LAMPIRAN

Lampiran 1.Ethical Clearence

UMY UNIVERSITAS MUHAMMADIYAH YOGYAKARTA Unggul & Islami | FAKULTAS KEDOKTERAN DAN ILMU KESEHATAN

Nomor : 615.3/EP-FKIK-UMY/XII/2018

KETERANGAN LOLOS UJI ETIK
ETHICAL APPROVAL

Komite Etik Penelitian Fakultas Kedokteran dan Ilmu Kesehatan Universitas Muhammadiyah Yogyakarta dalam upaya melindungi hak asasi dan kesejahteraan responden/subyek penelitian, telah mengkaji dengan teliti protokol berjudul :

The Ethics Committee of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta, with regards of the protection of human rights and welfare in research, has carefully reviewed the research protocol entitled :

"Uji Aktivitas Antagonisme Etil P- Metoksisnamat Senyawa Aktif Kencur (*Kaempferia galanga L*) terhadap Reseptor AcHM3 pada Organ Ileum *Cavia porcellus* Terisolasi : Studi *In Vitro* dan *In Silico*"

Peneliti Utama *Principal Investigator*	: Puguh Novi Arsito Catur Ageng pambudi
Nama Institusi *Name of the Institution*	: Program Studi Farmasi FKIK UMY
Negara *Country*	: Indonesia

Dan telah menyetujui protokol tersebut diatas.
And approved the above-mentioned protocol.

Yogyakarta, 22 Desember 2018
Ketua
Chairperson

Dr. dr. Titiek Hidayati, M.Kes., Sp.DLP., FISPH., FISCM.

*Peneliti Berkewajiban :
1. Menjaga kerahasiaan identitas subyek penelitian
2. Memberitahukan status penelitian apabila :
   a. Setelah masa berlakunya keterangan lolos uji etik (1 tahun sejak tanggal terbit), penelitian masih belum selesai, dalam hal ini *ethical clearance* harus diperpanjang
   b. Penelitian berhenti di tengah jalan
3. Melaporkan kejadian serius yang tidak diinginkan (*serious adverse events*).
4. Peneliti tidak boleh melakukan tindakan apapun pada responden/subyek sebelum penelitian lolos uji etik.

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Lampiran 2.Hasil determinasi tanaman

LABORATORIUM BIOLOGI

FAKULTAS MIPA
UNIVERSITAS AHMAD DAHLAN
Jl. Prof. Dr. Soepomo, Yogyakarta Telp. (0274) 563515

SURAT KETERANGAN
Nomor : 007/Lab.Bio/B/I/2019

Yang bertanda tangan di bawah ini Kepala Laboratorium Biologi Universitas Ahmad Dahlan menerangkan bahwa :

Nama	: Siti Lathifah R
NIM	: 20150350083
Prodi, PT	: Farmasi, Universitas Muhammadiyah Yogyakarta

Telah melakukan determinasi tanaman dengan bimbingan Hery Setiyawan, M.Si di Laboratorium Biologi Universitas Ahmad Dahlan, pada tanggal 21 Januari 2019

Tanaman tersebut adalah :
Kaempferia galanga Linn.

Demikian Surat Keterangan ini untuk dapat dipergunakan seperlunya.

Yogyakarta, 21 Januari 2019
Kepala Laboratorium Biologi

Drs. Hadi Sasongko, M.Si.

1. **Perhitungan Agonis $AChM_3$**

   BM $AChM_3$ = 240,1 g/mol.

   Pembuatan larutan stok $2x10^{-1}$ M:

   Berat $AChM_3$ yang dibutuhkan → $M = \frac{berat\ (g)}{BM}\ x\ \frac{1000}{volume\ (mL)}$

   $2x10^{-1}$ M $= \frac{x}{240{,}1\ g/mol}\ x\ \frac{1000}{10\ mL}$

   $X = \frac{2\ x\ 10^{-1}\ M\ x\ 240{,}1 \frac{g}{mol}\ x\ 10\ mL}{1000}$

   $X = 480{,}2\ x\ 10^{-3}$ g.

