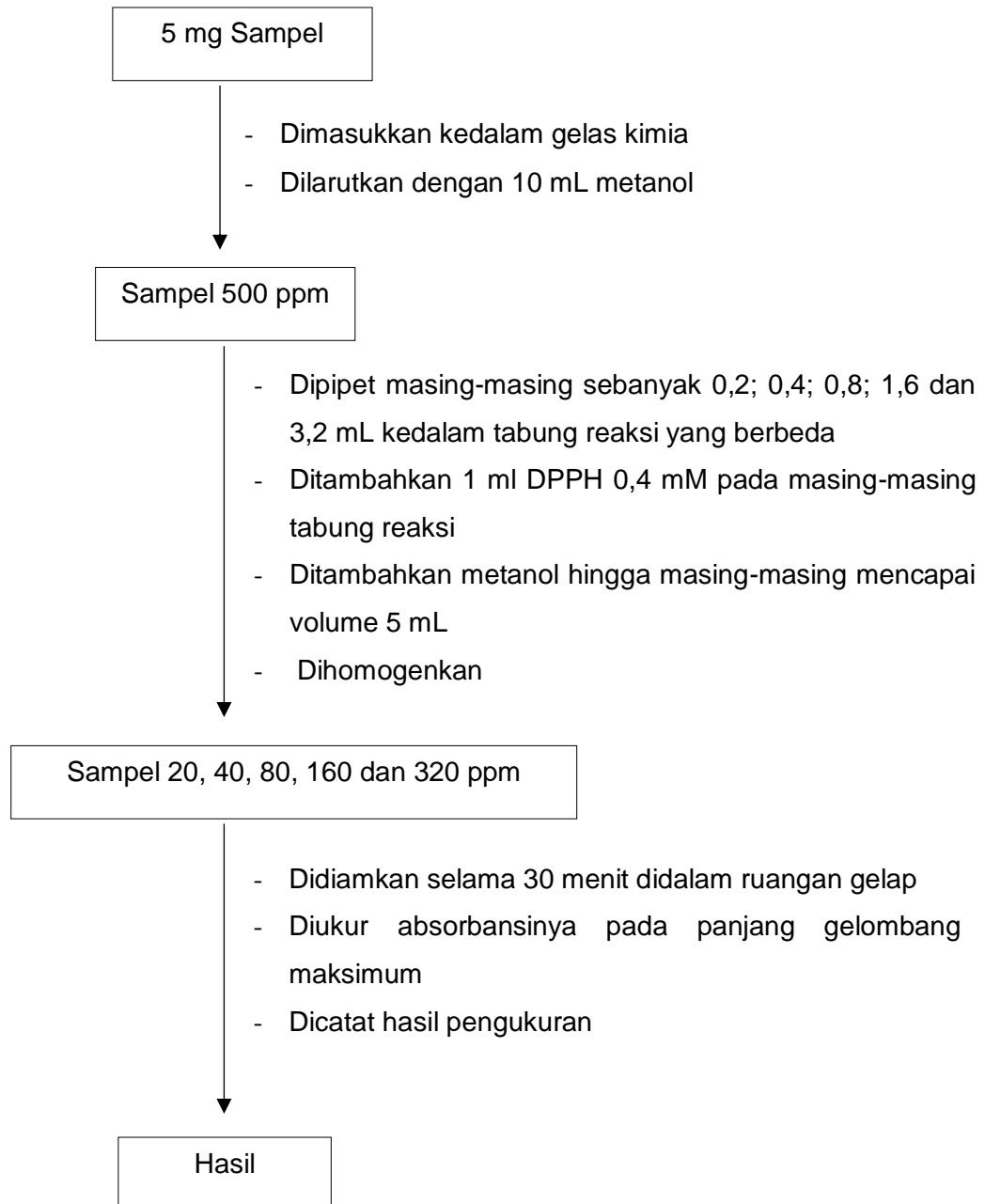
### 4.1 Pembuat Larutan DPPH (2,2-Diphenyl-1-Picrylhydrazyl) 0,4 mM



