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LAMPIRAN

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SURAT PERSETUJUAN

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Menyetujui mahasiswa tersebut dibawah ini :

N a m a	: **Sri Anggarini Rasyid**
Nomor Pokok	: C013191014
Program Studi	: Doktor Ilmu Kedokteran

Bermaksud melakukan penelitian dengan Judul :
Peranan Ekstrak dan Hidrolisat Protein Kerang Pokea (*Batissa violaceae celebensis* Martens 1897) sebagai Imunostimulan dan Anti Proliferasi Kanker secara *in Vitro* dan *in Vivo* pada Mencit BALB/c

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REKOMENDASI PERSETUJUAN ETIK

Nomor : 820/UN4.6.4.5.31/ PP36/ 2020

Tanggal: 23 Desember 2020

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH20110627	No Sponsor Protokol	
Peneliti Utama	**Sri Anggarini Rasyid,S.Si,MSi**	Sponsor	
Judul Peneliti	Potensi Kerang Pokea (Batissa Violaceae Celebensis Marten 1897) Sebagai Imunostimulan Dan Anti Proliferasi Sel Kanker Secara In Vitro Dan In Vivo		
No Versi Protokol	2	Tanggal Versi	15 Desember 2020
No Versi PSP		Tanggal Versi	
Tempat Penelitian	**RS Universitas Hasanuddin Makassar, Balai Besar Veteriner Maros, Laboratorium Biofarmaka dan Biofarmasi Prodi Farmasi STIKES Mandala Waluya Kendari**		
Jenis Review	[ ] Exempted [x] Expedited [ ] Fullboard Tanggal	Masa Berlaku **23 Desember 2020** sampai **23 Desember 2021**	Frekuensi review lanjutan
Ketua Komisi Etik Penelitian Kesehatan FKUH	Nama **Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)**	Tanda tangan	
Sekretaris Komisi Etik Penelitian Kesehatan FKUH	Nama **dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)**	Tanda tangan	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Lapor SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan

Analisis kadar flavonoid dan Fenol

Tests of Between-Subjects Effects

Dependent Variable: Absorbansi Flavanoid

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.011[a]	2	0.006	6.129	0.035
Intercept	0.242	1	0.242	263.336	0.000
Perlakuan	0.011	2	0.006	6.129	0.035
Error	0.006	6	0.001		
Total	0.259	9			
Corrected Total	0.017	8			

a. R Squared = .671 (Adjusted R Squared = .562)

Absorbansi Flavanoid

Duncan[a,b]

Perlakuan	N	Subset 1	Subset 2	Duncan Group
Fn_heksana	3	0.1203		b
EEtOH	3	0.1647	0.1647	ab
FEtOAc	3		0.2070	a
Sig.		0.124	0.138	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Absorbansi Fenol

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.129[a]	2	0.064	21.563	0.002
Intercept	1.106	1	1.106	370.851	0.000
Perlakuan	0.129	2	0.064	21.563	0.002
Error	0.018	6	0.003		
Total	1.253	9			
Corrected Total	0.147	8			

a. R Squared = .878 (Adjusted R Squared = .837)

Absorbansi Fenol

Duncan[a,b]

Perlakuan	N	Subset 1	Subset 2	Duncan Group
Fn_heksana	3	0.2333		b
EEtOH	3	0.3037		b
FEtOAc	3		0.5147	a
Sig.		0.166	1.000	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .003.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Uji Limfosit

Tests of Between-Subjects Effects

Dependent Variable: Ekstrak_Etanol_Limfosit

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.004[a]	4	0.001	3.718	0.042
Intercept	8.406	1	8.406	33714.022	0.000
Konsentrasi	0.004	4	0.001	3.718	0.042
Error	0.002	10	0.000		
Total	8.412	15			
Corrected Total	0.006	14			

a. R Squared = .598 (Adjusted R Squared = .437)

Ekstrak_Etanol_Limfosit

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Duncan
K10	3	0.0913		b
K500	3	0.0913		b
K100	3	0.0923		b
Kontrol	3	0.1173	0.1173	ab
K1000	3		0.1797	a
Sig.		0.409	0.051	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 0.001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Etil_Asetat_Limfosit

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.011[a]	4	0.003	38.035	0.000
Intercept	0.148	1	0.148	2123.679	0.000
Konsentrasi	0.011	4	0.003	38.035	0.000
Error	0.001	10	6.960E-5		
Total	0.159	15			
Corrected Total	0.011	14			

a. R Squared = .938 (Adjusted R Squared = .914)

Etil_Asetat_Limfosit

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan
K10	3	0.0763			c
K500	3	0.0777			c
K100	3	0.0820			c
Kontrol	3		0.1173		b
K1000	3			0.1430	a
Sig.		0.446	1.000	1.000	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 6.960E-5.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = .05.

