

## **BAB V**

### **KESIMPULAN DAN SARAN**

#### **A. Kesimpulan**

Berdasarkan hasil penelitian ini dapat disimpulkan :

Pertama, ekstrak etanolik daun seligi (*Phyllanthus buxifolius* (Bl.) M.A.) mempunyai pengaruh terhadap peningkatan kadar HDL dan penurunan kadar LDL pada serum darah tikus.

Kedua, ekstrak etanolik daun seligi pada dosis 300 mg/kg BB tikus mempunyai pengaruh paling efektif meningkatkan kadar HDL dan menurunkan kadar LDL serum darah tikus yang setara dengan kontrol positif simvastatin.

#### **B. Saran**

Saran yang dapat diberikan untuk penelitian yang lebih lanjut berdasarkan hasil penelitian tentang aktivitas ekstrak etanolik daun seligi terhadap peningkatan kadar HDL dan penurunan kadar LDL sebagai berikut :

Pertama, perlu adanya penelitian lebih lanjut mengenai daun seligi dan kandungan kimia di dalamnya dengan melakukan isolasi zat aktif murni yang dilanjutkan dengan pengujian aktivitas hiperlipidemia.

Kedua, perlu adanya penelitian lebih lanjut untuk mengetahui toksisitas senyawa yang terdapat pada ekstrak etanolik daun seligi.

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## Lampiran 1. Hasil determinasi tanaman



No : 003/DET/UPT-LAB/13/II/2013  
Hal : Surat Keterangan Determinasi Tumbuhan

Menerangkan bahwa :

Nama : Sri Agustiningsih  
NIM : 15092778 A  
Fakultas : Farmasi Universitas Setia Budi

Telah mendeterminasikan tumbuhan : **Seligi (*Phyllanthus buxifolius* (Bl.)M.A.**

Hasil determinasi berdasarkan : **Baker: Flora of Java**

1b – 2b – 3b – 4b – 12b – 13b – 14b – 17b – 18b – 19b – 20b – 21b – 22b – 23b – 24b – 25b – 26b – 27a – 28b – 29b – 30b – 31b – 32b – 74a – 75b – 76a – 77a – 78a – 79b – 80a – 81b – 86b – 87b – 97a – 98b – 99b – 100b – 143b – 147b – 156a. 99. Familia Euphorbiaceae. 1b – 3b – 4b – 6a – 7b – 8b – 10b – 13b – 15b – 25b – 26b – 27b – 28b – 29b – 30b – 31b – 32b – 33a – 34b.  
*Phyllanthus* L. 1b – 6d – 16b. ***Phyllanthus buxifolius* (Bl.)M.A.**

Deskripsi :

Habitus : Perdu menahun, tinggi 1 – 1,5 meter.  
Daun : tunggal, duduk daun berseling, helaihan daun asimetris, bangun bulat telur, panjang 1,5-3 cm, lebar 1-1,5 cm, ujung runcing, pangkal tumpul, tepi rata, bertulang menyirip, tepi rata, berwarna hijau tua.  
Bunga : tunggal, berwarna kuning, menggantung di ketiak daun, bertangkai pendek, benangsari banyak, pendek, kuning.  
Buah : bulat, diameter 5-10 mm, waktu masih muda berwarna hijau, setelah tua berwarna coklat.  
Biji : pipih, bentuk ginjal, berwarna coklat.

Pustaka :

Backer C.A. & Brink R.C.B. (1965): *Flora of Java* (Spermatophytes only).  
N.V.P. Noordhoff – Groningen – The Netherlands.

Surakarta, 13 Januari 2013  
Tim determinasi  
  
Dra.Kartinah Wiryosoendjojo, SU.

