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**LAMPIRAN**  
**A. Dokumentasi Penelitian**



Simplisia dan proses maserasi menggunakan Ethanol 96%

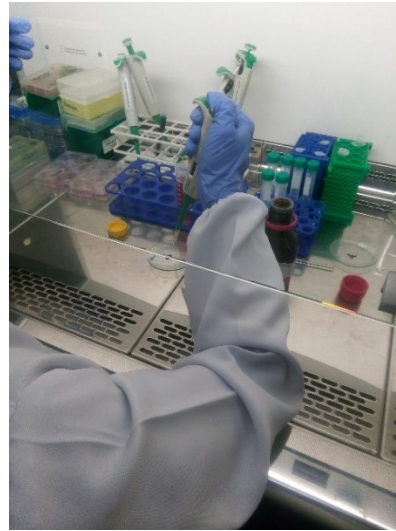


Proses penguapan menggunakan Alat *Rotatory Evaporator*

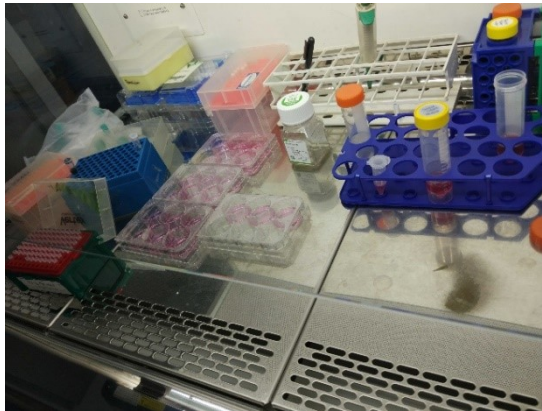




Proses Kultur Sel



Pembuatan Larutan Uji



Penggantian Medium



Pengukuran menggunakan ELISA READER

## B. LoA Accepted dari Jurnal Nasional



HN.LOA.hn50101.20201227

### ----- Letter of Acceptance -----

Health Notions states that the following article:

**Title:**

**IC50 and Cell Viability of Combination of Ethanol Extract Moringa oleifera Leave (EEMo) and Ethanol Extract Carica papaya Leave (EECP) on Breast Cancer Cells**

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Thank you very much

December 27, 2020

Editor in Chief  
  
Dr. Heru SWN, C.P.M.C.

### C. Perhitungan Pembuatan Medium, Konsentrasi dan Jumlah Sel

#### 1. Perhitungan Medium

- a. 10% FBS  $= \frac{10}{100} \times 50 = 5 \text{ mL}$
- b. 1% P/S  $= \frac{1}{100} \times 50 = 0,5 \text{ mL}$
- c. 1% Fungizone  $= \frac{1}{100} \times 50 = 0,5 \text{ mL}$
- d. 1% Gentamisin  $= \frac{1}{100} \times 50 = 0,5 \text{ mL}$
- e. DMEM = sampai 50 mL

#### 2. Perhitungan pembuatan konsentrasi

- a. 20 ppm  $= \frac{20 \text{ ppm}}{1000 \text{ ppm}} \times 10 \text{ mL} = 0,2 \text{ mL}$
- b. 40 ppm  $= \frac{40 \text{ ppm}}{1000 \text{ ppm}} \times 10 \text{ mL} = 0,4 \text{ mL}$
- c. 80 ppm  $= \frac{80 \text{ ppm}}{1000 \text{ ppm}} \times 10 \text{ mL} = 0,8 \text{ mL}$
- d. 160 ppm  $= \frac{160 \text{ ppm}}{1000 \text{ ppm}} \times 10 \text{ mL} = 1,6 \text{ mL}$

#### 3. Perhitungan Jumlah Sel yang digunakan

Diperoleh sel konfluen  $1,63 \times 10^7$  sel dari flash T25

Dibutuhkan  $5 \times 10^4$  untuk tiap sumuran pada *well plate* 96, jadi  $50.000 \times 84 = 4.200.000$

$$\frac{4.200.000}{16.300.000} \times 100 \mu\text{L} = 25,77 \mu\text{L}$$

Dipipet 25,77  $\mu\text{L}$  dari stok konfluen lalu di cukupkan sampai 8.400  $\mu\text{L}$  dengan medium, homogenkan kemudian dipipet 100  $\mu\text{L}$  untuk tiap sumuran ( $5 \times 10^4$  sel).