Tests of Between-Subjects Effects

Dependent Variable: N-Hexana_Limfosit

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.015[a]	4	0.004	51.680	0.000
Intercept	0.098	1	0.098	1327.168	0.000
Konsentrasi	0.015	4	0.004	51.680	0.000
Error	0.001	10	7.367E-5		
Total	0.114	15			
Corrected Total	0.016	14			

a. R Squared = .954 (Adjusted R Squared = .935)

N-HexanaLimfosit

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan
K10	3	0.0453			c
K100	3	0.0527			c
K500	3		0.0683		b
Kontrol	3			0.1173	a
K1000	3			0.1200	a
Sig.		0.320	1.000	0.712	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.367E-5.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: N-Hexana_MCF7

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.005[a]	4	0.001	1.352	0.317
Intercept	3.598	1	3.598	4028.646	0.000
Konsentrasi	0.005	4	0.001	1.352	0.317
Error	0.009	10	0.001		
Total	3.611	15			
Corrected Total	0.014	14			

a. R Squared = 0.351 (Adjusted R Squared = 0.091)

Tests of Between-Subjects Effects

Dependent Variable: Etanol_A549

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.023[a]	4	0.006	3.426	0.052
Intercept	2.893	1	2.893	1736.625	0.000
Konsentrasi	0.023	4	0.006	3.426	0.052
Error	0.017	10	0.002		
Total	2.933	15			
Corrected Total	0.039	14			

a. R Squared = 0.578 (Adjusted R Squared = 0.409)

Tests of Between-Subjects Effects

Dependent Variable: N-Hexana_Hepatoma

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.109[a]	4	0.027	4.436	0.026
Intercept	3.804	1	3.804	620.796	0.000
Konsentrasi	0.109	4	0.027	4.436	0.026
Error	0.061	10	0.006		
Total	3.974	15			
Corrected Total	0.170	14			

a. R Squared = 0.640 (Adjusted R Squared = 0.495)

N-Hexana_Hepatoma

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan
K1000	3	0.3757			c
K500	3	0.4460	0.4460		bc
Kontrol	3		0.5310	0.5310	ab
K100	3		0.5420	0.5420	ab
K10	3			0.6233	a
Sig.		0.297	0.182	0.198	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 0.006.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: Etil_Asetat_A549

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.030[a]	4	0.007	16.352	0.000
Intercept	2.838	1	2.838	6285.153	0.000
Konsentrasi	0.030	4	0.007	16.352	0.000
Error	0.005	10	0.000		
Total	2.872	15			
Corrected Total	0.034	14			

a. R Squared = 0.867 (Adjusted R Squared = 0.814)

Etil_Asetat_A549

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan
K1000	3	0.3720			c
K500	3	0.4040	0.4040		bc
K100	3		0.4310		b
Kontrol	3			0.4770	a
K10	3			0.4910	a
Sig.		0.095	0.151	0.439	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 0.000.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Tests of Between-Subjects Effects

Dependent Variable: N-Hexana_A549

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.013[a]	4	0.003	3.475	0.050
Intercept	2.849	1	2.849	3002.977	0.000
Konsentrasi	0.013	4	0.003	3.475	0.050
Error	0.009	10	0.001		
Total	2.871	15			
Corrected Total	0.023	14			

a. R Squared = 0.582 (Adjusted R Squared = 0.414)

N-Hexana_A549

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Duncan
K1000	3	0.3963		b
K500	3	0.4193	0.4193	ab
K100	3	0.4237	0.4237	ab
K10	3		0.4627	a
Kontrol	3		0.4770	a
Sig.		0.324	0.058	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 0.001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

DATA SEL WiDr

Etanol (ppm)

Tests of Between-Subjects Effects

Dependent Variable: Etanol (ppm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.055[a]	5	0.011	19.769	0.000
Intercept	4.286	1	4.286	7640.001	0.000
Konsentrasi	0.055	5	0.011	19.769	0.000
Error	0.007	12	0.001		
Total	4.348	18			
Corrected Total	0.062	17			

a. R Squared = 0.892 (Adjusted R Squared = 0.847)

Nilai sig.0.000 < 0.05 artinya terdapat perbedaan yang signifikan diantara konsentrasi dan kotrol, selanjutnya dilakukan uji lanjut DMRT.