**Lampiran 2. Surat keterangan pembelian hewan uji**

**"ABIMANYU FARM"**

✓ Mencit putih jantan ✓ Tikus Wistar ✓ Swis Webster ✓ Cacing ✓ Mencit Jepang ✓ Kelinci New Zaeland  
Ngampon RT 04 / RW 04. Mojosongo Kec. Jebres Surakarta. Phone 085 629 994 33 / Lab USB Ska

Menerangkan dengan sebenarnya bahwa Tikus Putih Jantan (*Rattus norvegicus*) yang dibeli oleh:

Nama	:	Sri Agustiningsih
Alamat	:	Universitas Setia Budi Surakarta
Fakultas	:	Farmasi
Nim	:	15092778 A
Keperluan	:	Praktikum Penelitian
Tanggal	:	18 April 2013
Jenis	:	Tikus Putih
Kelamin	:	Tikus Putih Jantan
Umur	:	± 3 - 4 bulan
Jumlah	:	30 ekor jantan

Atas kerja samanya, kami mengucapkan terima kasih dan mohon maaf jika dalam pelayanannya banyak kekurangan.

Surakarta, 23 Mei 2013

Hormat kami



**ABIMANYU FARM**

Sigit Pramono

**Lampiran 3. Foto tanaman seligi dan serbuk daun seligi****Gambar 6a. Foto tanaman seligi****Gambar 6b. Foto serbuk daun seligi**

**Lampiran 4. Foto alat dan hasil ekstrak etanolik daun seligi**



**Gambar 7a. Foto alat *waterbath***



**Gambar 7b. Foto alat *moisture balance***



Gambar 7c. Foto hasil ekstrak etanolik daun seligi

**Lampiran 5. Foto hewan uji****Gambar 8. Foto hewan uji**

**Lampiran 6. Foto reagen dan alat pengukur kadar HDL dan LDL**

**Gambar 9a. Foto reagen HDL *Precipitant***



**Gambar 9b. Foto reagen LDL *Precipitant***



Gambar 9c. Foto reagen kolesterol kit



Gambar 9d. Foto alat centrifuge



**Gambar 9d. Foto alat fotometer**

**Lampiran 7. Foto hasil identifikasi kandungan kimia serbuk daun seligi**



**Gambar 10a. Foto identifikasi flavonoid**



**Gambar 10b. Foto identifikasi saponin**



**Gambar 10c. Foto identifikasi polifenol**

**Lampiran 8. Hasil pembuatan ekstrak etanolik daun seligi**

Dari hasil penelitian diperoleh data sebagai berikut:

Berat awal serbuk (gram)	Berat wadah Kosong (gram)	Berat wadah + Zat (gram)	Bagian kental (gram)	Rendemen (%)
500	60,89	136,98	76,09	15,22

Perhitungan % rendemen ekstrak etanol daun seligi:

$$\begin{aligned}\% \text{ Rendemen} &= \frac{\text{Bagian kental (g)}}{\text{Berat awal serbuk (g)}} \times 100 \% \\ &= \frac{76,09}{500} \times 100 \% \\ &= 15,22 \% \text{ b/b}\end{aligned}$$

Jadi rendemen ekstrak etanol terhadap berat serbuk daun seligi adalah 15,22 % b/b.

### Lampiran 9. Perhitungan dosis sediaan

#### A. Perhitungan dosis ekstrak etanolik daun seligi

Variasi dosis dari hasil orientasi yang digunakan dalam penelitian ini adalah dosis I = 75 mg/kgBB (1/2 kali dosis); dosis II = 150 mg/kgBB (1 kali dosis); dosis III = 300 mg/kgBB (2 kali dosis). Dibuat larutan stok 3% = 3 g/100 mL = 300 mg/10 mL = 30 mg/mL. Perhitungan dosis pemberian pada binatang uji :

❖ Dosis I = 75 mg/kgBB atau 15 mg/ 200 g BB

No	BB Tikus (gram)	Dosis (mg)	Volume pemberian (ml)
1.	190	14,25	0,48
2.	185	13,87	0,46
3.	200	15,0	0,5
4.	195	14,6	0,49
5.	190	14,25	0,48

$$\text{Dosis} = \frac{\text{BB tikus (g)}}{200 (\text{g})} \times 15 \text{ mg}/200 \text{ g BB}$$

$$\text{Volume pemberian} = \frac{\text{Dosis (mg)}}{30 \text{ mg}} \times 1 \text{ ml}$$