**D. Indeks Kombinasi (IK) EEDK – EEDP pada kultur sel MCF-7 dengan  
*Compusyn system***

Summary Table

Experiment Name: EK+EP terhadap MCF-7  
 Date: 20 November 2020  
 File Name: C:\Users\TOSHIBA\Documents\Indeks Kombinasi EK-EP.cse  
 Description: Aktivitas Sitotoksik dari kombinasi ekstrak Kelor dan ekstrak Pepaya terhadap sel MCF-7

Drug: Ekstrak Kelor (EK) [ppm]  
 Drug: Ekstrak Pepaya (EP) [ppm]  
 Drug Combo: Kombinasi 1:3 (1:3) (EK+EP [1:3])  
 Drug Combo: Kombinasi 1:1 (1:1) (EK+EP [1:1])  
 Drug Combo: Kombinasi 3;1 (3:1) (EK+EP [3:1])

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Drug/Combo	Dm	m	r
EK	937.200	-0.4247	-0.8804
EP	169.129	-0.3325	-0.9301
1:3	399517.	-0.1102	-0.6811
1:1	87910.0	-0.1246	-0.9694
3:1	2.633E7	-0.0569	-0.4735

---

CI values at:

Combo	ED50	ED75	ED90	ED95
1:3	1878.23	2.32572	0.00292	3.12E-5
1:1	306.791	1.13725	0.00438	1.01E-4
3:1	59979.4	0.00549	5.5E-10	9.8E-15

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## E. Hasil Uji Fitokimia Ekstrak Etanol Daun Kelor dan Ekstrak Etanol Daun Pepaya



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### LEMBAR HASIL

No	Golongan Senyawa	Ekstrak Kelor	Ekstrak Pepaya	Pereaksi	Keterangan
1.	Flavonoid	+	+	AlCl <sub>3</sub> 10% (Aluminium klorida)	Hasil (+) Bercak berwarna kuning setelah disemprot AlCl <sub>3</sub> 10 %
2.	Tannin	+	+	FeCl <sub>3</sub> (Besi (III) klorida) 5%	Hasil (+) Bercak berwarna hitam setelah disemprot FeCl <sub>3</sub> 5%
3.	Terpenoid	+	+	H <sub>2</sub> SO <sub>4</sub>	Hasil (+) Bercak berwarna merah muda kecoklatan setelah disemprot H <sub>2</sub> SO <sub>4</sub>
4.	Alkaloid	+	+	Mayer, Dragendorf	Hasil (+) Endapan putih setelah ditambahkan pereaksi Mayer, Endapan Merah setelah ditambahkan pereaksi Dragendorf
5.	Saponin	-	-	Aquadest	Hasil (-) Tidak terbentuk busa yang stabil setelah dipanaskan dan dikocok

Makassar, 20/09 - 2020

Kepala Laboratorium Fitokimia

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NIP. 19641231 199002 1 005

### F. Viabilitas Sel MCF-7 dan Sel Vero

$$\text{selhidup \%} = \frac{\text{absorbansi perlakuan} - \text{absorbansi kontrol media}}{\text{absorbansi kontrolsel} - \text{absorbansi kontrol media}} \times 100\%$$