Tabel Uji Lanjut DMRT

Etanol (ppm)

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan Group
K500	3	0.4273			c
K100	3	0.4557			c
K10	3	0.4607			c
K250	3	0.4673			c
K1000	3		0.5217		b
Kontrol	3			0.5950	a
Sig.		0.079	1.000	1.000	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Hasil uji lanjut Duncan menunjukkan semua konsentrasi berbeda nyata terhadap kontrol. Konsentrasi 1000 berbeda nyata dengan konsentrasi lainnya dan memiliki nilai lebih tinggi dibandingkan dengan konsentrasi lainnya.

Etil Asetat

Tests of Between-Subjects Effects

Dependent Variable: Etil Asetat

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.066[a]	5	.013	25.425	0.000
Intercept	3.926	1	3.926	7505.931	0.000
Konsentrasi	.066	5	.013	25.425	0.000
Error	.006	12	.001		
Total	3.998	18			
Corrected Total	.073	17			

a. R Squared = .914 (Adjusted R Squared = .878)

Nilai sig.0.000 < 0.05 artinya terdapat perbedaan yang signifikan diantara konsentrasi dan kotrol, selanjutnya dilakukan uji lanjut DMRT.

Etil Asetat

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan Group
K500	3	.4190			c
K1000	3	.4240			c
K250	3	.4323			c
K100	3	.4513	.4513		bc
K10	3		.4803		b
Kontrol	3			.5950	a
Sig.		.134	.146	1.000	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .001.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Hasil uji lanjut Duncan menunjukkan semua konsentrasi berbeda nyata terhadap kontrol. Konsentrasi 10 dan 100 memiliki nilai yang lebih tinggi dibandingakan dengan konsentrasi lainnya.

N-Hexan

Tests of Between-Subjects Effects

Dependent Variable: N-Hexan

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.149[a]	5	0.030	8.486	0.001
Intercept	3.390	1	3.390	967.291	0.000
Konsentrasi	0.149	5	0.030	8.486	0.001
Error	0.042	12	0.004		
Total	3.581	18			
Corrected Total	0.191	17			

a. R Squared = .780 (Adjusted R Squared = .688)

Nilai sig.0.001 < 0.05 artinya terdapat perbedaan yang signifikan diantara konsentrasi dan kotrol, selanjutnya dilakukan uji lanjut DMRT.

N-Hexan

Duncan[a,b]

Konsentrasi	N	Subset 1	Subset 2	Subset 3	Duncan Group
K500	3	.3050			c
K1000	3	.3807	.3807		bc
K250	3	.4063	.4063		bc
K100	3		.4253		b
K10	3		.4917	.4917	ab
Kontrol	3			.5950	a
Sig.		.069	.054	.054	

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = .004.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0.05.

Hasil uji lanjut Duncan menunjukkan K10 tidak berbeda nyata terhadap kontrol, sedangkan konsentrasi lainnya berbeda nyata dengan kontrol. Konsentrasi 10 hanya berbeda nyata dengan konsentrasi 500, sedangkan dengan konsentrasi lainnya tidak berbeda nyata.

EKSPRESI KI-67 PADA LESI PRA KANKER KOLON MENCIT BALB/c PASCA PEMBERIAN EKSTRAK TERPURIFIKASI

Kelompok Perlakuan	Ekspresi Ki-67 Lesi Pra kanker kolon			Rata-rata Ekspresi Ki-67
	Mencit 1	Mencit 2	Mencit 3	
Kontrol (-)	7,6	2,4	4,8	4,9
Kontrol (+)	93,4	113,4	92,2	99,7
Et	27,8	4,2	1,4	11,1
FEa	26,2	13,8	27,4	22,5
FNH	5,6	30,2	46	27,3