### 4.2 Penentuan Panjang Gelombang Maksimum

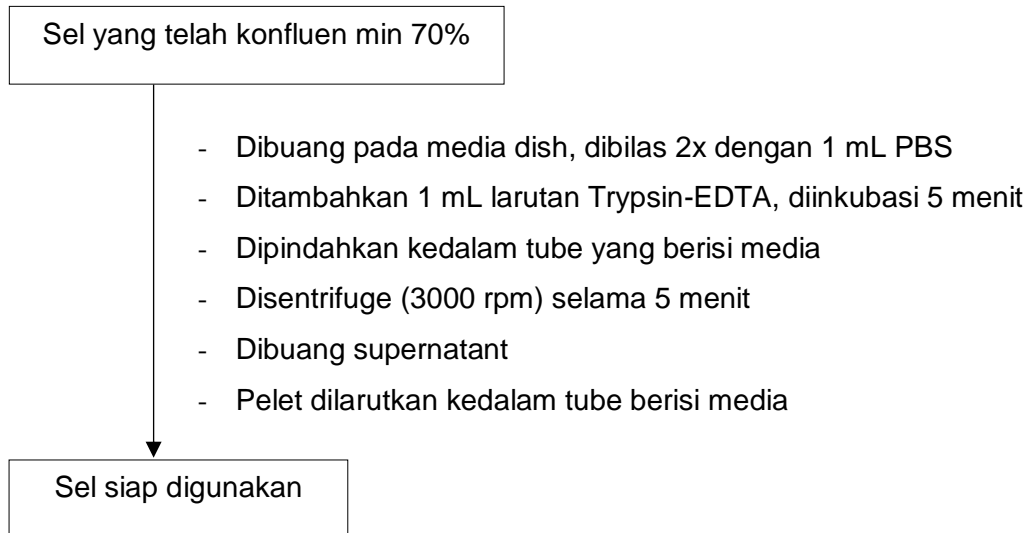


### 4.3 Pengukuran Daya Antioksidan Sampel

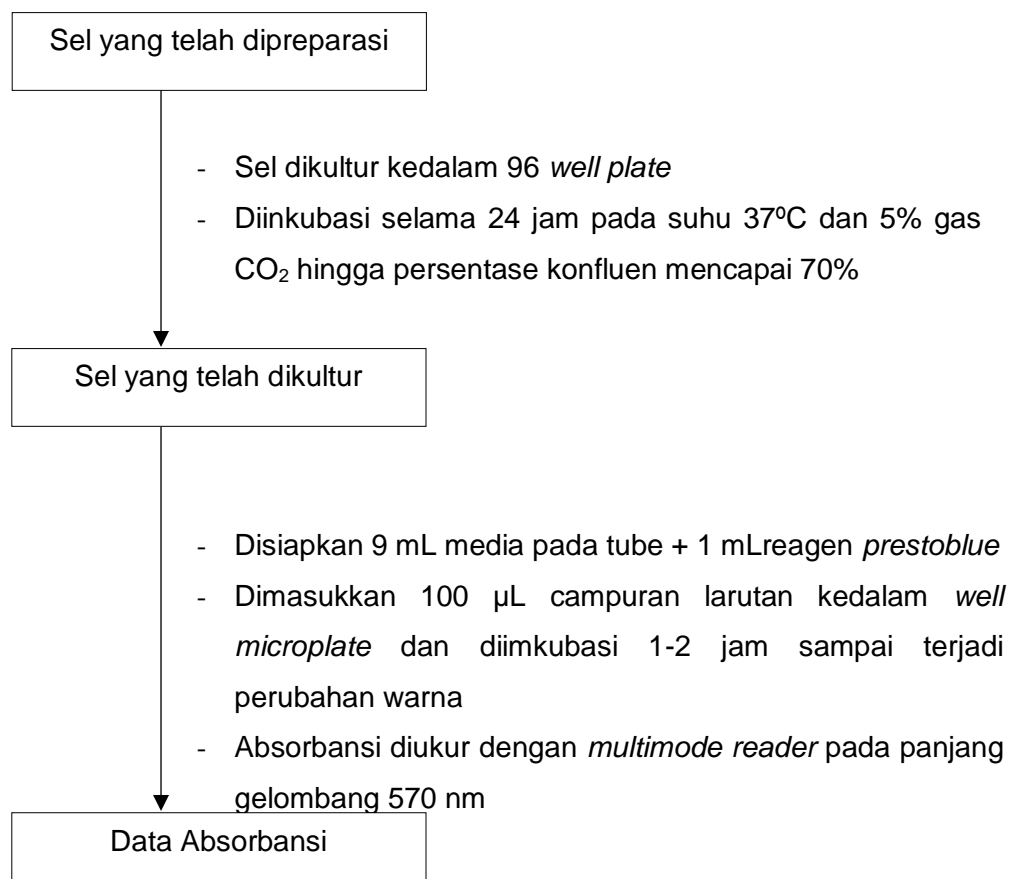


## Lampiran 5. Pengujian sitotoksik terhadap sel MCF-7

### 5.1 Preparasi Sel



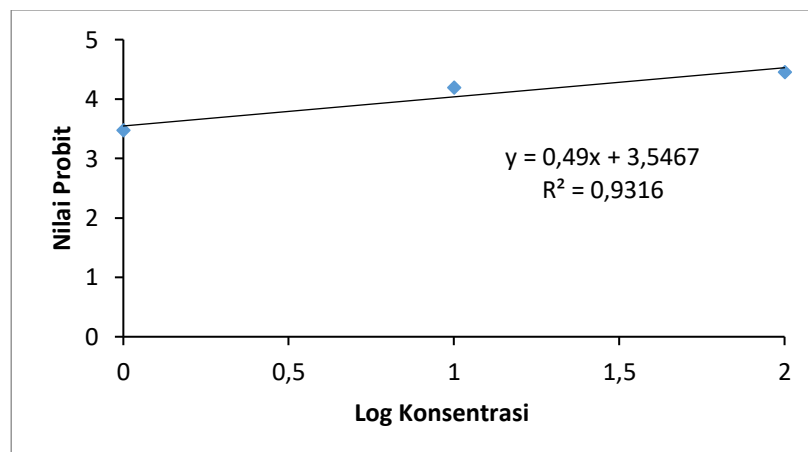
### 5.2 Pengujian sampel terhadap sel MCF-7



## Lampiran 6. Perhitungan nilai LC<sub>50</sub> dengan metode BSLT

### 6.1 Perhitungan nilai LC<sub>50</sub> ekstrak *n*-heksana *Padina sp*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	7	3,47
1	20	4,19
2	33	4,45



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,490 x + 3,546$$

$$y - \frac{3,546}{0,490} = x$$

$$5 - \frac{3,546}{0,490} = 2,967346$$

Jadi, Log x = 2,967346

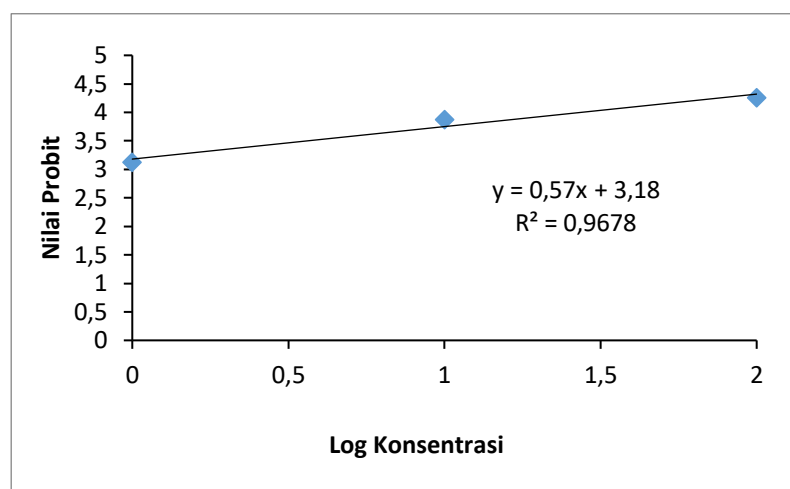
$$x = \text{antilog } 2,967346$$

$$x = 927,568 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 927,568 \text{ ppm}$$

## 6.2 Perhitungan nilai LC<sub>50</sub> ekstrak etil asetat *Padina sp*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	3	3,12
1	13	3,87
2	23	4,26



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,570 x + 3,180$$

$$y - \frac{3,180}{0,570} = x$$

$$5 - \frac{3,180}{0,570} = 3,192982$$

Jadi, Log x = 3,192982

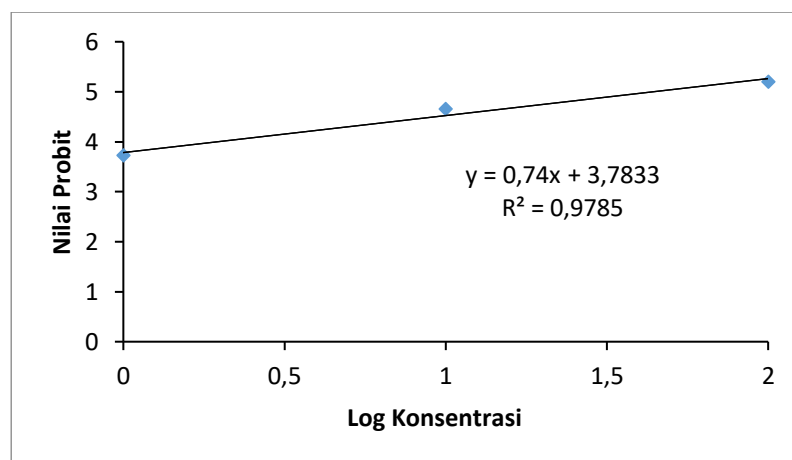
$$x = \text{antilog } 3,192982$$

$$x = 1559,490 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 1559,490 \text{ ppm}$$

### 6.3 Perhitungan nilai LC<sub>50</sub> ekstrak aseton *Padina sp*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	3	3,12
1	20	3,72
2	33	4,12



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,50 x + 3,1533$$

$$y - \frac{3,1533}{0,50} = x$$

$$5 - \frac{3,1533}{0,50} = 3,6934$$

Jadi, Log x = 3,6934

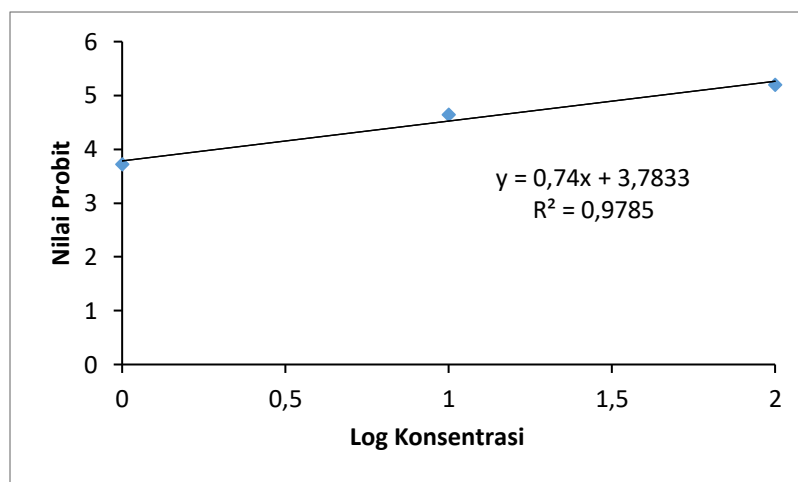
$$x = \text{antilog } 3,6934$$

$$x = 4936,282 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 4936,282 \text{ ppm}$$