❖ Dosis II = 150 mg/kgBB atau 30 mg/ 200 g BB

No	BB Tikus (gram)	Dosis (mg)	Volume pemberian (ml)
1.	190	28,5	0,95
2.	198	29,7	0,99
3.	194	29,1	0,97
4.	195	29,2	0,98
5.	200	30,0	1

$$\text{Dosis} = \frac{\text{BB tikus (g)}}{200 (\text{g})} \times 30 \text{ mg}/200 \text{ g BB}$$

$$\text{Volume pemberian} = \frac{\text{Dosis (mg)}}{30 \text{ mg}} \times 1 \text{ ml}$$

❖ Dosis III = 300 mg/kgBB atau 60 mg/ 200 g BB

No	BB Tikus (gram)	Dosis (mg)	Volume pemberian (ml)
1.	189	56,7	1,89
2.	192	57,6	1,92
3.	180	54,0	1,8
4.	198	59,4	1,98
5.	190	57,0	1,9

$$\text{Dosis} = \frac{\text{BB tikus (g)}}{200 (\text{g})} \times 60 \text{ mg}/200 \text{ g BB}$$

$$\text{Volume pemberian} = \frac{\text{Dosis (mg)}}{30 \text{ mg}} \times 1 \text{ ml}$$

### B. Penentuan dosis sediaan untuk obat simvastatin

Untuk obat simvastatin 10 mg konversi dosis dari manusia dengan berat badan 70 kg terhadap tikus yang berat badannya 200 gram = 0,018 (D.R. Laurence, 1964).

$$\text{Pemakaian untuk 1 hari} = 1 \times 10 \text{ mg} = 10 \text{ mg}$$

$$\begin{aligned}\text{Maka konversi ke dosis tikus} &= 0,018 \times 10 \text{ mg} / 200 \text{ g BB} \\ &= 0,18 \text{ mg} / 200 \text{ g BB}\end{aligned}$$

Dibuat larutan stok 0,01% = 0,01 g/100 mL = 1 mg/ 10 mL = 0,1 mg/mL dengan melarutkan 1 tablet yang mengandung 10 mg simvastatin ditambah suspensi CMC 0,5% sampai volume 100 mL.

No	BB Tikus (gram)	Dosis (mg)	Volume pemberian (ml)
1.	185	0,16	1,6
2.	190	0,17	1,7
3.	193	0,17	1,7
4.	200	0,20	2
5.	189	0,17	1,7

**Lampiran 10. Rata-rata kadar HDL serum darah tikus**

**Tabel 10. Kadar HDL serum darah tikus putih**

Kelompok	Replikasi	Hari ke – 0 (mg/dl)	Hari ke – 14 (mg/dl)	Hari ke – 28 (mg/dl)
Kontrol normal	1	94	75	83
	2	73	71	74
	3	95	88	87
	4	88	83	78
	5	82	73	79
Rata-rata		86,4	78,0	80,2
SD		9,17	7,21	4,97
Kontrol positif	1	86	32	69
	2	98	39	74
	3	110	41	83
	4	95	44	81
	5	81	32	75
Rata-rata		94,0	37,6	76,4
SD		11,25	5,41	5,64
Kontrol negatif	1	97	44	46
	2	84	33	42
	3	99	36	65
	4	82	29	57
	5	94	30	54
Rata-rata		91,2	34,4	52,8
SD		7,73	6,02	9,09
Dosis I 75 mg/kg BB	1	98	47	54
	2	75	34	51
	3	72	30	44
	4	92	39	46
	5	94	33	52
Rata-rata		86,2	36,6	49,4
SD		11,84	6,65	4,23
Dosis II 150 mg/kg BB	1	86	35	61
	2	85	33	66
	3	78	28	52
	4	95	41	59
	5	87	35	56
Rata-rata		86,2	34,4	58,8
SD		6,06	4,67	5,26
Dosis III 300 mg/kg BB	1	79	26	62
	2	91	45	78
	3	96	36	77
	4	94	43	73
	5	77	29	68
Rata-rata		87,4	35,8	71,6
SD		8,79	8,35	6,65