A. UJI ANOVA

Descriptives

Ekspresi ki-67

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
kontrol (-)	3	4.9333	2.60256	1.50259	-1.5318	11.3985	2.40	7.60
kontrol (+)	3	99.6667	11.90854	6.87540	70.0842	129.2491	92.20	113.40
et	3	11.1333	14.50149	8.37244	-24.8904	47.1570	1.40	27.80
fea	3	22.4667	7.52950	4.34716	3.7624	41.1710	13.80	27.40
fNh	3	27.2667	20.35911	11.75434	-23.3082	77.8415	5.60	46.00
Total	15	33.0933	37.05447	9.56742	12.5733	53.6134	1.40	113.40

Test of Homogeneity of Variances

Ekspresi ki-67

Levene Statistic	df1	df2	Sig.
2.358	4	10	.124

ANOVA

Ekspresi ki-67

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17562.336	4	4390.584	26.447	.000
Within Groups	1660.133	10	166.013		
Total	19222.469	14			

Ekspresi ki-67

Tukey HSD[a]

Kelompok Perlakuan	N	Subset for alpha = 0.05	
		1	2
kontrol (-)	3	4.9333	
et	3	11.1333	
fea	3	22.4667	
fNh	3	27.2667	
kontrol (+)	3		99.6667
Sig.		.282	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

B. UJI DUNCAN

Data

Duncan[a,b]

Perlakuan	N	Subset	
		1	2
A	3	4.9333	
C	3	11.1333	
D	3	22.4667	
E	3	27.2667	
B	3		99.6667
Sig.		.076	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 166.013.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0,05.

EKSPRESI CD-8 PADA LESI PRA KANKER KOLON MENCIT BALB/c PASCA PEMBERIAN EKSTRAK TERPURIFIKASI

Kelompok Perlakuan	Ekspresi CD-8 Lesi Pra kanker kolon			Rata-rata Ekspresi Ki-67
	Mencit 1	Mencit 2	Mencit 3	
Kontrol (-)	28,8	6,4	24,2	19,80
Kontrol (+)	7,2	7	7	7,07
Et	88	82	90	86,67
FEa	77	88	72	79,00
FNH	81	81	110	90,67
Total Kelompok	282	264,4	303,2	
Rata-rata	56,4	52,88	60,64	

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Kelompok Perlakuan	Statistic	df	Sig.	Statistic	df	Sig.
Ekspresi CD-8	Kontrol (-)	.312	3	.	.896	3	.374
	Kontrol (+)	.385	3	.	.750	3	.000
	Et	.292	3	.	.923	3	.463
	FEa	.263	3	.	.955	3	.593
	FNH	.385	3	.	.750	3	.000

a. Lilliefors Significance Correction

A.Uji homogenitas dan uji anova

Test of Homogeneity of Variances

Ekspresi CD-8

Levene Statistic	df1	df2	Sig.
5.531	4	10	.013

ANOVA

Ekspresi CD-8

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19122.256	4	4780.564	47.366	.000
Within Groups	1009.280	10	100.928		
Total	20131.536	14			

Ekspresi CD-8

Tukey HSD[a]

Kelompok Perlakuan	N	Subset for alpha = 0.05	
		1	2
Kontrol (+)	3	7.0667	
Kontrol (-)	3	19.8000	
FEa	3		79.0000
Et	3		86.6667
FNH	3		90.6667
Sig.		.555	.629

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

C. UJI DUNCAN

Data

Duncan[a,b]

Perlakuan	N	Subset	
		1	2
B	3	7.0667	
A	3	19.8000	
D	3		79.0000
C	3		86.6667
E	3		90.6667
Sig.		.152	.204

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 100.928.

a. Uses Harmonic Mean Sample Size = 3.000.

b. Alpha = 0,05.

