

BAB V

KESIMPULAN DAN SARAN

A. Kesimpulan

1. Ekstrak etanol rimpang *Curcuma zedoaria* mempunyai aktivitas sitotoksik terhadap sel kanker payudara T47D berupa sitotoksik moderat dengan nilai IC_{50} sebesar 107 $\mu\text{g/ml}$
2. Nilai indeks selektivitas ekstrak etanol rimpang *Curcuma zedoaria* terhadap sel Vero dibandingkan dengan sel kanker payudara T47D sebesar 4.21 >3 selektif
3. Ekstrak etanol *Curcuma zedoaria* mampu mengekspresikan jumlah protein 53 pada kultur sel T47D

B. Saran

1. Perlu dilakukan penelitian lebih lanjut ke senyawa murni atau isolat dari rimpang temu putih agar nilai sitotoksiknya lebih kecil dan diharapkan poten mampu menghambat pertumbuhan sel kanker payudara T47D
2. Perlu dilakukan uji dengan menggunakan beberapa antibodi untuk mengetahui dan membandingkan jumlah protein yang terekspresi.
3. Perlu dilakukan penelitian dengan parameter yang berbeda ke arah floctometri, penuaan sel, apoptosis dan proliferasi.

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Zampieri, L., et al. (2000) *Differential Modulation by Estradiol of P-glycoprotein Drug Resistance Protein Expression in Cultured MCF7 and T47D Breast Cancer Cells*. *Anticancer Res.*

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Lampiran 1. Hasil determinasi ekstrak etanol rimpang temu putih



KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

BADAN PENELITIAN DAN PENGEMBANGAN KESEHATAN

BALAI BESAR PENELITIAN DAN PENGEMBANGAN

TANAMAN OBAT DAN OBAT TRADISIONAL

Jalan Raya Lawu No. 11 Tawangmangu, Karanganyar, Jawa Tengah 57792

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Nomor : YK.01.03/2/ 1883 /2019 22 Mei 2019
Hal : Keterangan Determinasi

Yth. Dekan Fakultas Farmasi
Universitas Setia Budi
Jalan Let. Jend. Sutoyo
Solo

Merujuk surat Saudara nomor: 4247/A10 – 4/21.12.2018 tanggal 21 Desember 2018 hal permohonan determinasi, dengan ini kami sampaikan bahwa hasil determinasi sampel tanaman sebagai berikut:

Nama Sampel : Temu Putih
Sampel : Sampel segar
Spesies : *Curcuma zedoaria* (Christm.) Roscoe
Sinonim : *Amomum zedoaria* Christm., *Curcuma pallida* Lour.
Familia : Zingiberaceae
Nama Pemohon : Ana Maria Ulfa
Penanggung Jawab Identifikasi : Anshary Maruzy, S.Si.

Hasil determinasi tersebut hanya mencakup sampel tumbuhan yang telah dikirimkan ke B2P2TOOT.


Atas perhatian Saudara, kami sampaikan terima kasih.

Plh. Kepala Balai Besar Penelitian
dan Pengembangan Tanaman
Obat dan Obat Tradisional,




Nita Supriyati, M. Biotech, Apt
NIP-197811152002122001

Lampiran 2. Etical clearent



HEALTH RESEARCH ETHICS COMMITTEE
KOMISI ETIK PENELITIAN KESEHATAN
Dr. Moewardi General Hospital
RSUD Dr. Moewardi

School of Medicine Sebelas Maret University
Fakultas Kedokteran Universitas sebelas Maret



ETHICAL CLEARANCE
KELAIKAN ETIK

Nomor : 209 / II /HREC / 2019

The Health Research Ethics Committee Dr. Moewardi General Hospital / School of Medicine Sebelas Maret
 Komisi Etik Penelitian Kesehatan RSUD Dr. Moewardi / Fakultas Kedokteran Universitas Sebelas Maret

Maret University Of Surakarta, after reviewing the proposal design, herewith to certify
 Surakarta, setelah menilai rancangan penelitian yang diusulkan, dengan ini menyatakan

That the research proposal with topic :
 Bahwa usulan penelitian dengan judul

uji aktivitas sitotoksik ekstrak etanol rimpang temu putih (curcuma zedoaria) dan pengaruhnya terhadap jumlah protein 53 pada kultur sel T47D

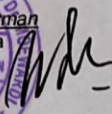
Principal investigator : Ana Maria Ulfa
 Peneliti Utama : 21154487A

Location of research : Laboratorium Parasitologi Universitas Gadjah Mada Yogyakarta
 Lokasi Tempat Penelitian

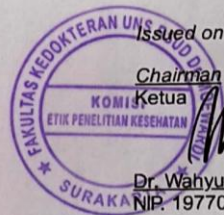
Is ethically approved
 Dinyatakan layak etik