   X = 480,2 mg.

   Dilakukan pengenceran dan dibuat seri kadar dari konsentrasi $2x10^{-2}$ hingga $2x10^{-8}$.

2. **Perhitungan Atropin Sulfat**

   BM Atropin Sulfat = 676,8 g/mol

   → Pembuatan larutan Atropin konsentrasi $2x10^{-6}$ M

   Berat Atropin Sulfat yang dibutuhkan:

   $2x10^{-6}$ M $= \frac{x\ (g)}{676{,}8\ g/mol}\ x\ \frac{1000}{10\ mL}$

   $X = \frac{2\ x\ 10^{-6}\ M\ x\ 676{,}8 \frac{g}{mol}\ x\ 10\ mL}{1000}$

   $X = 13{,}54\ x\ 10^{-6}$ g.

   $X = 13{,}54\ x\ 10^{-3}$ mg.

   Sediaan injeksi yang tersedia = 0,25 mg/mL.

→ Volume sediaan yang diambil =

$$V = \frac{13{,}54 \times 10^{-3}\ g}{0{,}25\ g/mL} = 54{,}16 \times 10^{-3}\ mL \sim 54{,}16\ \mu L \text{ ad } 10\ mL.$$

→ Dosis Atropin volume 100 µL

$$\frac{13{,}54 \times 10^{-3}\ mg}{10\ mL} = \frac{13{,}54\ \mu g}{10.000\ \mu L} = 13{,}54 \times 10^{-4}\ \mu g/\ \mu L$$

$13{,}54 \times 10^{-4}$ µg/ µL x 100 µL = 0,1354 µg.

→ Konsentrasi larutan Atropin 2×10^{-6} M sebanyak 100 µL dalam buffer krebs 20 mL:

2×10^{-6} M x 0,1 mL = M_2 x 20 mL

$$M_2 = \frac{2 \times 10^{-6} M \times 0{,}1\ mL}{20\ mL}$$

$M_2 = 10^{-8}$ M ~ 10^{-2} µM.

→ Dosis Atropin volume 500 µL

$$\frac{13{,}54 \times 10^{-3}\ mg}{10\ mL} = \frac{13{,}54\ \mu g}{10.000\ \mu L} = 13{,}54 \times 10^{-4}\ \mu g/\ \mu L$$

$13{,}54 \times 10^{-4}$ µg/ µL x 500 µL = $67{,}7 \times 10^{-2}$ µg.

→ Konsentrasi larutan Atropin 2×10^{-6} M sebanyak 500 µL dalam buffer krebs 20 mL:

2×10^{-6} M x 0,5 mL = M_2 x 20 mL

$$M_2 = \frac{2 \times 10^{-6} M \times 0{,}5\ mL}{20\ mL}$$

$M_2 = 5 \times 10^{-8}$ M ~ 5×10^{-2} µM.

3. Perhitungan EPMS

BM EPMS = 260,2 g/mol

→ Pembuatan larutan stok EPMS konsentrasi $2x10^{-1}$ M

Berat EPMS yang dibutuhkan:

$2x10^{-1}$ M $= \frac{x\ (g)}{260{,}2\ g/mol}\ x\ \frac{1000}{5\ mL}$

$X = \frac{2\ x\ 10^{-1}\ M\ x\ 260{,}2\ \frac{g}{mol} x\ 5\ mL}{1000}$

X = 206,2 mg

Dilakukan pengenceran hingga mencapai konsentrasi $2x10^{-2}$ M.

→ Dosis EPMS volume 100 µL

$\frac{206{,}2\ mg}{5\ mL} = \frac{206.200\ \mu g}{5.000\ \mu L} = 41{,}24\ \mu g/\ \mu L$

41,24 µg/ µL x 100 µL = 4124 µg ~ 4,124 mg.

Dilakukan pengenceran 10x hingga mencapai konsentrasi $2x10^{-2}$ M dengan dosis yang didapatkan yaitu 0,4124 mg.