**Lampiran 11. Rata-rata kadar LDL serum darah tikus**

**Tabel 11. Kadar LDL serum darah tikus putih**

Kelompok	Replikasi	Hari ke - 0 (mg/dl)	Hari ke - 14 (mg/dl)	Hari ke - 28 (mg/dl)
Kontrol normal	1	52-15 = 37	50-14 = 36	48-14 = 34
	2	49-13 = 36	53-12 = 41	50-10 = 40
	3	39-12 = 27	50-13 = 37	49-12 = 37
	4	55-16 = 39	53-17 = 36	50-16 = 34
	5	57-12 = 45	60-13 = 47	60-11 = 49
Rata-rata		36,8	39,4	38,8
SD		6,49	4,72	6,22
Kontrol positif	1	43-14 = 29	114-47 = 67	42-12 = 30
	2	57-19 = 38	138-42 = 96	60-9 = 51
	3	48-10 = 38	119-44 = 75	44-11 = 33
	4	78-12 = 66	147-51 = 96	76-15 = 61
	5	65-19 = 46	141-45 = 96	59-12 = 47
Rata-rata		43,4	86,0	44,4
SD		13,99	13,98	12,88
Kontrol negatif	1	83-18 = 65	156-47 = 109	152-42 = 110
	2	59-15 = 44	137-47 = 90	132-43 = 89
	3	70-19 = 51	146-44 = 102	139-40 = 99
	4	64-18 = 46	168-40 = 128	164-36 = 128
	5	74-12 = 62	139-45 = 94	137-41 = 96
Rata-rata		53,6	104,6	104,4
SD		9,44	14,99	15,20
Dosis I 75 mg/kg BB	1	67-14 = 53	138-44 = 94	72-18 = 54
	2	83-13 = 70	161-49 = 112	93-28 = 65
	3	70-13 = 57	142-44 = 98	78-29 = 49
	4	59-12 = 47	138-45 = 93	68-28 = 40
	5	64-16 = 48	151-46 = 105	84-26 = 58
Rata-rata		55,0	100,4	53,2
SD		9,30	8,01	9,41
Dosis II 150 mg/kg BB	1	59-15 = 44	130-45 = 85	68-21 = 47
	2	61-17 = 44	126-47 = 79	62-20 = 42
	3	58-14 = 44	137-47 = 90	70-21 = 49
	4	82-14 = 68	153-43 = 110	78-19 = 59
	5	72-17 = 55	165-49 = 116	88-20 = 68
Rata-rata		51,0	96,0	53,0
SD		10,63	16,14	10,41
Dosis III 300 mg/kg BB	1	84-14 = 70	162-47 = 115	81-14 = 67
	2	71-10 = 61	148-45 = 103	70-13 = 57
	3	59-11 = 48	126-44 = 82	54-12 = 42
	4	60-18 = 42	134-46 = 88	63-14 = 49
	5	67-15 = 52	140-42 = 98	68-12 = 56
Rata-rata		54,6	97,2	54,2
SD		11,03	12,91	9,36

## Lampiran 12. Hasil analisa statistik kadar HDL tikus dengan uji

### *Kolmogorov-Smirnov dan ANOVA*

#### A. Hasil analisa statistik penurunan kadar HDL tikus

##### NPar Tests

		Notes
Output Created		21-Jun-2013 19:03:01
Comments		
Input	Active Dataset Filter Weight Split File N of Rows in Working Data File	DataSet0 <none> <none> <none> 30
Missing Value Handling	Definition of Missing Cases Used	User-defined missing values are treated as missing. Statistics for each test are based on all cases with valid data for the variable(s) used in that test.
Syntax		NPAR TESTS /K-S(NORMAL)=prlkn /STATISTICS DESCRIPTIVES /MISSING ANALYSIS.
Resources	Processor Time Elapsed Time Number of Cases Allowed <sup>a</sup>	0:00:00.016 0:00:00.016 196608

a. Based on availability of workspace memory.