Issued on : 19 Feb 2019



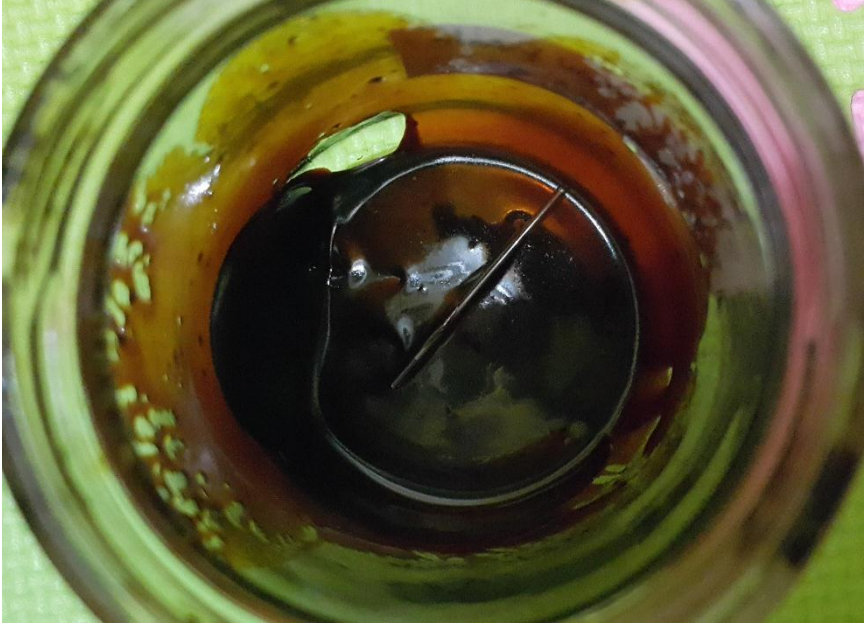
Chairman
 Ketua







Dr. Wahyu Dwi Atmoko, SpF
 NIP. 19770224 201001 1 004











Lampiran 3. Hasil simplisia dan serbuk, ekstrak etanol rimpang temu putih






Simplisia temu putih	 A photograph showing several pieces of ginger rhizome (temu putih) that have been sliced into thin, yellowish, circular slices. The slices are piled together, showing their fibrous texture and light brown color.
Serbuk rimpang temu putih	 A photograph of a pile of fine, light brown powder (serbuk) spread out on a white surface. The powder has a granular, slightly clumpy appearance.
Ekstrak etanol rimpang temu putih	 A photograph looking down into a glass jar containing a dark brown, viscous liquid (ekstrak etanol). A small metal spoon is resting inside the jar, partially submerged in the liquid. The jar has a pink lid.


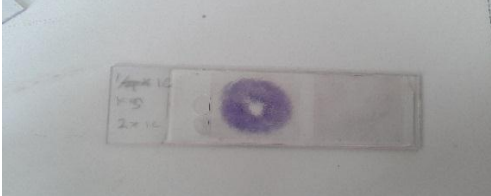
Lampiran 4. Alat yang digunakan waktu praktikum

No	Nama	Gambar
1.	Timbangan analitik	 A photograph of an analytical balance scale. It features a stainless steel weighing pan on a central column, enclosed in a clear glass draft shield. The base is a light-colored metal with a digital display and control buttons.
2.	Evaporator	 A photograph of a rotary evaporator in a laboratory setting. It consists of a rotating glass flask held by a motor, connected to a condenser and a collection flask. The entire assembly is mounted on a base with a control panel.
3.	Autoclaf	 A photograph of a laboratory autoclave. It is a white, rectangular unit with a green lid and a control panel on top. The brand name 'Heraeus' is visible on the front.
4.	Micro pipet	 A photograph of a single-channel micro pipette. It has a blue body with a white plunger and a white tip. The brand name 'Accumax' is printed on the side.




No	Nama	Gambar
5.	Elisa Reader	
6.	Vortek	
7.	Incubator CO ₂	
8.	Laminar Air Flow	

No	Nama	Gambar
9.	Microskop Inverted	 A photograph of an inverted microscope on a laboratory bench. The microscope is white and black, with a large objective lens at the bottom. It is surrounded by various lab supplies like a blue folder and a stack of papers.
10.	Well Plate 96	 A photograph of a clear plastic 96-well microplate. The plate is filled with a clear liquid, and the individual wells are visible in a grid pattern.
11.	Well Plate 6	 A photograph of a clear plastic 6-well microplate. The wells are labeled with handwritten green ink: 'Y1', 'Y2', 'Y3', 'Y4', 'Y5', and 'Y6'. The plate contains a clear liquid.
12.	Filter	 A photograph of a laboratory setup for filtering. A glass bottle is placed on a metal surface, with a red liquid being poured through a filter into it. A petri dish with red agar is also visible in the foreground.




No	Nama	Gambar
13.	Clonical tube	
14.	Ependrof	
15.	Counter	
16.	Haemositometer	
17.	Aliquot	

No	Nama	Gambar
18.	Microtube	
19.	Cover Slipe	

Lampiran 5. Penetapan Kadar air serbuk rimpang temu putih

No	Nama	Gambar
1.	Kadar air 1	
2.	Kadar air 2	
3.	Kadar air 3	



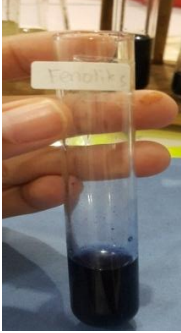
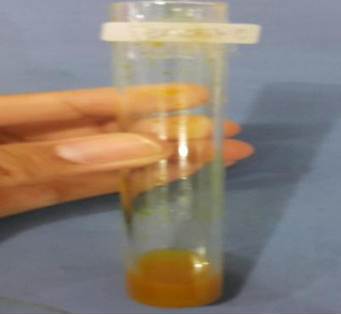
Lampiran 6. Penetapan Susut pengeringan serbuk rimpang temu putih