→ Konsentrasi larutan EPMS $2x10^{-2}$ M sebanyak 100 µL dalam buffer krebs 20 mL:

$2x10^{-2}$ M x 0,1 mL = M_2 x 20 mL

$M_2 = \frac{2\ x\ 10^{-2} M\ x\ 0{,}1\ mL}{20\ mL}$

$M_2 = 10^{-4}$ M ~ 100 µM.

→ Dosis EPMS volume 200 µL

$\frac{206{,}2\ mg}{5\ mL} = \frac{206.200\ \mu g}{5.000\ \mu L} = 41{,}24\ \mu g/\ \mu L.$

41,24 µg/ µL x 200 µL = 8248 µg ~ 8,248 mg.

Dilakukan pengenceran 10x hingga mencapai konsentrasi $2x10^{-2}$ M dengan dosis yang didapatkan yaitu 0,8248 mg.

→ Konsentrasi larutan EPMS $2x10^{-2}$ M sebanyak 200 µL dalam buffer krebs 20 mL:

$2x10^{-2}$ M x 0,2 mL = M_2 x 20 mL

$M_2 = \frac{2 \times 10^{-2} M \times 0.2 mL}{20 mL}$

$M_2 = 2 \times 10^{-4}$ M ~ 200 µM.

Lampiran 4 Uji statistik ANOVA

Case Processing Summary

		Cases					
		Valid		Missing		Total	
	Merk	N	Percent	N	Percent	N	Percent
nilai	Kontrol	10	100.0%	0	.0%	10	100.0%
	atropin 100	5	100.0%	0	.0%	5	100.0%
	atropin 200	5	100.0%	0	.0%	5	100.0%
	EPMS 100	5	100.0%	0	.0%	5	100.0%
	EPMS 200	5	100.0%	0	.0%	5	100.0%

Descriptives

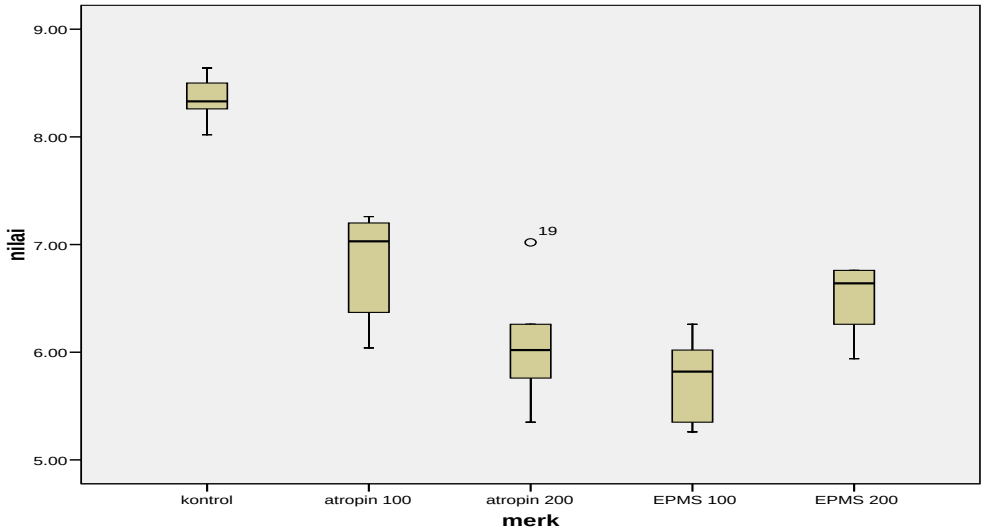
	Merk			Statistic	Std. Error
nilai	kontrol	Mean		8.3330	.06527
		95% Confidence Interval for Mean	Lower Bound	8.1854	
			Upper Bound	8.4806	
		5% Trimmed Mean		8.3333	
		Median		8.3300	
		Variance		.043	
		Std. Deviation		.20640	
		Minimum		8.02	
		Maximum		8.64	
		Range		.62	
		Interquartile Range		.31	
		Skewness		-.279	.687
		Kurtosis		-.589	1.334
	atropin 100	Mean		6.7800	.24341
		95% Confidence Interval for Mean	Lower Bound	6.1042	
			Upper Bound	7.4558	
		5% Trimmed Mean		6.7944	
		Median		7.0300	
		Variance		.296	
		Std. Deviation		.54429	
		Minimum		6.04	
		Maximum		7.26	
		Range		1.22	
		Interquartile Range		1.03	
		Skewness		-.708	.913
		Kurtosis		-2.072	2.000
	atropin 200	Mean		6.0820	.27883
		95% Confidence Interval for Mean	Lower Bound	5.3079	
			Upper Bound	6.8561	
		5% Trimmed Mean		6.0706	
		Median		6.0200	
		Variance		.389	
		Std. Deviation		.62347	
		Minimum		5.35	
		Maximum		7.02	
		Range		1.67	