#### 1. Absorbansi MCF-7 dan Nilai Viabilitas Sel pada 48 jam setelah pemberian ekstrak

NO	perlakuan	R	absorban MCF Pepaya	absorban MCF Kelor	absorban MCF 3:1	absorban MCF 1:1	absorban MCF 1:3	kontrol media
		1	1.717	1.717	1.717	1.717	1.717	0.3119
1	negatif	2	1.5866	1.5866	1.5866	1.5866	1.5866	0.2972
		Total						
		rata	1.6518	1.6518	1.6518	1.6518	1.6518	0.3046
		1	1.3030	1.5133	1.4530	1.3642	1.5263	
2	20 ppm	2	1.3047	1.9323	1.3636	1.5860	1.4845	
		Total						
		rata	1.30385	1.7228	1.4083	1.4751	1.5054	
		1	1.2934	1.5367	1.2983	1.5020	1.2854	
3	40 ppm	2	1.2275	1.4623	1.3106	1.4157	1.6231	
		Total						
		rata	1.2605	1.4995	1.3045	1.4589	1.4543	
		1	1.1564	1.5368	1.3275	1.3313	1.4215	
4	80 ppm	2	1.2270	1.3475	1.4251	1.4622	1.3816	
		Total						
		rata	1.1917	1.4422	1.3763	1.3968	1.4016	

		1	0.9593	1.4588	1.3280	1.3488	1.3002
5	160 ppm	2	0.9481	1.3806	1.3283	1.4078	1.3342
		Total					
		rata	0.9537	1.4197	1.3282	1.3783	1.3172

Konsentrasi	Rata- Rata Perlakuan MCF7				
	Pepaya	Kelor	3;1	1;1	1;3
0 ppm	1.6518	1.6518	1.6518	1.6518	1.6518
20 ppm	1.3039	1.7228	1.4083	1.4751	1.5054
40 ppm	1.2605	1.4995	1.3045	1.4589	1.4543
80 ppm	1.1917	1.4422	1.3763	1.3968	1.4016
160 ppm	0.9537	1.4197	1.3282	1.3783	1.3172

Konsentrasi	Persen sel yang hidup tiap Perlakuan MCF7				
	Pepaya	Kelor	3;1	1;1	1;3
0 ppm	100	100	100	100	100
20 ppm	74	105	82	87	89
40 ppm	71	89	74	86	85
80 ppm	66	84	80	81	81
160 ppm	48	83	76	80	75