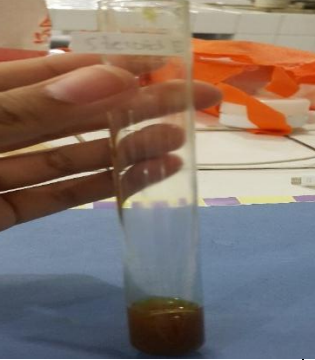

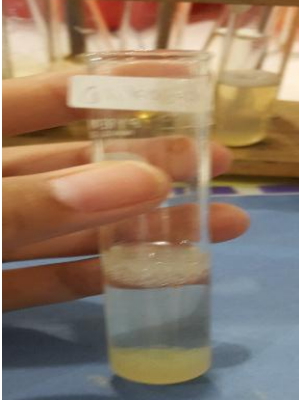

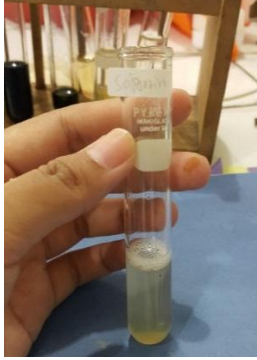
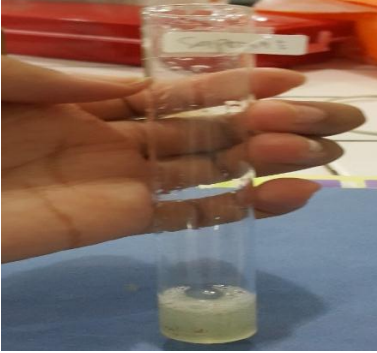
No	Nama	Gambar
1.	Susut kering 1	 A digital scale with a white top and a control panel. The display shows 7.5% moisture content, 105, and 14:41. The scale is labeled 'OHRAUS' and 'MS 23'. A yellow warning triangle with 'SSS' is visible on the top.
2.	Susut kering 2	 A digital scale with a white top and a control panel. The display shows 9.5% moisture content, 105, and 07:58. The scale is labeled 'OHRAUS' and 'MS 23'. A yellow warning triangle with 'SSS' is visible on the top.
3.	Susut kering 3	 A digital scale with a white top and a control panel. The display shows 9.2% moisture content, 105, and 09:30. The scale is labeled 'OHRAUS' and 'MS 23'. A yellow warning triangle with 'SSS' is visible on the top.



Lampiran 7. Uji bebas etanol



Lampiran 8. Identifikasi senyawa yang terkandung dalam rimpang temu putih

Identifikasi	Serbuk	Ekstrak
1. Flavonoid		
2. Fenolik		

Identifikasi	Serbuk	Ekstrak
3. Tanin		
4. Steroid		
5. Glikosida		
6. Saponin		

Identifikasi	Serbuk	Ekstrak
7.		

Lampiran9 . Pembuatan media DMEM

DMEM	1 sachet
NaH ₂ CO ₃	3.7 gram
Hepes.....	2 gram
Aquabidest.....	ad 1000 ml

Lampiran 10. Pembuatan larutan dapar phosfat PBS

NaCl	8 gram
KCl	0.2 gram
KH ₂ PO ₄	0.2 gram
Na ₂ HPO ₄	1.15 gram
Aquabides	ad 1000ml

Lampiran 11. Pembuatan larutan MTT assay

Stok MTT	1ml
Media kultur (DMEM)	10 ml

Lampiran 12. Pembuatan larutan stopper

HCL.....0.01 N

SDS.10% Ad 100 ml

Lampiran 13. Perhitungan rimpang kering terhadap rimpang basah

Rendemen berat rimpang kering terhadap rimpang basah

$$\text{Rendemen (\% } \frac{b}{b} \text{)} = \frac{\text{Berat kering}}{\text{Berat basah}} \times 100\%$$

$$\text{Rendemen (\% } \frac{b}{b} \text{)} = \frac{340 \text{ g}}{3500 \text{ g}} \times 100\% = 9.7 \%$$

Perhitungan Lost On Drying (LOD) pengeringan rimpang temu putih basah

$$\text{Rendemen (\%)} = \frac{\text{Berat basah} - \text{Berat kering}}{\text{Berat basah}} \times 100\%$$

$$\text{Rendemen (\%)} = \frac{3500 \text{ g} - 340 \text{ g}}{3500 \text{ g}} \times 100\% = 90.28 \%$$

Lampiran 14. Perhitungan rendemen hasil ekstrak rimpang temu putih

Rendemen hasil ekstrak etanol rimpang temu putih

$$\text{Rendemen (\%)} = \frac{\text{Berat ekstrak}}{\text{Berat serbuk}} \times 100\%$$

$$\text{Rendemen (\%)} = \frac{29 \text{ g}}{250 \text{ g}} \times 100\% = 11.6 \%$$

Lampiran 15. Perhitungan kadar air

$$\text{Rendemen (\%)} = \frac{\text{Berat air yang didapat ml}}{\text{berat ekstrak yang didapat gram}} \times 100\%$$

$$= \frac{1.1 \text{ ml}}{10 \text{ g}} \times 100\% = 11 \%$$

$$\text{Rendemen (\%)} = \frac{\text{Berat air yang didapat ml}}{\text{berat ekstrak yang didapat gram}} \times 100\%$$

$$= \frac{1.2 \text{ ml}}{10 \text{ g}} \times 100\% = 12 \%$$

$$\text{Rendemen (\%)} = \frac{\text{Berat air yang didapat ml}}{\text{berat ekstrak yang didapat gram}} \times 100\%$$

$$= \frac{1.1 \text{ ml}}{10 \text{ g}} \times 100\% = 11 \%$$

Lampiran 2. Perhitungan Panen sel

Uji sitotoksik

A. Kultur sel Vero

Jumlah sel terhitung $= \frac{A+B+C+D}{4} \times 10^4 = 90 \times 10^4$

Volume panen sel yang di transfer $= \frac{100}{90} = 1.1 \text{ ml ad } 10 \text{ media stok}$