[DataSet0]

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
kelompok perlakuan	30	3.50	1.737	1	6

### One-Sample Kolmogorov-Smirnov Test

		kelompok perlakuan
N		30
Normal Parameters <sup>a,b</sup>	Mean	3.50
	Std. Deviation	1.737
Most Extreme Differences	Absolute	.139
	Positive	.139
	Negative	-.139
Kolmogorov-Smirnov Z		.764
Asymp. Sig. (2-tailed)		.604

a. Test distribution is Normal.

b. Calculated from data.

### T-Test

#### Notes

Output Created		21-Jun-2013 19:03:35
Comments		
Input	Active Dataset Filter Weight Split File	DataSet0 <none> <none> <none>
	N of Rows in Working Data File	30
Missing Value Handling	Definition of Missing  Cases Used	User defined missing values are treated as missing.  Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=prlkn(1 2) /MISSING=ANALYSIS /VARIABLES=HDL /CRITERIA=CI(.95).
Resources	Processor Time Elapsed Time	0:00:00.016 0:00:00.015

[DataSet0]

#### Group Statistics

	kelompok perlakuan	N	Mean	Std. Deviation	Std. Error Mean
kadar HDL	normal	5	8.40	6.465	2.891
	positif	5	56.40	7.987	3.572

**Independent Samples Test**

		Levene's Test for Equality of Variances	t-test for Equality of Means							95% Confidence Interval of the Difference	
			F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
kadar HDL	Equal variances assumed	.373	.558	-10.445	8	.000	-48.000	4.596	-58.598	-37.402	
	Equal variances not assumed			-10.445	7.667	.000	-48.000	4.596	-58.678	-37.322	

## B. Hasil analisa statistik peningkatan kadar HDL tikus

### NPar Tests

Notes		
Output Created		13-Jun-2013 13:57:15
Comments		
Input	Active Dataset Filter Weight Split File N of Rows in Working Data File	DataSet0 <none> <none> <none>
Missing Value Handling	Definition of Missing Cases Used	30 User-defined missing values are treated as missing. Statistics for each test are based on all cases with valid data for the variable(s) used in that test.
Syntax		NPAR TESTS /K-S(NORMAL)=prlkn /STATISTICS DESCRIPTIVES /MISSING ANALYSIS.
Resources	Processor Time Elapsed Time Number of Cases Allowed <sup>a</sup>	0:00:00.016 0:00:00.015 196608

a. Based on availability of workspace memory.

### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
kelompok perlakuan	30	3.50	1.737	1	6

### One-Sample Kolmogorov-Smirnov Test

	kelompok perlakuan
N	30
Normal Parameters <sup>a,b</sup>	Mean Std. Deviation
Most Extreme Differences	Absolute Positive Negative
Kolmogorov-Smirnov Z	.764
Asymp. Sig. (2-tailed)	.604

a. Test distribution is Normal.

b. Calculated from data.

**Oneway****Notes**

Output Created		13-Jun-2013 13:57:39
Comments		
Input	Active Dataset Filter Weight Split File	DataSet0 <none> <none> <none>
Missing Value Handling	N of Rows in Working Data File Definition of Missing Cases Used	30 User-defined missing values are treated as missing. Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax	ONEWAY hdl BY prlkn /STATISTICS DESCRIPTIVES HOMOGENEITY /MISSING ANALYSIS /POSTHOC=SNK ALPHA(0.05).	
Resources	Processor Time Elapsed Time	0:00:00.141 0:00:00.141

**Descriptives**

kadar HDL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
normal	5	2.20	5.263	2.354	-4.33	8.73	-5	8
positif	5	38.80	3.493	1.562	34.46	43.14	35	43
negatif	5	18.40	12.178	5.446	3.28	33.52	2	29
uji 1	5	12.80	5.586	2.498	5.86	19.74	7	19
uji 2	5	24.40	5.683	2.542	17.34	31.46	18	33
uji 3	5	35.80	4.438	1.985	30.29	41.31	30	41
Total	30	22.07	14.295	2.610	16.73	27.40	-5	43