Merk			Statistic	Std. Error
	Interquartile Range		1.09	
	Skewness		.696	.913
	Kurtosis		.876	2.000
EPMS 100	Mean		5.7420	.19205
	95% Confidence Interval for Mean	Lower Bound	5.2088	
		Upper Bound	6.2752	
	5% Trimmed Mean		5.7400	
	Median		5.8200	
	Variance		.184	
	Std. Deviation		.42944	
	Minimum		5.26	
	Maximum		6.26	
	Range		1.00	
	Interquartile Range		.84	
	Skewness		-.059	.913
	Kurtosis		-2.281	2.000
EPMS 200	Mean		6.4720	.16157
	95% Confidence Interval for Mean	Lower Bound	6.0234	
		Upper Bound	6.9206	
	5% Trimmed Mean		6.4856	
	Median		6.6400	
	Variance		.131	
	Std. Deviation		.36128	
	Minimum		5.94	
	Maximum		6.76	
	Range		.82	
	Interquartile Range		.66	
	Skewness		-.951	.913
	Kurtosis		-.906	2.000

Tests of Normality

		Kolmogorov-Smirnov(a)			Shapiro-Wilk		
	Merk	Statistic	df	Sig.	Statistic	df	Sig.
nilai	Kontrol	.162	10	.200(*)	.943	10	.582
	atropin 100	.277	5	.200(*)	.863	5	.239
	atropin 200	.188	5	.200(*)	.974	5	.900
	EPMS 100	.219	5	.200(*)	.926	5	.567
	EPMS 200	.279	5	.200(*)	.849	5	.191

* This is a lower bound of the true significance.
a Lilliefors Significance Correction



Oneway

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
	Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound	Upper Bound
kontrol	10	8.3330	.20640	.06527	8.1854	8.4806	8.02	8.64
atropin 100	5	6.7800	.54429	.24341	6.1042	7.4558	6.04	7.26
atropin 200	5	6.0820	.62347	.27883	5.3079	6.8561	5.35	7.02
EPMS 100	5	5.7420	.42944	.19205	5.2088	6.2752	5.26	6.26
EPMS 200	5	6.4720	.36128	.16157	6.0234	6.9206	5.94	6.76
Total	30	6.9570	1.11199	.20302	6.5418	7.3722	5.26	8.64

Descriptives

Test of Homogeneity of Variances

nilai

Levene Statistic	df1	df2	Sig.
2.739	4	25	.051

ANOVA

nilai

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.476	4	7.869	44.883	.000
Within Groups	4.383	25	.175		
Total	35.859	29			