B. Kultur sel T47D

Jumlah sel terhitung $= \frac{A+B+C+D}{4} \times 10^4 = 225 \times 10^4$

Volume panen sel yang di transfer $= \frac{100}{225} = 0.44 \text{ ml ad } 10 \text{ media stok}$

Uji immunositokimia

A. Kultur sel T47D

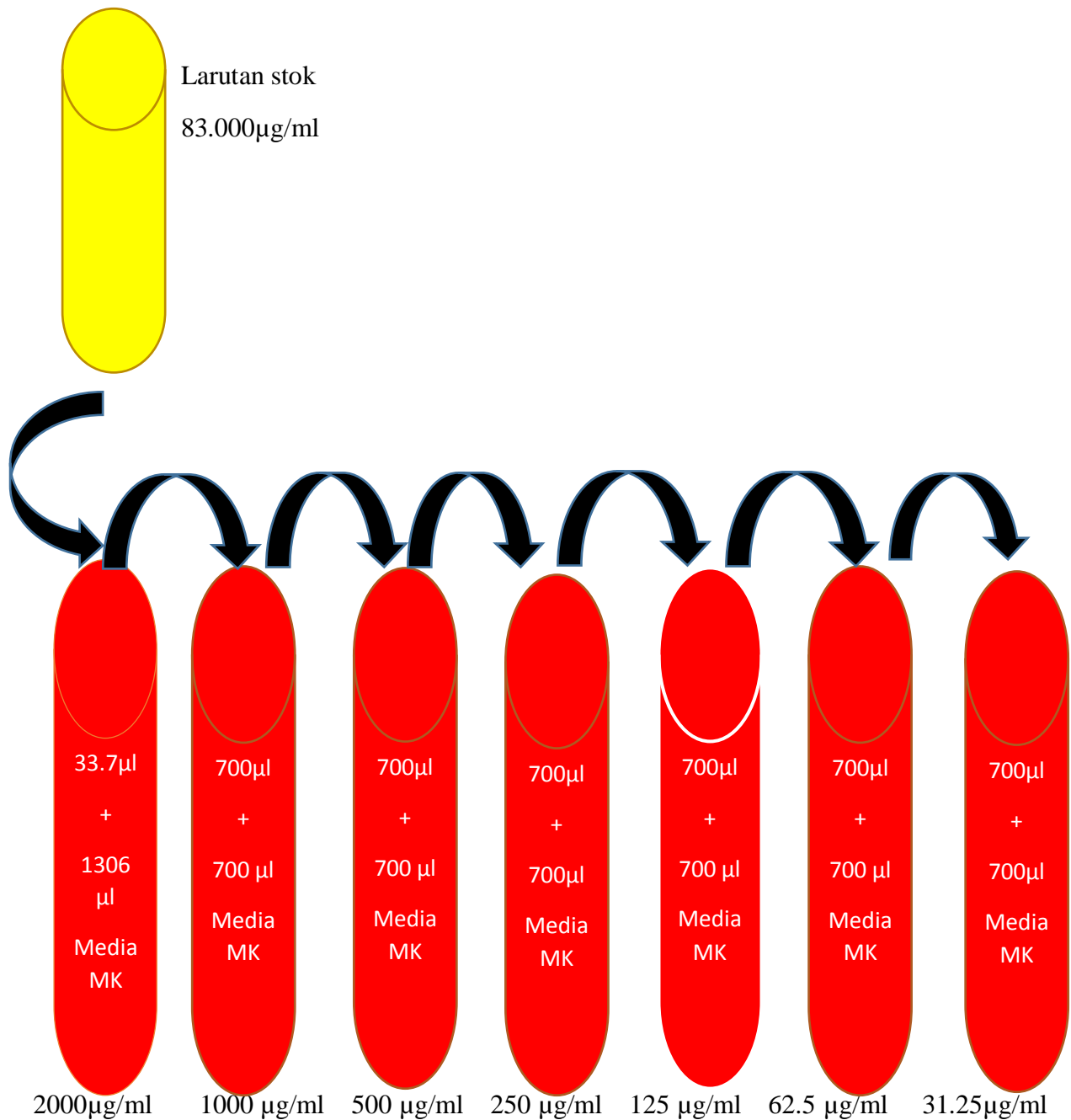
Jumlah sel terhitung $= \frac{A+B+C+D}{4} \times 10^4 = 600 \times 10^4$

Volume panen sel yang di transfer $= \frac{100}{600} = 0.166 \text{ ml ad } 10 \text{ media stok}$



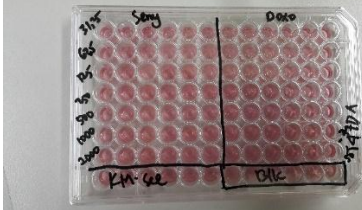
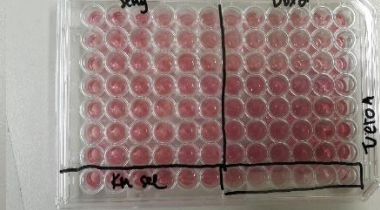






Lampiran 3. Perhitungan pembuatan larutan untuk uji sitotoksik

Uji Sitotoksik

Pembuatan larutan stok	
Konsentrasi yang dibuat = 8.3 mg/100 μ l = 8300 μ g/100 μ l =83000 μ g/ml	
Pembuatan seri konsentrasi	
1. Konsentrasi 2000 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 2000 = V_2 \times 83000$ $V_2 = 33.7 \mu$ l *) Dipipet 33.7 μ l dari larutan Stok + 1366.3 μ l media DMEM	5. Konsentrasi 125 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 250 = V_2 \times 500$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 4 dan + 700 μ l media DMEM
2. Konsentrasi 1000 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 1000 = V_2 \times 2000$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 1 dan + 700 μ l media DMEM	6. Konsentrasi 62.5 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 250 = V_2 \times 500$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 5 dan + 700 μ l media DMEM
3. Konsentrasi 500 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 500 = V_2 \times 1000$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 2 dan + 700 μ l media DMEM	6. Konsentrasi 31.25 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 250 = V_2 \times 500$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 6 dan + 700 μ l media DMEM
4. Konsentrasi 250 μ l $V_1 \times N_1 = V_2 \times N_2$ $1400 \times 250 = V_2 \times 500$ $V_2 = 700 \mu$ l *) Dipipet 700 μ l dari larutan Konsentrasi 3 dan + 700 μ l media DMEM	