**Test of Homogeneity of Variances**

kadar HDL

Levene Statistic	df1	df2	Sig.
5.080	5	24	.003

**ANOVA**

kadar HDL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4840.267	5	968.053	21.401	.000
Within Groups	1085.600	24	45.233		
Total	5925.867	29			

**Post Hoc Tests**  
**Homogeneous Subsets**
**kadar HDL**Student-Newman-Keuls<sup>a</sup>

kelompok perlakuan	N	Subset for alpha = 0.05			
		1	2	3	4
normal	5	2.20			
uji 1	5		12.80		
negatif	5			18.40	
uji 2	5				24.40
uji 3	5				35.80
positif	5				38.80
Sig.		1.000	.200	.171	.487

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5,000.

**Lampiran 13. Hasil analisa statistik kadar LDL tikus dengan uji**

***Kolmogorov-Smirnov dan ANOVA***

**A. Hasil analisa statistik peningkatan kadar LDL tikus**

**NPar Tests**

**Notes**

Output Created		21-Jun-2013 18:08:42
Comments		
Input	Active Dataset Filter Weight Split File N of Rows in Working Data File	DataSet0 <none> <none> <none> 30
Missing Value Handling	Definition of Missing Cases Used	User-defined missing values are treated as missing. Statistics for each test are based on all cases with valid data for the variable(s) used in that test.
Syntax		NPAR TESTS /K-S(NORMAL)=prlkn /STATISTICS DESCRIPTIVES /MISSING ANALYSIS.
Resources	Processor Time Elapsed Time Number of Cases Allowed <sup>a</sup>	0:00:00.016 0:00:00.031 196608

a. Based on availability of workspace memory.

[DataSet0]

**Descriptive Statistics**

	N	Mean	Std. Deviation	Minimum	Maximum
kelompok perlakuan	30	3.50	1.737	1	6

### One-Sample Kolmogorov-Smirnov Test

		kelompok perlakuan
N		30
Normal Parameters <sup>a,b</sup>	Mean	3.50
	Std. Deviation	1.737
Most Extreme Differences	Absolute	.139
	Positive	.139
	Negative	-.139
Kolmogorov-Smirnov Z		.764
Asymp. Sig. (2-tailed)		.604

a. Test distribution is Normal.

b. Calculated from data.

### T-Test

#### Notes

Output Created		21-Jun-2013 18:11:57
Comments		
Input	Active Dataset Filter Weight Split File	DataSet0 <none> <none> <none>
	N of Rows in Working Data File	30
Missing Value Handling	Definition of Missing  Cases Used	User defined missing values are treated as missing.  Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=prlkn(1 2) /MISSING=ANALYSIS /VARIABLES=LDL /CRITERIA=CI(.95).
Resources	Processor Time Elapsed Time	0:00:00.031 0:00:00.032

[DataSet0]

#### Group Statistics

	kelompok perlakuan	N	Mean	Std. Deviation	Std. Error Mean
kadar LDL	normal	5	2.60	5.128	2.293
	positif	5	42.60	11.216	5.016

### Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	Lower	Upper
kadar LDL	Equal variances assumed	4.674	.063	-7.252	8	.000	-40.000	5.515	-52.719	-27.281
	Equal variances not assumed			-7.252	5.602	.000	-40.000	5.515	-53.731	-26.269

## B. Hasil analisa statistik penurunan kadar LDL tikus

### NPar Test

#### Notes

Output Created		21-Jun-2013 11:24:46
Comments		
Input	Active Dataset Filter Weight Split File N of Rows in Working Data File	DataSet0 <none> <none> <none> 30
Missing Value Handling	Definition of Missing Cases Used	User-defined missing values are treated as missing. Statistics for each test are based on all cases with valid data for the variable(s) used in that test.
Syntax		NPAR TESTS /K-S(NORMAL)=prlkn /STATISTICS DESCRIPTIVES /MISSING ANALYSIS.
Resources	Processor Time Elapsed Time Number of Cases Allowed <sup>a</sup>	0:00:00.031 0:00:00.023 196608

a. Based on availability of workspace memory.