**2. Absorbansi Vero dan Nilai Viabilitas Sel pada 48 jam setelah pemberian ekstrak**

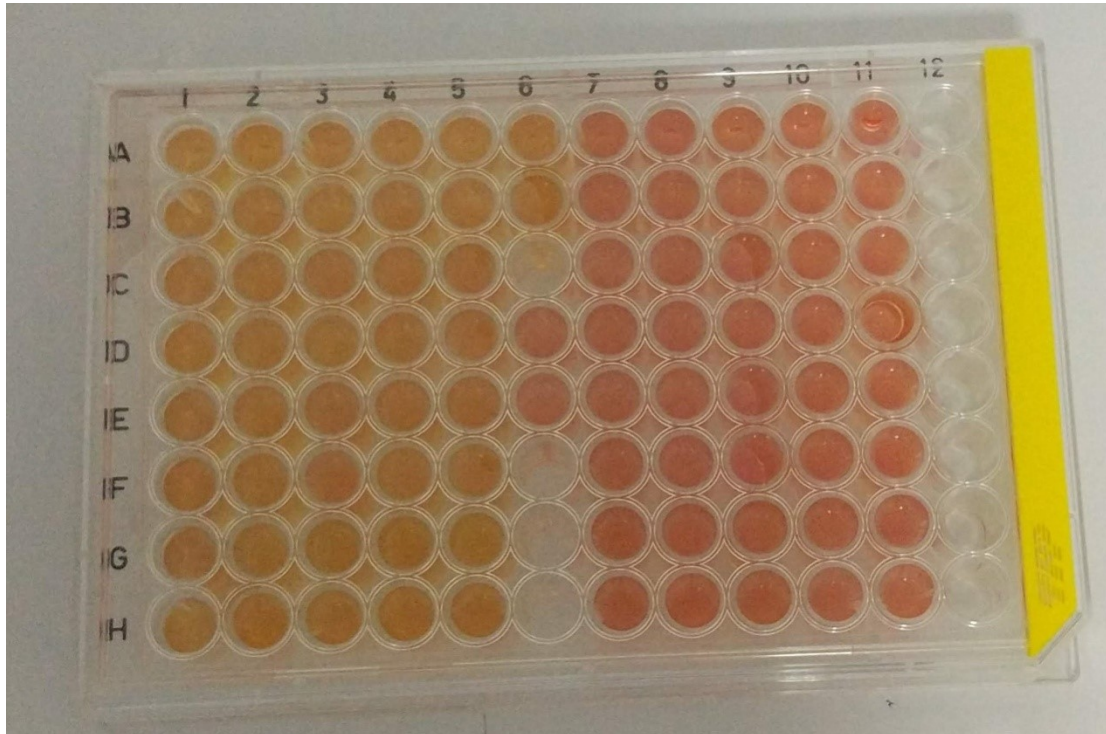
NO	perlakuan	R	absorban Vero Pepaya	absorban Vero Kelor	absorban Vero 3:1	absorban Vero 1:1	absorban Vero 1:3	kontrol media
		1	0.8168	0.8168	0.8168	0.8168	0.8168	0.3119
1	negatif	2	0.7138	0.7138	0.7138	0.7138	0.7138	0.2972
		Total						
		rata	0.7653	0.7653	0.7653	0.7653	0.7653	0.3046
		1	1.5064	1.0396	1.1926	1.1134	0.939	
2	20 ppm	2	1.2039	1.2424	1.0754	1.2573	1.1855	
		Total						
		rata	1.35515	1.141	1.134	1.18535	1.06225	
		1	1.0753	1.0106	1.1475	1.1143	1.2584	
3	40 ppm	2	0.9742	0.9005	1.0458	1.0669	1.2199	
		Total						
		rata	1.0248	0.9556	1.0967	1.0906	1.2392	
		1	1.0692	0.9147	0.8522	1.0725	1.1259	
4	80 ppm	2	1.1048	0.7695	0.9007	0.9199	1.2383	
		Total						
		rata	1.0870	0.8421	0.8765	0.9962	1.1821	
		1	1.186	0.9861	1.0296	1.0194	0.812	
5	160 ppm	2	1.0912	1.0951	1.0786	0.8149	0.8548	
		Total						
		rata	1.1386	1.0406	1.0541	0.9172	0.8334	



Konsentrasi	Rata- Rata Perlakuan Vero				
	Pepaya	Kelor	3;1	1;1	1;3
0 ppm	0.7653	0.7653	0.7653	0.7653	0.7653
20 ppm	1.3552	1.1410	1.1340	1.1854	1.0623
40 ppm	1.0248	0.9556	1.0967	1.0906	1.2392
80 ppm	1.0870	0.8421	0.8765	0.9962	1.1821
160 ppm	1.1386	1.0406	1.0541	0.9172	0.8334

Konsentrasi	Persen sel yang hidup tiap Perlakuan Vero				
	Pepaya	Kelor	3;1	1;1	1;3
0 ppm	100	100	100	100	100
20 ppm	228	182	180	191	164
40 ppm	156	141	172	171	203
80 ppm	170	117	124	150	190
160 ppm	181	160	163	133	115

### G. *Microplate-96* sumuran



Keterangan: *microplate-96* sumuran yang berisi sel dan larutan uji

1. 1 – 5 = kultur sel MCF-7
2. 7 – 11 = kultur sel vero
3. A – B = 20  $\mu\text{g/mL}$
4. C – D = 40  $\mu\text{g/mL}$
5. E – F = 80  $\mu\text{g/mL}$
6. G – H = 160  $\mu\text{g/mL}$
7. Kolom 1 dan 7 = Ekstrak Tunggal Pepaya
8. Kolom 2 dan 8 = Ekstrak Tunggal Kelor
9. Kolom 3 dan 9 = Ekstrak Kombinasi 3:1
10. Kolom 4 dan 10 = Ekstrak Kombinasi 1:1
11. Kolom 5 dan 11 = Ekstrak Kombinasi 1:3
12. Kolom 6 (A – B) = Kontrol MCF
13. Kolom 6 (D – E) = Kontrol Vero