Lampiran 4. Ilustrasi Pembuatan seri konsentrasi

Lampiran 5. Degradasi warna saat sebelum pemberian sampel, sesudah pemberian sampel dan sesudah MTT

NO	Keterangan	T47D	Vero
1.	Platting sel		
2.	Treatment		
3.	MTT Assay		
4.	SDS stoper		
5.	Baca Elisa reader		

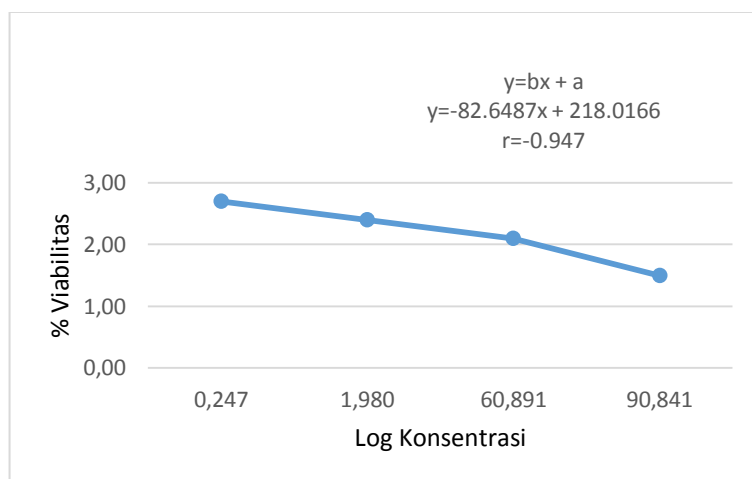
Lampiran 6. Perhitungan IC₅₀ ekstrak rimpang temu putih

A. Perhitungan IC₅₀ Ekstrak temu putih terhadap kultur sel T47D

C	LOG C	ABS	MS	MK	P	KM	% VIABILITAS
500	2,70	0,11	0,51	0,11	0,00	0,40	0,25
250	2,40	0,12	0,51	0,11	0,01	0,40	1,98
125	2,10	0,35	0,51	0,11	0,25	0,40	60,89
31,25	1,49	0,47	0,51	0,11	0,37	0,40	90,84
Rata rata							
MEDIA SEL	0,508	0,502	0,498	0,517	0,526	0,515	0,511
MEDIA KONTROL	0,102	0,102	0,104	0,112	0,112	0,113	0,1075

C	KONSENTRASI EKSTRAK RIMPANG TEMU PUTIH CURCUMA ZEDOARIA
MK	NILAI RATA RATA MEDIA KONTROL
MS	NILAI RATA RATA MEDIA SEL
P	NILAI RATA RATA ABSORBANSI DIKURANGI KONTROL MEDIA
KM	MDIA SEL DIKURANGI KONTROL MEDIA
% VIABILITAS	P/KM*100

r	-0,94712
a	218,0166
b	-82,6487
	50
antilog	2,0329
ic 50	107,8699
Y=bx+a	

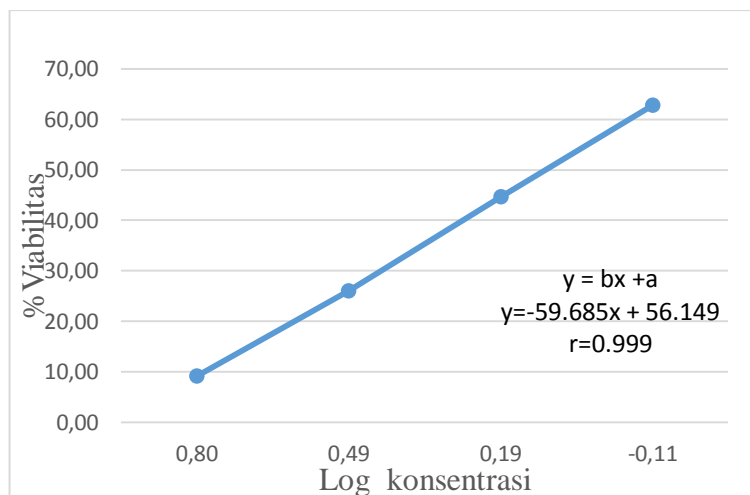


B. Perhitungan IC₅₀ Doxorubicin terhadap kultur sel T47D

C	LOG C	ABS	MS	MK	P	KM	%VIABILITAS	
6,3	0,8	0,1	0,5	0,1	0,0	0,4	9,1	
3,1	0,5	0,2	0,5	0,1	0,1	0,4	26,0	
1,6	0,2	0,3	0,5	0,1	0,2	0,4	44,6	
0,8	-0,1	0,4	0,5	0,1	0,3	0,4	62,8	
MEDIA SEL		0,508	0,502	0,498	0,517	0,526	0,515	0,511
MEDIA KONTROL		0,102	0,102	0,104	0,112	0,112	0,113	0,108
Rata rata								