#### 6.4 Perhitungan nilai LC<sub>50</sub> ekstrak metanol *Padina sp*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	3	3,12
1	13	3,87
2	27	4,39



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,635 x + 3,158$$

$$y - \frac{3,158}{0,635} = x$$

$$5 - \frac{3,158}{0,635} =$$

Jadi, Log x = 2,900787

$$x = \text{antilog } 2,900787$$

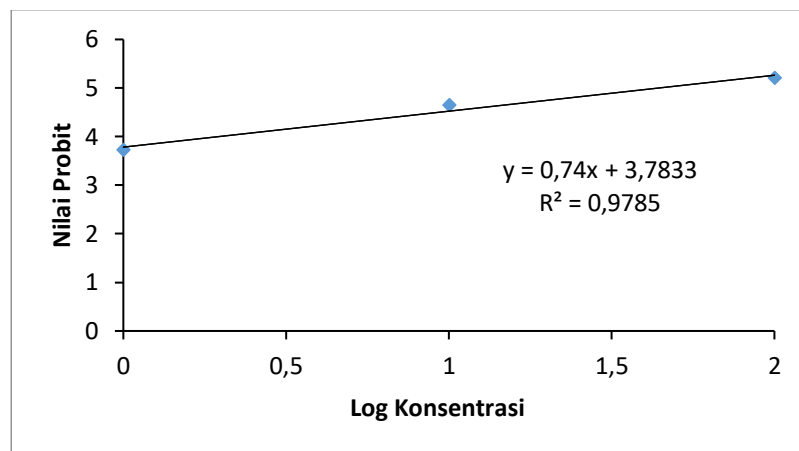
$$x = 795,768 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 795,768 \text{ ppm}$$



### 6.5 Perhitungan nilai LC<sub>50</sub> ekstrak *n*-heksana *C. aurantifolia*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	10	3,72
1	35	4,65
2	58	5,2



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,740 x + 3,043$$

$$y - \frac{3,043}{0,740} = x$$

$$5 - \frac{3,043}{0,740} = 2,644594$$

Jadi, Log x = 2,644594

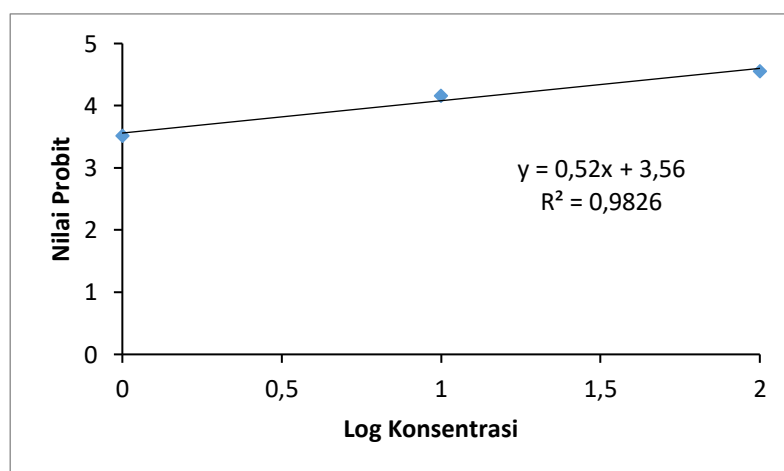
$$x = \text{antilog } 2,644594$$

$$x = 441,157 \text{ ppm}$$

**LC<sub>50</sub> sampel = 441,157 ppm**

### 6.6 Perhitungan nilai LC<sub>50</sub> ekstrak etil asetat *C. aurantifolia*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	7	3,52
1	20	4,16
2	33	4,56



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,520 x + 3,560$$

$$y - \frac{3,560}{0,520} = x$$

$$5 - \frac{3,560}{0,520} = 2,769231$$

Jadi, Log x = 2,769231

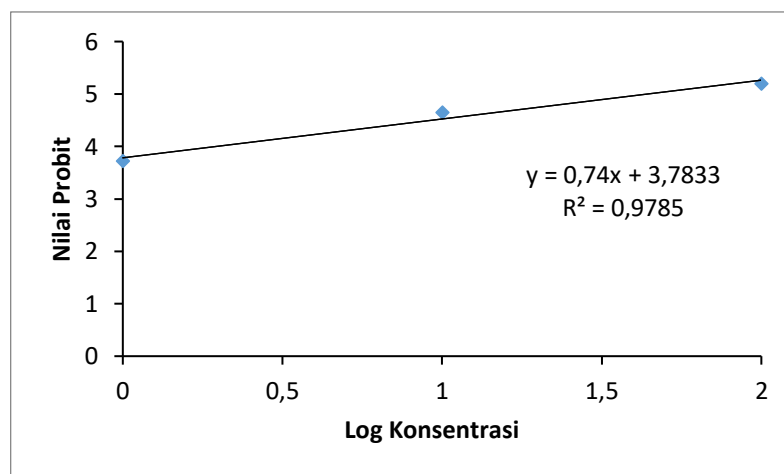
$$x = \text{antilog } 2,769231$$

$$x = 587,801 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 587,801 \text{ ppm}$$

### 6.7 Perhitungan nilai LC<sub>50</sub> ekstrak aseton *C. aurantifolia*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	7	3,12
1	20	4,01
2	23	4,36



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,620 x + 3,210$$

$$y - \frac{3,210}{0,620} = x$$

$$5 - \frac{3,210}{0,620} = 2,887096$$

Jadi, Log x = 2,887096

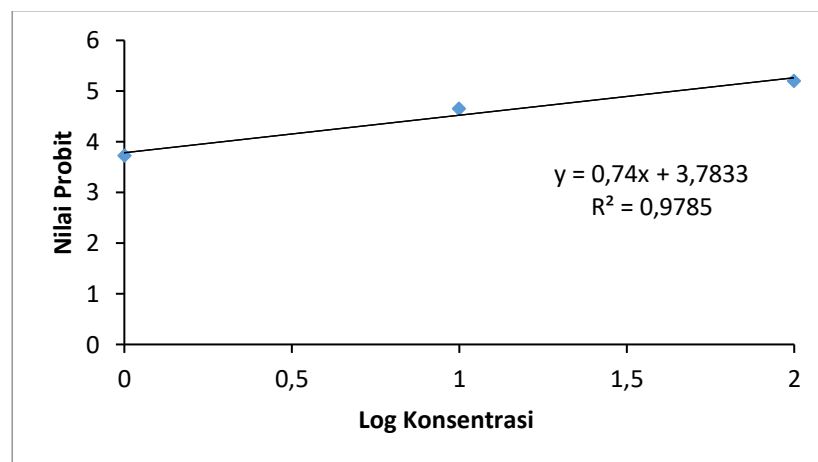
$$x = \text{antilog } 2,887096$$

$$x = 771,075 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 771,075 \text{ ppm}$$