Post Hoc Tests

Multiple Comparisons

	(I) merk	(J) merk	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
			Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
Tukey HSD	kontrol	atropin 100	1.55300(*)	.22934	.000	.8795	2.2265
		atropin 200	2.25100(*)	.22934	.000	1.5775	2.9245
		EPMS 100	2.59100(*)	.22934	.000	1.9175	3.2645
		EPMS 200	1.86100(*)	.22934	.000	1.1875	2.5345
	atropin 100	kontrol	-1.55300(*)	.22934	.000	-2.2265	-.8795
		atropin 200	.69800	.26482	.094	-.0797	1.4757
		EPMS 100	1.03800(*)	.26482	.005	.2603	1.8157
		EPMS 200	.30800	.26482	.772	-.4697	1.0857
	atropin 200	kontrol	-2.25100(*)	.22934	.000	-2.9245	-1.5775
		atropin 100	-.69800	.26482	.094	-1.4757	.0797
		EPMS 100	.34000	.26482	.703	-.4377	1.1177
		EPMS 200	-.39000	.26482	.589	-1.1677	.3877
	EPMS 100	kontrol	-2.59100(*)	.22934	.000	-3.2645	-1.9175
		atropin 100	-1.03800(*)	.26482	.005	-1.8157	-.2603
		atropin 200	-.34000	.26482	.703	-1.1177	.4377
		EPMS 200	-.73000	.26482	.073	-1.5077	.0477
	EPMS 200	kontrol	-1.86100(*)	.22934	.000	-2.5345	-1.1875
		atropin 100	-.30800	.26482	.772	-1.0857	.4697
		atropin 200	.39000	.26482	.589	-.3877	1.1677
		EPMS 100	.73000	.26482	.073	-.0477	1.5077
LSD	kontrol	atropin 100	1.55300(*)	.22934	.000	1.0807	2.0253
		atropin 200	2.25100(*)	.22934	.000	1.7787	2.7233
		EPMS 100	2.59100(*)	.22934	.000	2.1187	3.0633
		EPMS 200	1.86100(*)	.22934	.000	1.3887	2.3333
	atropin 100	kontrol	-1.55300(*)	.22934	.000	-2.0253	-1.0807
		atropin 200	.69800(*)	.26482	.014	.1526	1.2434
		EPMS 100	1.03800(*)	.26482	.001	.4926	1.5834
		EPMS 200	.30800	.26482	.256	-.2374	.8534
	atropin	kontrol	-2.25100(*)	.22934	.000	-2.7233	-1.7787

200						
	atropin 100	-.69800(*)	.26482	.014	-1.2434	-.1526
	EPMS 100	.34000	.26482	.211	-.2054	.8854
	EPMS 200	-.39000	.26482	.153	-.9354	.1554
EPMS 100	kontrol	-2.59100(*)	.22934	.000	-3.0633	-2.1187
	atropin 100	-1.03800(*)	.26482	.001	-1.5834	-.4926
	atropin 200	-.34000	.26482	.211	-.8854	.2054
	EPMS 200	-.73000(*)	.26482	.011	-1.2754	-.1846
EPMS 200	kontrol	-1.86100(*)	.22934	.000	-2.3333	-1.3887
	atropin 100	-.30800	.26482	.256	-.8534	.2374
	atropin 200	.39000	.26482	.153	-.1554	.9354
	EPMS 100	.73000(*)	.26482	.011	.1846	1.2754

Dependent Variable: nilai

* The mean difference is significant at the .05 level.

Homogeneous Subsets

nilai

		N	Subset for alpha = .05		
	merk	1	2	3	1
Tukey HSD(a,b)	EPMS 100	5	5.7420		
	atropin 200	5	6.0820	6.0820	
	EPMS 200	5	6.4720	6.4720	
	atropin 100	5		6.7800	
	kontrol	10			8.3330
	Sig.		.053	.070	1.000

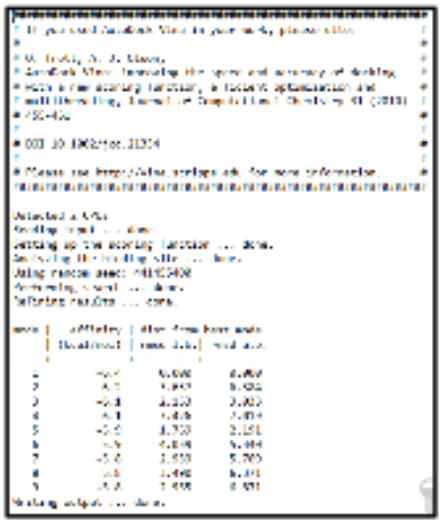
Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 5.556.