## H. Hasil IC<sub>50</sub> menggunakan Software Graphpad Prism

### 1. Nilai IC<sub>50</sub> Kultur Sel MCF-7

data mcf jam ke 48 gabungan.pzfx:Nonlin fit of Normalize of Transform of Data 1 - GraphPad Prism (Viewer mode) 8.4.3 (686)

	A	B	C	D	E	F	G	H	I
Nonlin fit Table of results									
	pepaya	Kelor	3:1	1:1	1:3				
1	<b>log(inhibitor) vs. normalized response</b>								
2	<b>Best-fit values</b>								
3	LogIC50	1.408	1.455	0.9079	1.080	1.387			
4	IC50	25.60	28.54	8.090	12.02	24.40			
5	<b>Std. Error</b>								
6	LogIC50	0.07912	0.3385	0.1814	0.2539	0.2036			
7	<b>95% CI (asymptotic)</b>								
8	LogIC50	1.229 to 1.587	0.6897 to 2.221	0.4976 to 1.318	0.5054 to 1.654	0.9268 to 1.848			
9	IC50	16.96 to 38.66	4.895 to 166.4	3.145 to 20.81	3.202 to 45.10	8.449 to 70.48			
10	<b>Goodness of Fit</b>								
11	Degrees of Freedom	9	9	9	9	9			
12	R squared	0.9067	0.3389	0.8233	0.6454	0.6246			
13	Sum of Squares	994.2	18904	2520	6686	6463			
14	Sy.x	10.51	45.83	16.73	27.26	26.80			
15									
16	<b>Number of points</b>								
17	# of X values	10	10	10	10	10			
18	# Y values analyzed	10	10	10	10	10			
19									
20									
21									

log(inhibitor) vs. normalized response  
Least squares fit

## 2. Nilai IC<sub>50</sub> Kultur Sel Vero

data vero ke 48 gabungan.pzfx:Nonlin fit of Normalize of Transform of Data 1 - GraphPad Prism (Viewer mode) 8.4.3 (686)

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Table of results

Nonlin fit Table of results		A	B	C	D	E	F	G	H	I
		Pepaya	Kelor	3:1	1:1	1:3				
1	<b>log(inhibitor) vs. normalized response</b>									
2	<b>Best-fit values</b>									
3	LogIC50	2.042	1.901	2.239	2.044	2.098				
4	IC50	110.0	79.59	173.4	110.6	125.2				
5	<b>Std. Error</b>									
6	LogIC50	0.3863	0.4043	0.4391	0.3736	0.4148				
7	<b>95% CI (asymptotic)</b>									
8	LogIC50	1.168 to 2.916	0.9864 to 2.815	1.246 to 3.232	1.199 to 2.889	1.159 to 3.036				
9	IC50	14.71 to 823.2	9.691 to 653.6	17.62 to 1708	15.80 to 774.5	14.43 to 1087				
10	<b>Goodness of Fit</b>									
11	Degrees of Freedom	9	9	9	9	9				
12	R squared	-1.024	-0.7965	-0.6099	-0.5759	-0.5405				
13	Sum of Squares	24638	29191	25901	23004	27107				
14	Sy.x	52.32	56.95	53.65	50.56	54.88				
15										
16	<b>Number of points</b>									
17	# of X values	10	10	10	10	10				
18	# Y values analyzed	10	10	10	10	10				
19										
20										
21										

log(inhibitor) vs. normalized response  
Least squares fit

Activate Windows  
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Nonlin fit of Normalize of Transf Table of results

9:16 AM  
26-Jan-21