### 6.8 Perhitungan nilai LC<sub>50</sub> ekstrak metanol *C. aurantifolia*

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	0	3,12
1	7	3,82
2	15	4,26



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,570 x + 3,1633$$

$$y - \frac{3,1633}{0,570} = x$$

$$5 - \frac{3,1633}{0,570} = 3,222280$$

Jadi, Log x = 3,222280

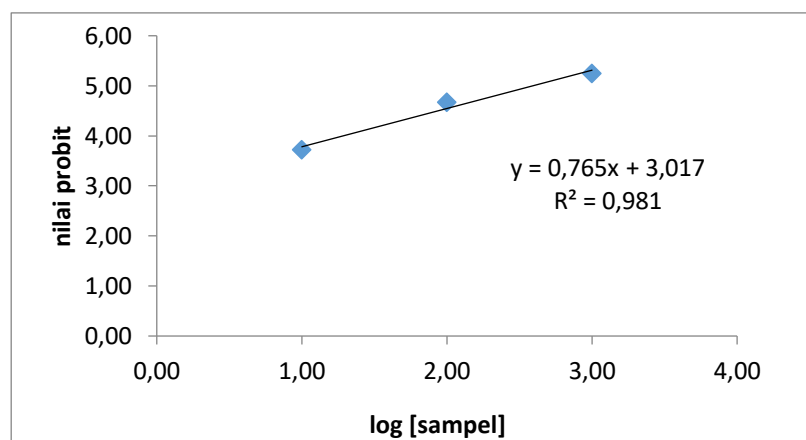
$$x = \text{antilog } 3,222280$$

$$x = 1668,322 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 1668,322 \text{ ppm}$$

### 6.9 Perhitungan nilai LC<sub>50</sub> ekstrak *n*-heksana KPC

Log Konsentrasi (X)	% kematian	Nilai Probit (Y)
1	10	3,72
2	37	4,67
3	60	5,25



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,765 x + 3,017$$

$$y - \frac{3,017}{0,765} = x$$

$$5 - \frac{3,017}{0,765} = 2,5921$$

Jadi, Log x = 2,5921

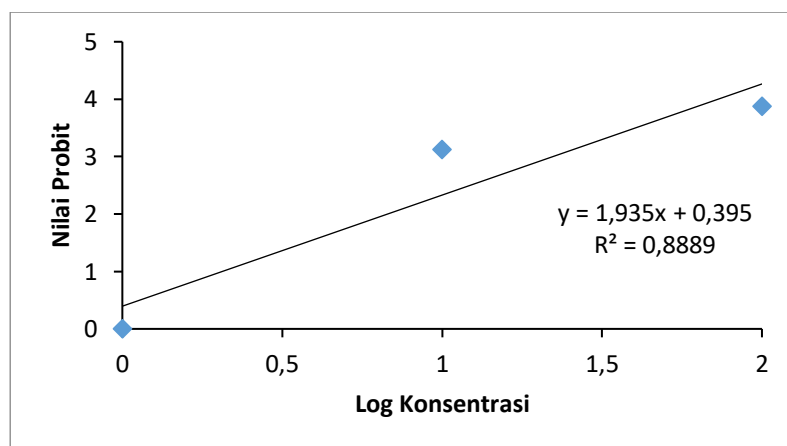
$$x = \text{antilog } 2,5921$$

$$x = 390,930 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 390,930 \text{ ppm}$$

### 6.10 Perhitungan nilai LC<sub>50</sub> ekstrak etil asetat KPC

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
0	0	0
1	3	3,12
2	13	3,87



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 1,935 x + 0,395$$

$$y - \frac{0,395}{1,935} = x$$

$$5 - \frac{0,395}{1,935} = 2,3798$$

Jadi, Log x = 2,3798

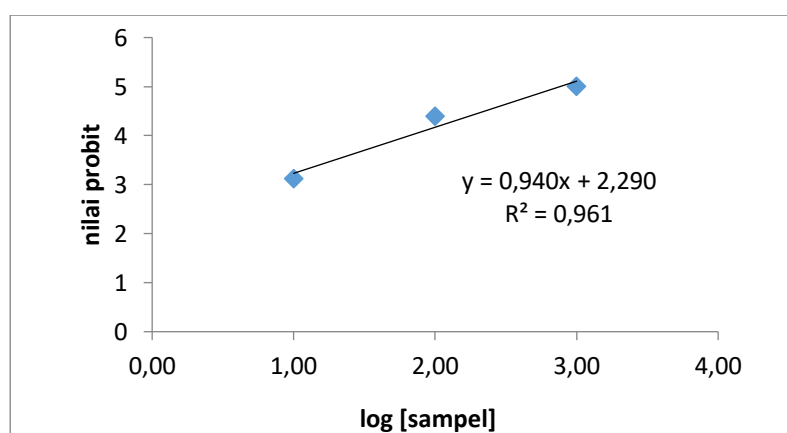
$$x = \text{antilog } 2,3798$$

$$x = 239,798 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 239,798 \text{ ppm}$$

### 6.11 Perhitungan nilai LC<sub>50</sub> ekstrak aseton KPC

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
1	3	3,12
2	27	4,39
3	50	5,00



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,940 x + 2,290$$

$$y - \frac{2,290}{0,940} = x$$

$$5 - \frac{2,290}{0,940} = 2,8829$$

Jadi, Log x = 2,8829

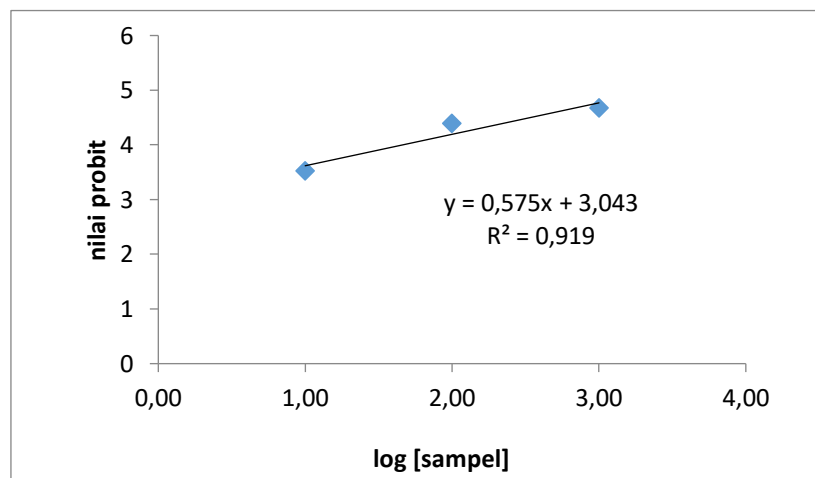
$$x = \text{antilog } 2,8829$$

$$x = 763,659 \text{ ppm}$$

$$\text{LC}_{50} \text{ sampel} = 763,659 \text{ ppm}$$

### 6.12 Perhitungan nilai LC<sub>50</sub> ekstrak metanol KPC

Log Konsentrasi (X)	% Kematian	Nilai Probit (Y)
1	7	3,52
2	27	4,39
3	37	4,67



Untuk LC<sub>50</sub> (x), nilai probit adalah 5 (y), dimasukkan ke persamaan regresi

$$y = 0,575 x + 3,043$$

$$y - \frac{3,043}{0,575} = x$$

$$5 - \frac{3,043}{0,575} = 3,404378$$

Jadi, Log x = 3,404378

$$x = \text{antilog } 3,404378$$

$$x = 2532,084 \text{ ppm}$$

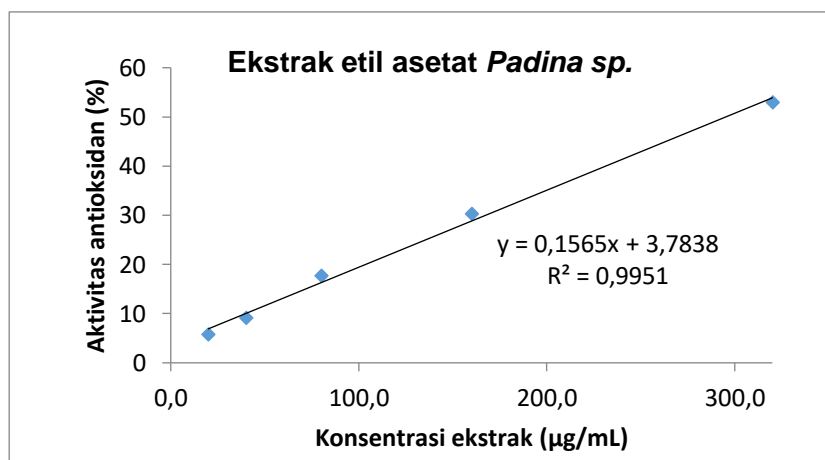
$$\text{LC}_{50} \text{ sampel} = 2532,084 \text{ ppm}$$