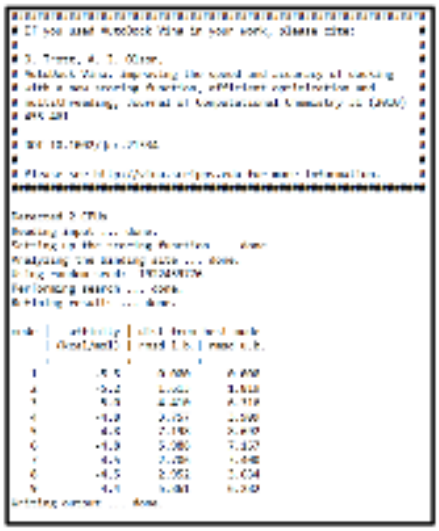
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Lampiran 5. Hasil Uji *In-Silico*

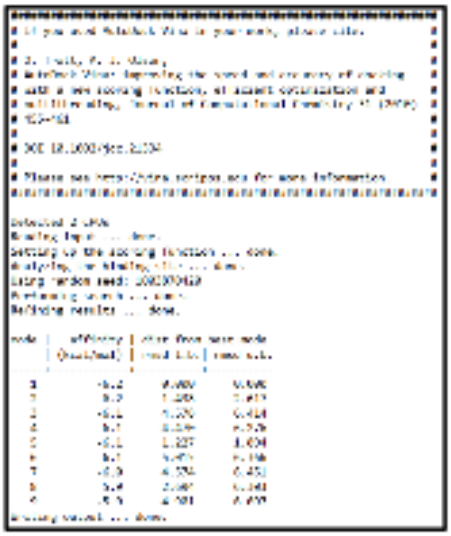
1. Uji Validasi Native Ligand (Tiotropium) dengan Reseptor AChM$_3$



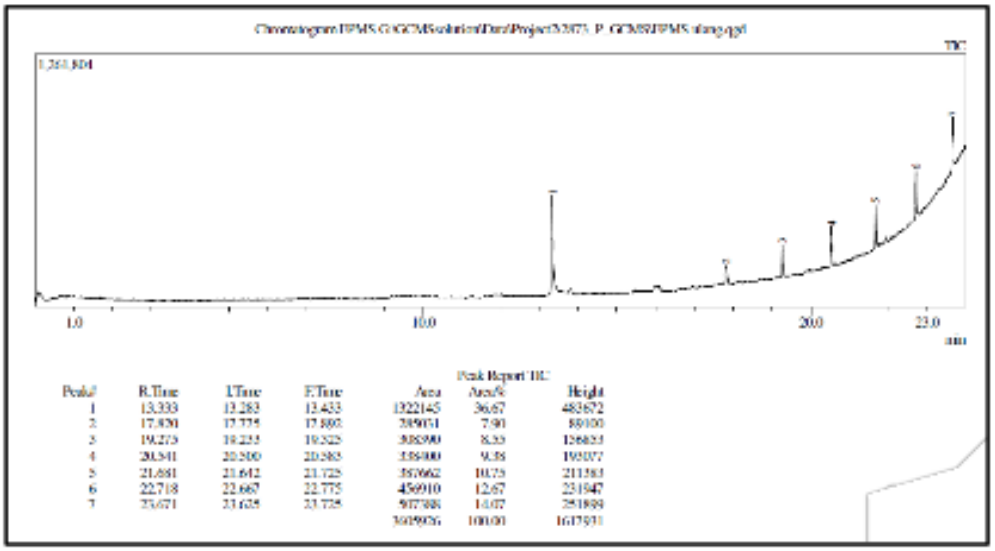
2. Uji Validasi EPMS dengan Reseptor AChM$_3$



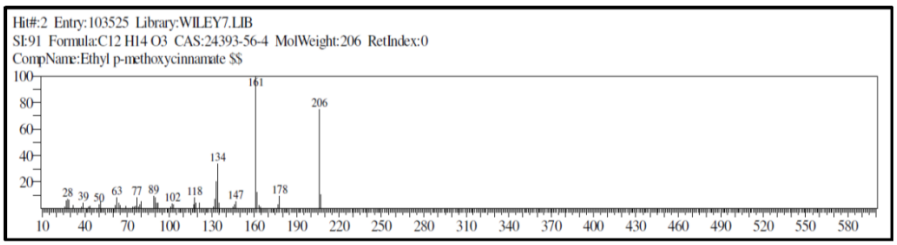
3. Uji Validasi Atropin dengan Reseptor AChM$_3$