DOKUMENTASI

Proses Maserasi

Proses Penyaringan

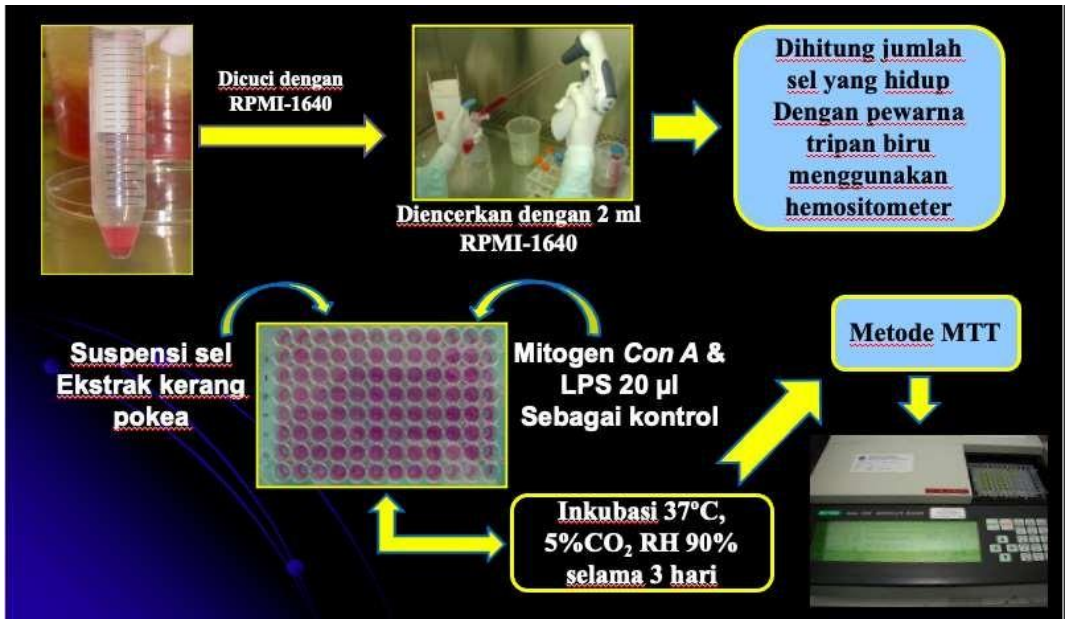
Proses Evaporasi

Ekstrak Kental

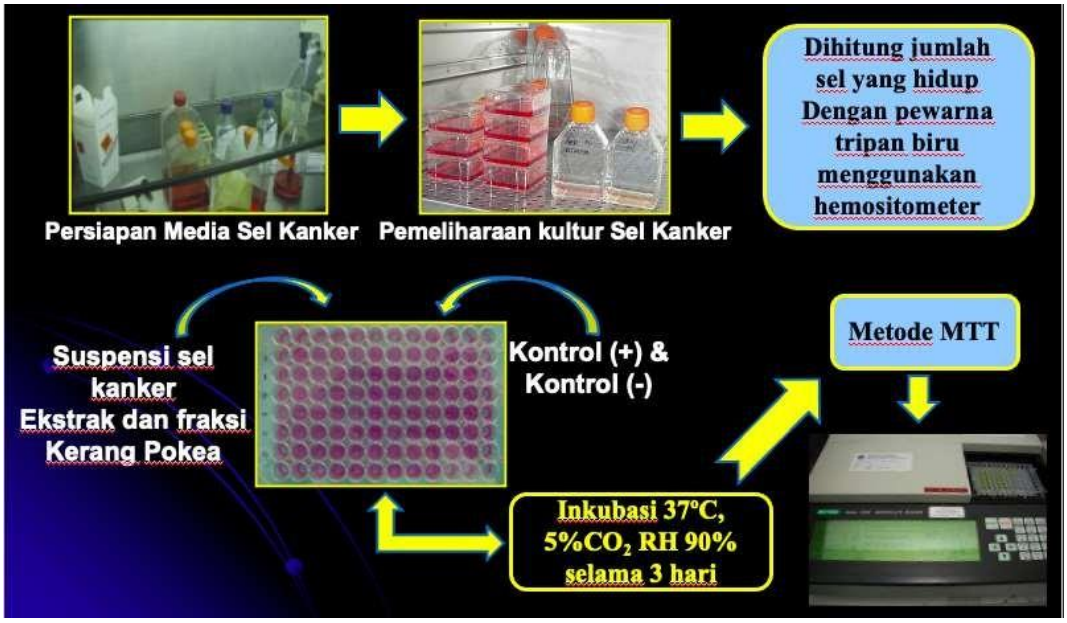
Proses Fraksinasi

Uji *In Vitro*

Isolasi Sel Limfosit



Pengujian Aktivitas Ekstrak Kerang Pokea Terhadap Proliferasi sel Limfosit



Pengujian Aktivitas Anti Proliferasi Sel Kanker secara *in vitro*

Uji in vivo



Proses Persiapan AOM & DSS



Injeksi AOM Pemberian Ekstrak Pengambilan organ kolon mencit


Persiapan IHC

Proses Pembacaan HE dan IHC Ki-67 dan CD-8