## Lampiran 7. Perhitungan aktivitas antioksidan dengan metode DPPH

### 7.1 Aktivitas antioksidan ekstrak etil asetat *Padina sp.*

No	Konsentrasi ( $\mu\text{g/mL}$ )	Absorbansi (A) $\lambda = 516 \text{ nm}$	Aktivitas Antioksidan (%)
1	20	0,705	12,639
2	40	0,655	18,835
3	80	0,565	29,988
4	160	0,360	55,390
5	320	-0,017	102,107
6	kontrol	0,807	-



$$y = 0,2989x + 6,7276$$

$$50 = 0,2989(\text{IC}_{50}) + 6,7276$$

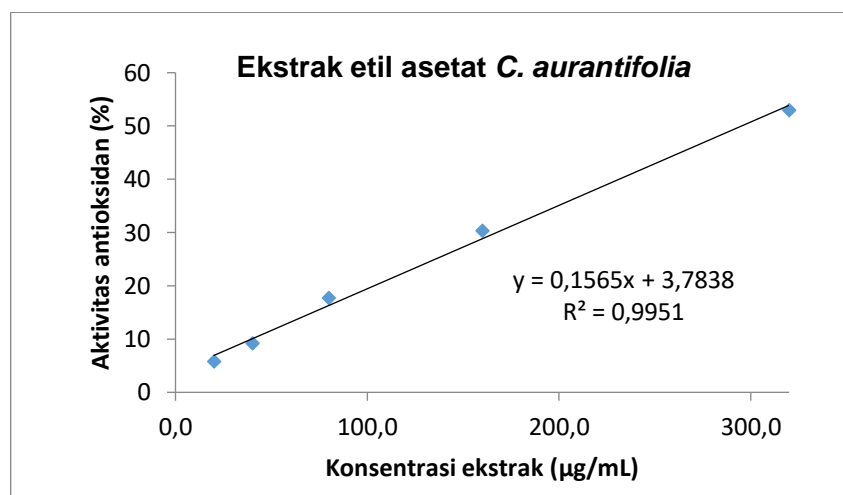
$$\text{IC}_{50} = \frac{50 - 6,7276}{0,2989}$$

$$\text{IC}_{50} = 144,77 \mu\text{g/mL}$$

**Nilai  $\text{IC}_{50}$  sampel 144,77  $\mu\text{g/mL}$**

## 7.2 Aktivitas antioksidan ekstrak etil asetat *C. aurantifolia*

No	Konsentrasi ( $\mu\text{g/mL}$ )	Absorbansi (A) $\lambda = 516 \text{ nm}$	Aktivitas Antioksidan (%)
1	20	0,749	5,786
2	40	0,722	9,182
3	80	0,654	17,736
4	160	0,554	30,314
5	320	0,374	52,956
6	kontrol	0,795	-



$$y = 0,1565x + 3,7841$$

$$50 = 0,1565 (\text{IC}_{50}) + 3,7841$$

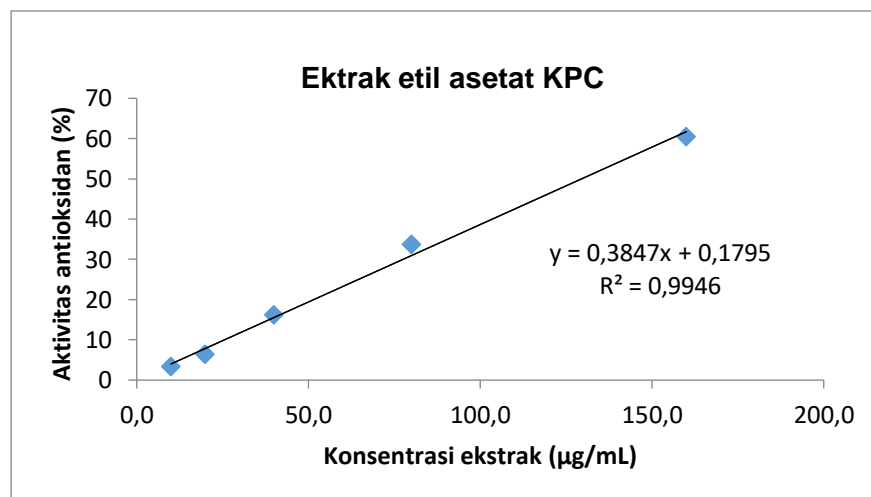
$$\text{IC}_{50} = \frac{50 - 3,7841}{0,1565}$$

$$\text{IC}_{50} = 295,31 \mu\text{g/mL}$$

**Nilai  $\text{IC}_{50}$  sampel 295,31  $\mu\text{g/mL}$**

### 7.3 Aktivitas antioksidan ekstrak etil KPC

No	Konsentrasi ( $\mu\text{g/mL}$ )	Absorbansi (A) $\lambda = 515 \text{ nm}$	Aktivitas Antioksidan (%)
1	10,0	0,979	1,90
2	20	0,917	8,12
3	40	0,844	15,43
4	80	0,661	33,77
5	160	0,393	60,62
6	kontrol	0,998	-



$$y = 0,3847x + 0,1795$$

$$50 = 0,3847 (\text{IC}_{50}) + 0,1795$$

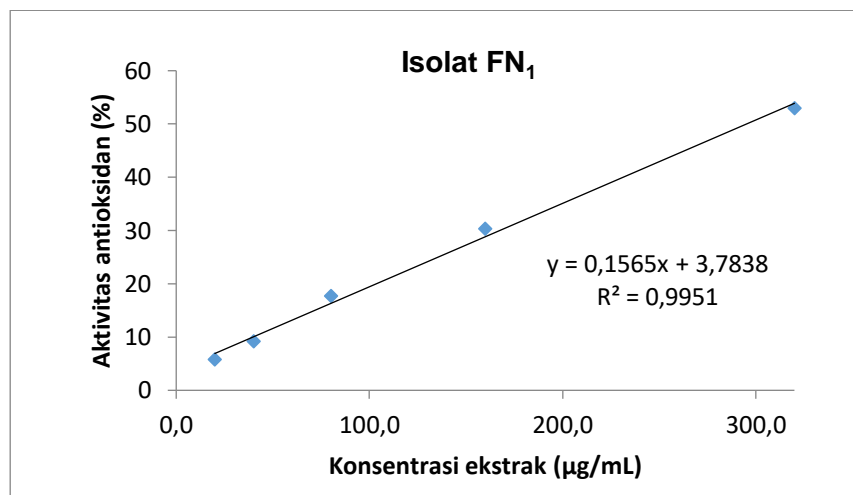
$$\text{IC}_{50} = \frac{50 - 0,3847}{0,1795}$$