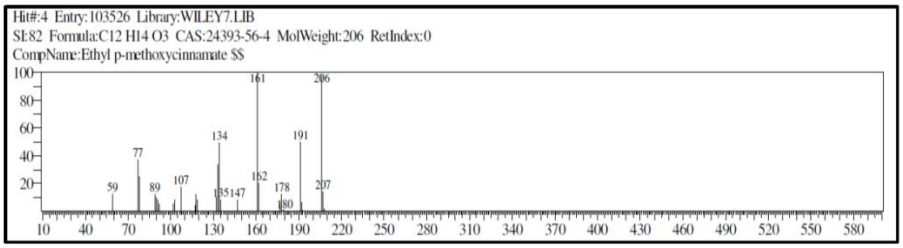


Lampiran 6. Hasil Uji GCMS



Gambar 6. Uji *Gas Chromatography* senyawa EPMS





Lampiran 7. Hasil uji *in vitro*

Nilai pD2 Atropin

	Nilai pD2		
	(-) Atropin	(+)Atropin 100	(+)Atropin 200
1	7,82	6,37	5,76
2	7,61	7,03	6,02
3	8,42	7,26	6,26
4	8,33	7,20	7,02
5	8,55	6,04	5,35
6	7,37		
7	8,02		
8	7,76		
9	8,02		
10	8,33		
Mean	8,02	6,78	6,08
SEM	0,12	0,17	0,20

Nilai EC50 Atropin

EC50 (-) Atropin	EC (+)Atropin 100	EC (+)Atropin 200
1,51E-08	4,27E-07	1,74E-06
2,45E-08	9,33E-08	9,55E-07
3,80E-09	5,50E-08	5,50E-07
4,68E-09	6,31E-08	9,55E-08
2,82E-09	9,12E-07	4,47E-06
4,27E-08		
9,55E-09		
1,74E-08		
9,55E-09		
4,68E-09		
1,35E-08	3,10E-07	1,56E-06
3,92E-09	1,66E-07	7,75E-07

Nilai pD2 EPMS

	Nilai pD2		
	(-) EPMS	(+) EPMS 100	(+) EPMS 200
1	9,70	5,35	6,76
2	8,26	5,82	6,76
3	8,88	5,26	5,94
4	8,64	6,26	6,26
5	8,50	6,02	6,64
6	8,26		
7	9,03		
8	9,55		
9	7,92		
10	8,76		
Mean	8,75	5,74	6,47
SEM	0,18	0,14	0,11

Nilai EC 50 EPMS

EC50 (-) EPMS	EC (+)EPMS 100	EC (+)EPMS 200
2,00E-10	4,47E-06	1,74E-07
5,50E-09	1,51E-06	1,74E-07
1,32E-09	5,50E-06	1,15E-06
2,29E-09	5,50E-07	5,50E-07
3,16E-09	9,55E-07	2,29E-07
5,50E-09		
9,33E-10		
2,82E-10		
1,20E-08		
1,74E-09		
3,29E-09	2,60E-06	4,55E-07
1,14E-09	9,99E-07	1,87E-07

Lampiran 8. Proses penelitian

Gambar 1. Perajangan rimpang kencur

Gambar 2. Proses maserasi

Gambar 3. Proses pemanasan menggunakan *Rotary Vacuum Evaporator* untuk memekatkan ekstrak

Gambar 4. Kristal Kencur setelah dilakukan pencucian

Gambar 5. Preparasi organ trakea

Lampiran 9. Turnitin

CATUR AGENG PAMBUDI UJI AKTIVITAS ANTAGONISME ETIL P-METOKSISINAMAT SENYAWAAKTIF KENCUR (Kaempferia galanga L) TERHADAP RESEPTOR ASETILKOLIN MUSKARINIK 3 PADA ORGAN ILEUM Cavia porcellus TERISOLASI: S

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