C	KONSENTRASI EKSTRAK RIMPANG TEMU PUTIH CURCUMA ZEDOARIA
MK	NILAI RATA RATA MEDIA KONTROL
MS	NILAI RATA RATA MEDIA SEL
P	NILAI RATA RATA ABSORBANSI DIKURANGI KONTROL MEDIA
KM	MDIA SEL DIKURANGI KONTROL MEDIA
% VIABILITAS	P/KM*100

R	-0,99981
A	56,14982
B	-59,6851
	50
antilog	IC =1,25
Y=bx+a	



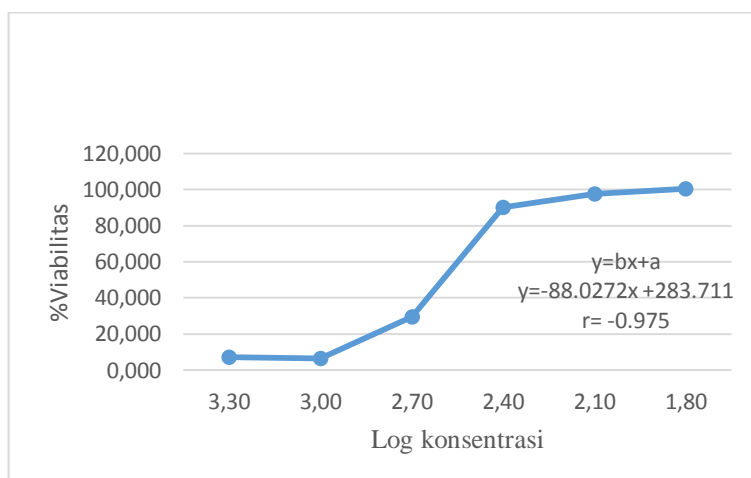
C. Perhitungan IC₅₀ Ekstrak temu putih terhadap kultur sel Vero

C	LOG C	ABS	MS	MK	P	KM	% VIABILITAS
500	2,7	0,262	0,669	0,093	0,169	0,576	29,369
250	2,4	0,613	0,669	0,093	0,520	0,576	90,278
125	2,1	0,655	0,669	0,093	0,562	0,576	97,598
62,5	1,8	0,671	0,669	0,093	0,578	0,576	100,405
31,25	1,5	0,607	0,669	0,093	0,514	0,576	89,178

MEDIA SEL	0,67	0,639	0,656	0,674	0,662	0,712	0,669
MEDIA KONTROL	0,103	0,104	0,096	0,093	0,084	0,077	0,093
Rata rata							

C	KONSENTRASI EKSTRAK RIMPANG TEMU PUTIH CURCUMA ZEDOARIA
MK	NILAI RATA RATA MEDIA KONTROL
MS	NILAI RATA RATA MEDIA SEL
P	NILAI RATA RATA ABSORBANSI DIKURANGI KONTROL MEDIA
KM	MDIA SEL DIKURANGI KONTROL MEDIA
% VIABILITAS	P/KM*100

r	-0,93752
a	283,7114
b	-88,0272
	50
	2,654989
Antilog	451,8445
Y=bx+a	

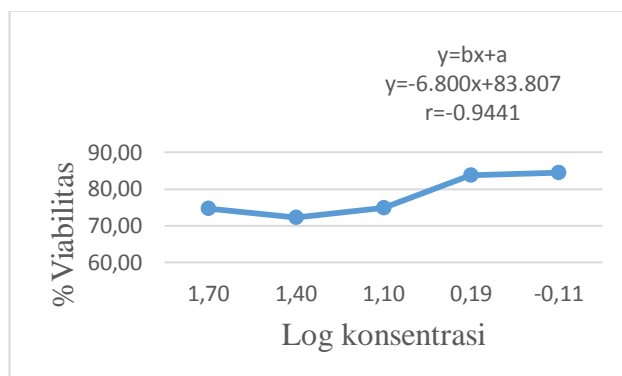


D. Perhitungan IC₅₀ Doxorubicin terhadap kultur sel Vero

C	LOG C	ABS	MS	MK	P	KM	%VIABILITAS
50	1,699	0,523	0,669	0,093	0,430	0,576	74,711
25	1,398	0,510	0,669	0,093	0,417	0,576	72,309
12,5	1,097	0,524	0,669	0,093	0,431	0,576	74,855
1,562	0,194	0,575	0,669	0,093	0,482	0,576	83,681
0,781	-0,107	0,579	0,669	0,093	0,486	0,576	84,375

MEDIA SEL	0,67	0,639	0,656	0,674	0,662	0,712	0,669
MEDIA KONTROL	0,103	0,104	0,096	0,093	0,084	0,077	0,093
Rata rata							