$$\text{IC}_{50} = 128,60 \mu\text{g/mL}$$

**Nilai  $\text{IC}_{50}$  sampel 128,60  $\mu\text{g/mL}$**

#### 7.4 Aktivitas antioksidan isolat FN<sub>1</sub>

No	Konsentrasi (µg/mL)	Absorbansi (A) λ = 515 nm	Aktivitas Antioksidan (%)
1	20	0,925	4,05
2	40	0,914	5,19
3	80	0,879	8,82
4	160	0,843	12,55
5	320	0,772	19,92
6	kontrol	0,964	-



$$y = 0,0523x + 3,6221$$

$$50 = 0,0523 (IC_{50}) + 3,6221$$

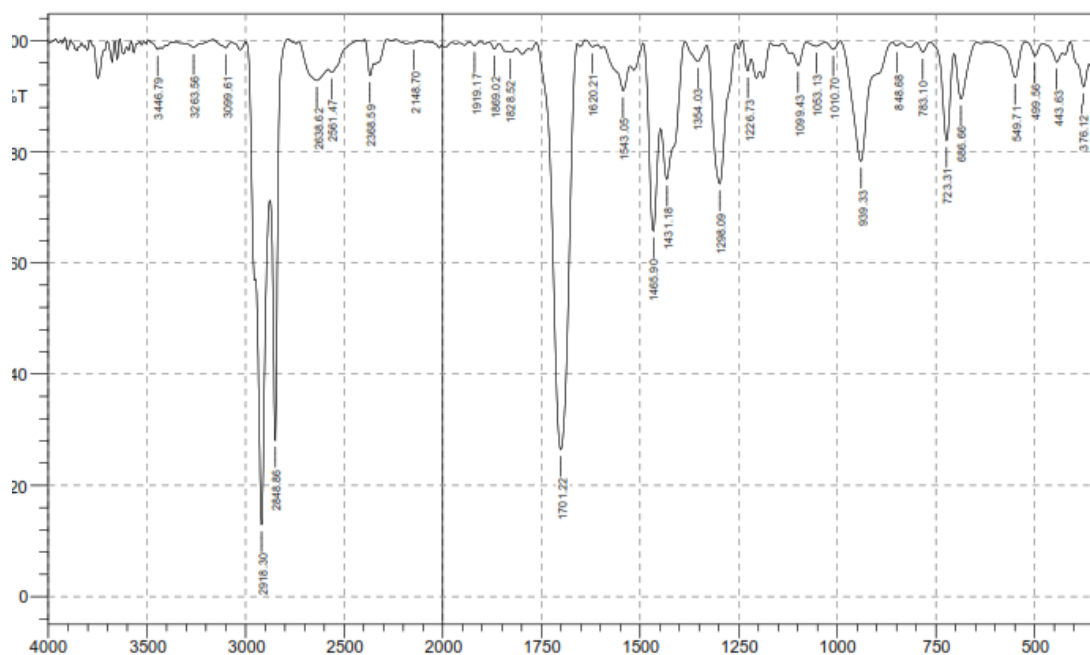
$$IC_{50} = \frac{50 - 0,0523}{3,6221}$$

$$IC_{50} = 886,76 \mu\text{g/mL}$$

**Nilai IC<sub>50</sub> sampel 886,76 µg/mL**

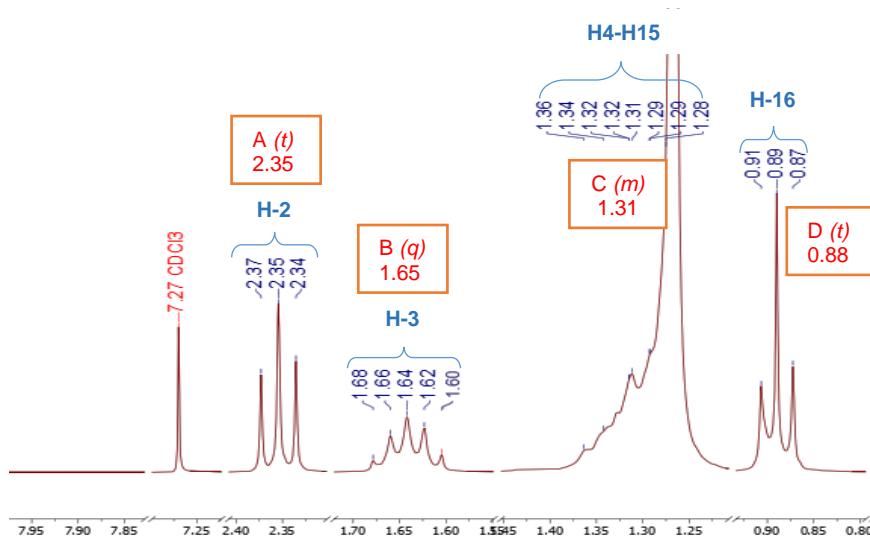
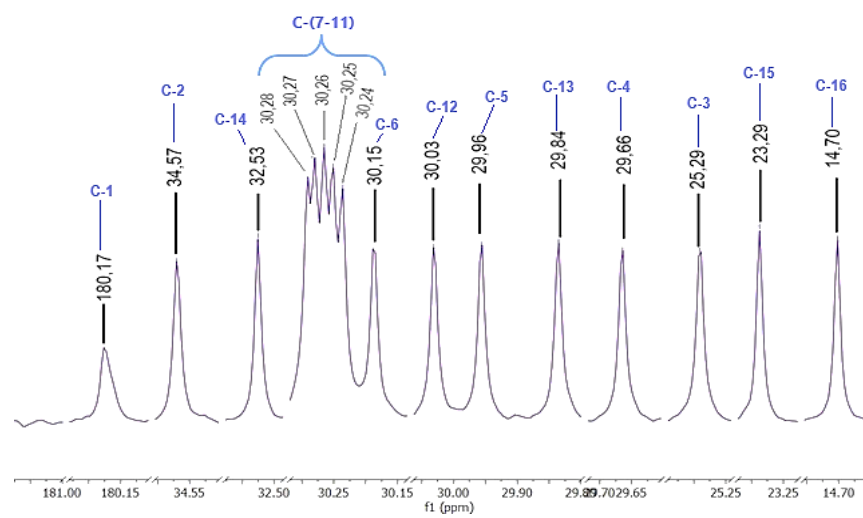
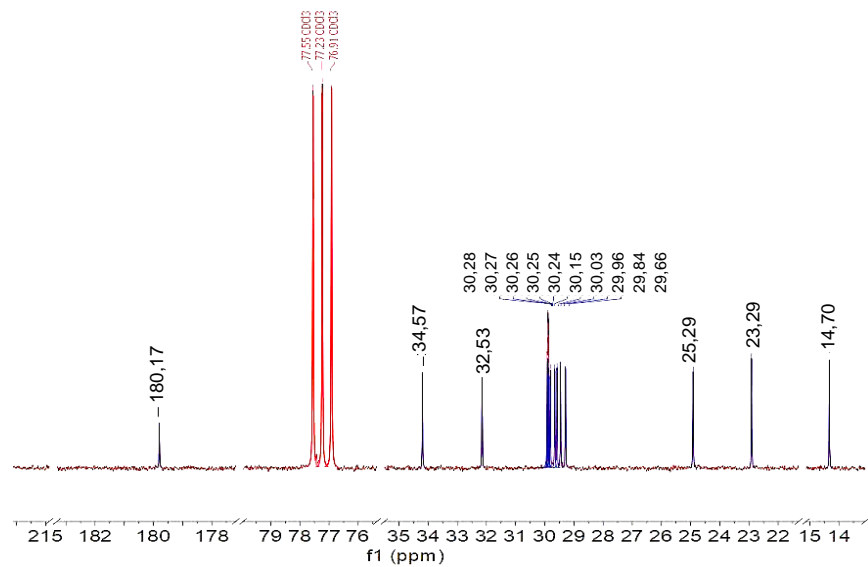
Lampiran 8. Spektrum FT-IR Isolat FN<sub>1</sub>

SHIMADZU



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	352.97	94.809	2.703	360.69	341.4	0.315	0.14
2	376.12	91.686	5.242	408.91	362.62	1.025	0.55
3	443.63	96.15	2.144	478.35	428.2	0.456	0.152
4	499.56	97.27	2.312	522.71	482.2	0.242	0.174
5	549.71	93.39	6.422	613.36	522.71	0.876	0.802
6	686.66	89.515	8.786	704.02	648.08	1.337	1.024
7	723.31	82.002	16.081	763.81	705.95	1.91	1.531
8	783.1	97.891	1.791	798.53	765.74	0.167	0.122
9	848.68	99.081	0.583	858.32	837.11	0.061	0.03
10	939.33	78.329	21.532	991.41	860.25	5.116	5.02
11	1010.7	98.421	1.406	1029.99	991.41	0.142	0.113
12	1053.13	98.992	0.697	1076.28	1029.99	0.136	0.073
13	1099.43	95.549	3.105	1114.86	1080.14	0.42	0.231
14	1226.73	94.575	3.209	1242.16	1219.01	0.337	0.166
15	1298.09	74.215	25.072	1330.88	1257.59	4.596	4.375
16	1354.03	96.264	3.138	1384.89	1330.88	0.551	0.42
17	1431.18	75.105	12.949	1446.61	1386.82	4.536	2.316
18	1465.9	65.721	24.665	1490.97	1448.54	4.368	2.653
19	1543.05	90.978	5.381	1591.27	1525.69	1.584	0.752
20	1620.21	98.893	0.741	1639.49	1606.7	0.088	0.046
21	1701.22	26.411	73.008	1762.94	1660.71	21.738	21.496
22	1828.52	97.924	0.414	1840.09	1815.02	0.209	0.028
23	1869.02	98.474	1.11	1884.45	1857.45	0.101	0.055
24	1919.17	99.123	0.692	1932.67	1903.74	0.063	0.04
25	2148.7	99.558	0.145	2164.13	2108.2	0.066	0.013
26	2368.59	93.715	3.88	2395.59	2351.23	0.737	0.356
27	2561.47	94.325	1.168	2580.76	2436.09	1.716	0.236
28	2638.62	92.833	3.938	2727.35	2582.68	3.452	1.717
29	2848.86	28.162	51.73	2875.86	2785.21	13.462	8.046
30	2918.3	12.915	50.291	2949.16	2877.79	30.046	16.197
31	3099.61	98.694	1.119	3165.19	3070.68	0.259	0.225
32	3263.56	98.741	0.757	3300.2	3224.98	0.27	0.106
33	3446.79	98.431	0.655	3491.16	3431.36	0.215	0.061

### Lampiran 9. Spektrum $^1\text{H-NMR}$ dan $^{13}\text{C-NMR}$ Isolat $\text{FN}_1$



## Lampiran 10. Hasil Pengujian antikanker terhadap sel MCF-7

### 10.1 Gambar Layout Plate

PLATE 1	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Keterangan:

	Media		Sampel -1
	Media + sel		Sampel -2
	Kontrol Positif		Sampel -3
	Kontrol Negatif: Pelarut		Sampel -4

### 10.2 Hasil Uji Antiproliferasi

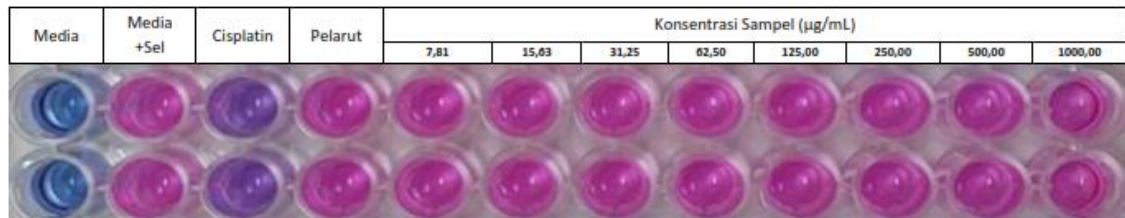
Sampel	IC <sub>50</sub> (µg/mL)
Isolat FN <sub>1</sub>	>1.000

### 10.3 Absorbansi Hasil isolat FN<sub>1</sub> terhadap sel MCF-7

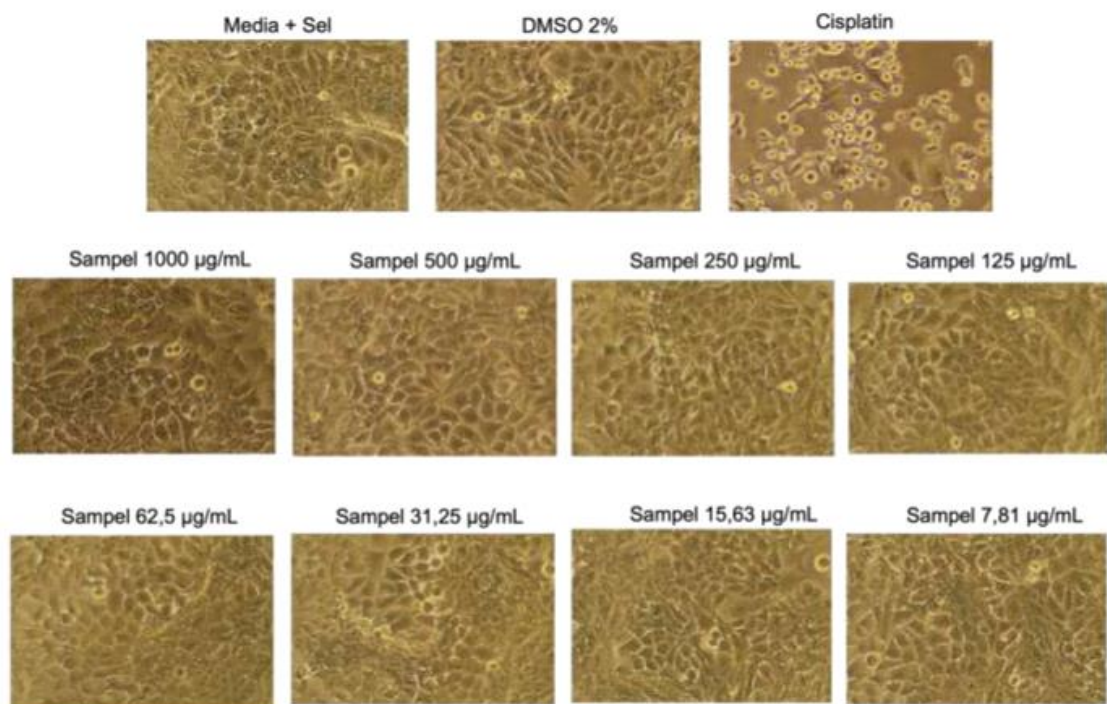
	Media	Media + Sel	Cisplatin	Pelarut	Konsentrasi Sampel (µg/mL)							
					7,81	15,63	31,25	62,50	125,00	250,00	500,00	1000,00
Absorbansi 570nm	0,4882	0,7953	0,6384	0,8006	0,8001	0,8019	0,7751	0,7755	0,7968	0,7880	0,7948	0,8021
	0,4944	0,7955	0,6482	0,8014	0,7871	0,7815	0,7680	0,7927	0,7845	0,8082	0,8101	0,8236
Absorbansi 600nm	0,6193	0,1983	0,4219	0,2041	0,2082	0,2059	0,2161	0,2140	0,2324	0,4780	0,3910	0,6126
	0,6188	0,2004	0,4536	0,2067	0,2031	0,2015	0,1999	0,1950	0,2010	0,2021	0,2148	0,2373
Selisih Absorbansi	-0,1311	0,5970	0,2165	0,5965	0,5919	0,5960	0,5590	0,5595	0,5644	0,3100	0,2038	0,1895
	-0,1244	0,5951	0,1946	0,5947	0,5840	0,5800	0,5681	0,5977	0,5835	0,6061	0,5953	0,5863
% Sel hidup		100,19	47,59	100,12	99,49	100,06	94,94	95,01	95,09	60,52	45,84	43,86
		99,93	44,56	99,88	98,40	97,84	96,20	100,29	98,33	101,45	99,96	98,71
Rata-rata % sel hidup		100,06	46,08	100,00	98,94	98,95	95,57	97,65	97,01	80,98	72,90	71,29
SEM		0,13	1,51	0,12	0,55	1,11	0,63	2,64	1,32	20,47	27,06	27,43
Normalisasi data % sel hidup		100,06	46,08	100,00	98,94	98,95	95,57	97,65	97,01	80,98	72,90	71,29