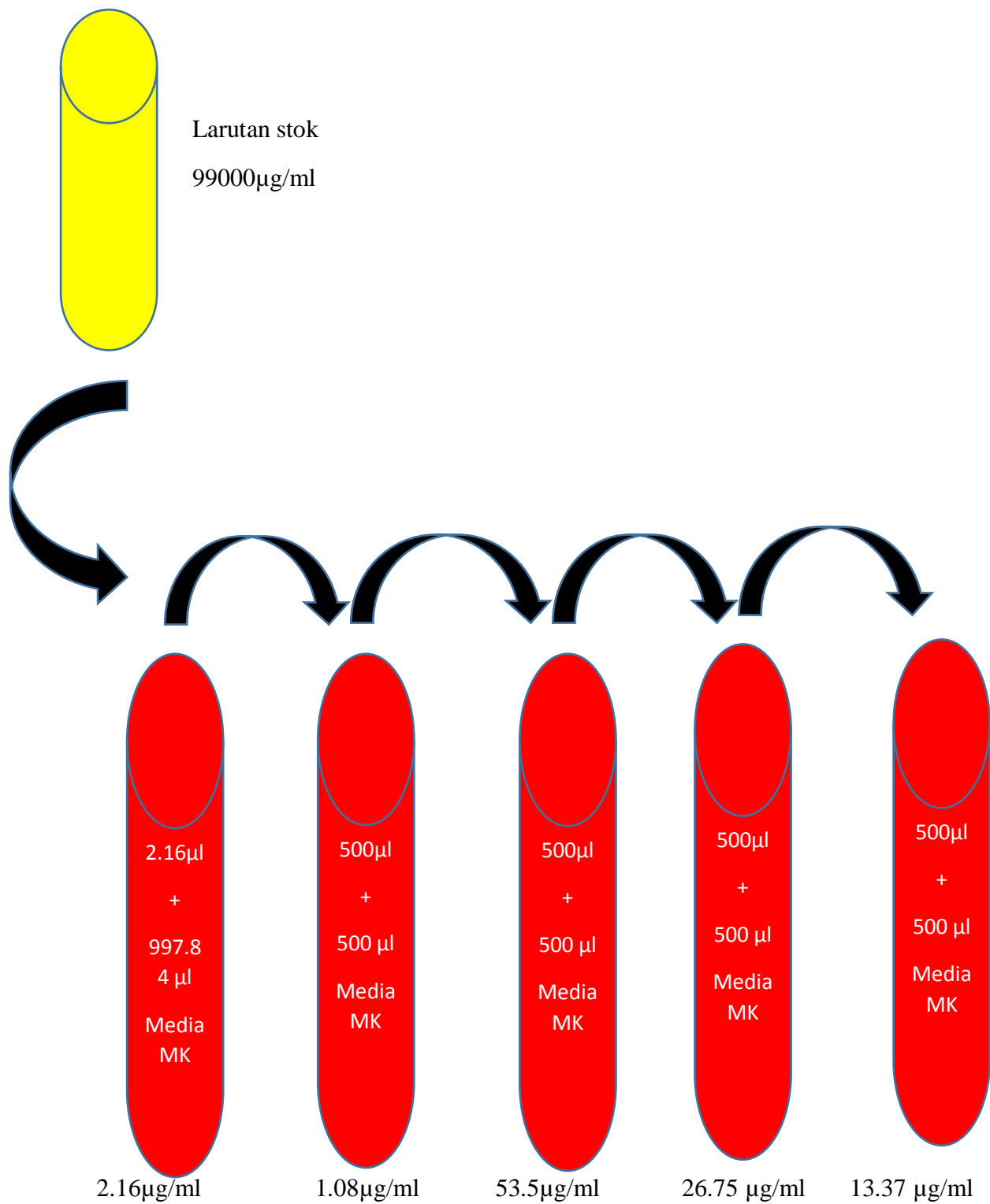
C	KONSENTRASI EKSTRAK RIMPANG TEMU PUTIH CURCUMA ZEDOARIA
MK	NILAI RATA RATA MEDIA KONTROL
MS	NILAI RATA RATA MEDIA SEL
P	NILAI RATA RATA ABSORBANSI DIKURANGI KONTROL MEDIA
KM	MEDIA SEL DIKURANGI KONTROL MEDIA
% VIABILITAS	$P/KM*100$
R	-0,94418
A	83,80739
B	-6,80032
50	4,971443
ANTILOG	93635,99