Keterangan: Konsentrasi cisplatin yang digunakan dalam uji sebesar 53 µM.

#### 10.4 Well Plate hasil pengujian isolat FN<sub>1</sub> terhadap sel MCF-7



#### 10.5 Morfologi sel MCF-7 terhadap hasil uji isolat FN<sub>1</sub>





### Lampiran 11. Dokumentasi Penelitian



Sampel *Padina sp.*



Sampel *Citrus aurantifolia*



Pengeringan sampel *Padina sp.*



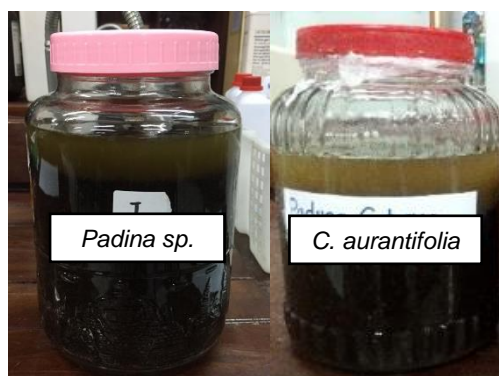
Pengeringan *Citrus aurantifolia*



Serbuk *Padina sp.*



Serbuk *C. aurantifolia*



Maserasi masing-masing Sampel



Penyaringan hasil maserasi *Padina sp* dan *C. aurantifolia*



Proses Evaporasi



Ekstrak *n*-heksan *Padina sp*



Ekstrak etil asetat *Padina sp*



Ekstrak aseton *Padina sp*



Ekstrak Metanol *Padina sp*



Ekstrak *n*-heksana *C. aurantifolia*



Ekstrak etil asetat *C. aurantifolia*



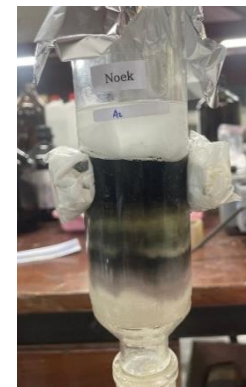
Ekstrak aseton *C. aurantifolia*



Ekstrak Metanol *C. aurantifolia*



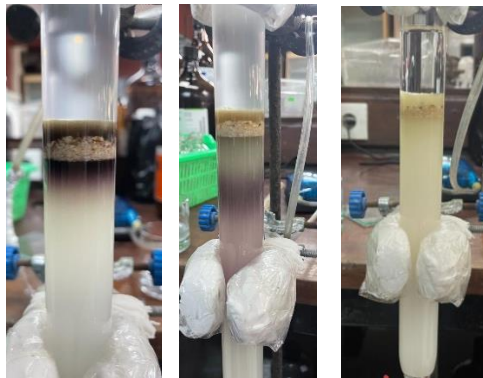
Ekstrak etil *Padina sp* dan *C. aurantifolia* digabung (1:1)



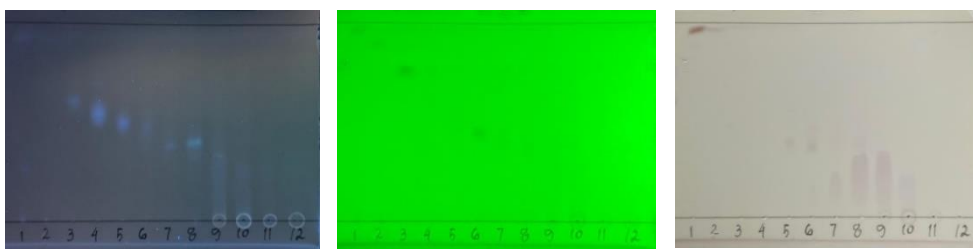
Proses Fraksinasi metode KKV



Fraksi-fraksi hasil KKV

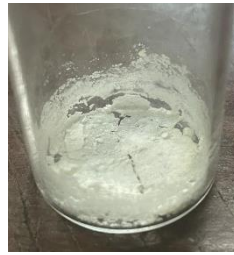
Proses Fraksinasi  
Dengan metode KKT

Proses Fraksinasi menggunakan Kromatotron

Hasil analisis KLT Fraksi gabungan kromatotron dengan eluen EtOAc : CHCl<sub>3</sub> :  
*n*-heksana (0,2 : 1,3 : 8,5)



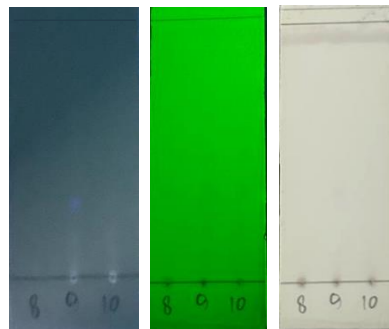
Fraksi gabungan 8



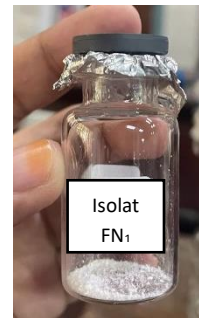
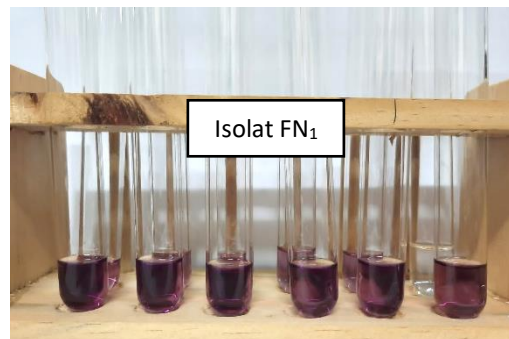
Fraksi gabungan 9



Fraksi gabungan 10



Hasil KLT 3 Fraksi gabungan  
dengan eluen  $\text{CHCl}_3$  : *n*-heksana (3 : 7)

Isolat FN<sub>1</sub>Uji aktivitas antioksidan isolat FN<sub>1</sub> dengan menggunakan metode DPPH

Media	Media +Sel	Cisplatin	Pelarut	Konsentrasi Sampel (µg/mL)							
				7,81	15,63	31,25	62,50	125,00	250,00	500,00	1000,00

Hasil uji aktivitas antikanker isolat FN<sub>1</sub> terhadap sel MCF-7 pada *Well Plate*