Lampiran 7. Perhitungan pembuatan larutan untuk uji Imunositokimia

Uji Imunositokimia

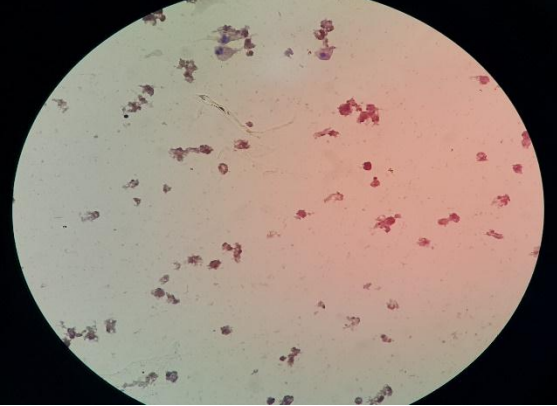

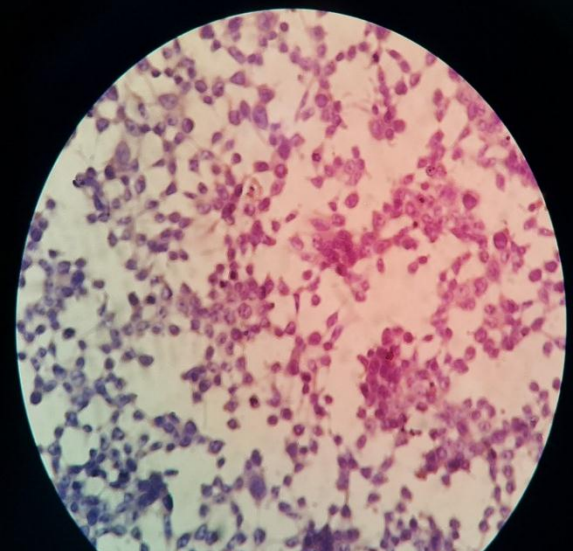
Pembuatan larutan stok	
Konsentrasi yang dibuat = 9.9 mg/100 μ l = 9900 μ g/100 μ l =99000 μ g/ml	
Pembuatan seri konsentrasi	
1.	Konsentrasi 2 x IC ₅₀ 214 μ l $V_1 \times N_1 = V_2 \times N_2$ $1000 \times 214 = V_2 \times 99000$ $V_2 = 2.16 \mu\text{l}$ *) Dipipet 2.16 μ l dari larutan Stok + 997.84 μ l media DMEM
2.	Konsentrasi 1 x IC ₅₀ 107 μ l $V_1 \times N_1 = V_2 \times N_2$ $1000 \times 107 = V_2 \times 214$ $V_2 = 500 \mu\text{l}$ *) Dipipet 500 μ l dari larutan Konsentrasi 1 dan + 500 μ l media DMEM
3.	Konsentrasi 1/2 x IC ₅₀ 53.5 μ l $V_1 \times N_1 = V_2 \times N_2$ $1000 \times 53.5 = V_2 \times 107$ $V_2 = 500 \mu\text{l}$ *) Dipipet 500 μ l dari larutan Konsentrasi 2 dan + 500 μ l media DMEM
4.	Konsentrasi 1/4 x IC ₅₀ 26.75 μ l $V_1 \times N_1 = V_2 \times N_2$ $1000 \times 26.75 = V_2 \times 53.5$ $V_2 = 500 \mu\text{l}$ *) Dipipet 500 μ l dari larutan Konsentrasi 3 dan + 500 μ l media DMEM
5.	Konsentrasi 1/8 x IC ₅₀ 13.37 μ l $V_1 \times N_1 = V_2 \times N_2$ $1000 \times 13.37 = V_2 \times 26.75$ $V_2 = 500 \mu\text{l}$ *) Dipipet 500 μ l dari larutan Konsentrasi d4an + 500 μ l media DMEM

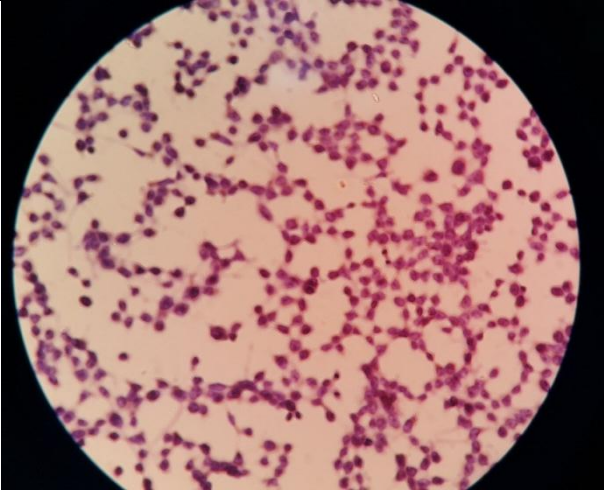
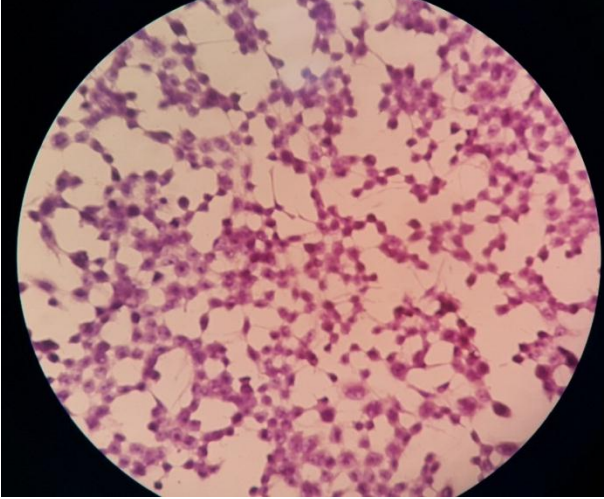
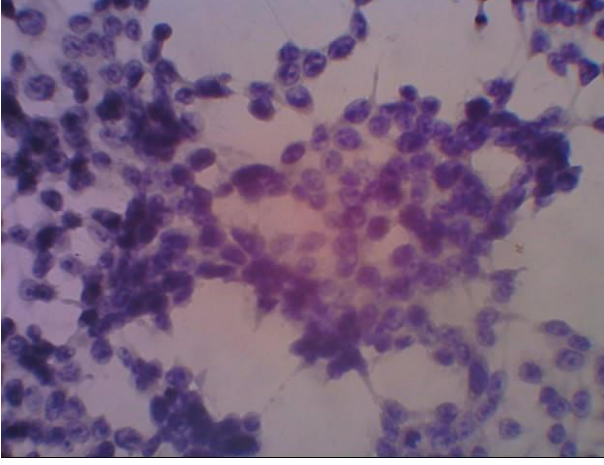
Lampiran 8. Ilustrasi pembuatan seri konsentrasi

Lampiran 9. Langkah pengujian Imunositokimia

No	Nama	gambar
1.	Plating	
2.	Tratmen	
3.	ICC	

Lampiran 10. Perhitungan jumlah protein yang mengekspresikan antibodi P53

No	Nama	Gambar
1.	2 X IC ₅₀	
2.	1 X IC ₅₀	
3.	1/2 X IC ₅₀	

No	Nama	Gambar
4.	1/4 X IC ₅₀	
5.	1/8 X IC	
6.	Kontrol sel	

Lampiran 11. Hasil perhitungan jumlah protein 53

No	Konsentrasi	Jumlah warna coklat	Jumlah warna ungu	%
1.	2XIC ₅₀	6	6	100
2.	1XIC ₅₀	14	14	100
3.	1/2XIC ₅₀	148	424	35
4.	1/4XIC ₅₀	272	405	67
5.	1/8XIC ₅₀	389	418	93
6.	KS	0	437	0