[DataSet0]

#### Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
kelompok perlakuan	30	3.50	1.737	1	6

#### One-Sample Kolmogorov-Smirnov Test

		kelompok perlakuan
N		30
Normal Parameters <sup>a,b</sup>	Mean Std. Deviation	3.50 1.737
Most Extreme Differences	Absolute Positive Negative	.139 .139 -.139
Kolmogorov-Smirnov Z		.764
Asymp. Sig. (2-tailed)		.604

a. Test distribution is Normal.

b. Calculated from data.

**Oneway****Notes**

Output Created		21-Jun-2013 11:25:02
Comments		
Input	Active Dataset Filter Weight Split File N of Rows in Working Data File	DataSet0 <none> <none> <none> 30
Missing Value Handling	Definition of Missing Cases Used	User-defined missing values are treated as missing. Statistics for each analysis are based on cases with no missing data for any variable in the analysis.
Syntax		ONEWAY LDL BY prlkn /STATISTICS DESCRIPTIVES HOMOGENEITY /MISSING ANALYSIS /POSTHOC=SNK ALPHA(0.05).
Resources	Processor Time Elapsed Time	0:00:00.109 0:00:00.117

[DataSet0]

**Descriptives**

kadar LDL

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
nomal	5	.60	1.673	.748	-1.48	2.68	-2	2
positif	5	41.60	5.727	2.561	34.49	48.71	35	49
negatif	5	.20	1.924	.860	-2.19	2.59	-2	3
uji 1	5	47.20	4.712	2.107	41.35	53.05	40	53
uji 2	5	43.00	6.205	2.775	35.30	50.70	37	51
uji 3	5	43.00	3.873	1.732	38.19	47.81	39	48
Total	30	29.27	21.211	3.873	21.35	37.19	-2	53

**Test of Homogeneity of Variances**

kadar LDL

Levene Statistic	df1	df2	Sig.
2.742	5	24	.043

### ANOVA

kadar LDL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12587.867	5	2517.573	131.352	.000
Within Groups	460.000	24	19.167		
Total	13047.867	29			

#### Post Hoc Tests

#### Homogeneous Subsets

**kadar LDL**

Student-Newman-Keuls<sup>a</sup>

kelompok perlakuan	N	Subset for alpha = 0.05	
		1	2
negatif	5	.20	
nomal	5	.60	
positif	5		41.60
uji 2	5		43.00
uji 3	5		43.00
uji 1	5		47.20
Sig.		.886	.208

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 5.000.

**Lampiran 14. Konversi perhitungan dosis berbagai jenis hewan dan manusia**

**Tabel 12. Konversi perhitungan dosis berbagai jenis hewan dan manusia**

Diketahui	20 g mencit	200 g tikus	400 g marmot	1,5 kg Kelinci	2 kg kucing	4 kg Kera	12 kg anjing	70 kg manusia
<b>20 g mencit</b>	1,0	7,0	12,29	27,8	29,7	64,1	123,2	387,9
<b>200 g tikus</b>	0,14	1,0	1,74	3,3	4,2	9,2	17,8	56,0
<b>400 g marmot</b>	0,08	0,57	1,0	2,25	2,4	5,2	10,2	31,5
<b>1,5 kg Kelinci</b>	0,04	0,25	0,44	1,0	1,08	2,4	4,5	14,2
<b>2 kg kucing</b>	0,03	0,23	0,41	0,92	1,0	2,2	4,1	13,0
<b>4 kg Kera</b>	0,016	0,11	0,19	0,42	0,45	1,0	1,9	6,1
<b>12 kg anjing</b>	0,008	0,006	0,10	0,22	0,24	0,52	1,0	3,1
<b>70 kg manusia</b>	0,0026	0,018	0,031	0,07	0,026	0,16	0,32	1,0

(Soehardjono, 1993)

**Lampiran 15. Daftar volume maksimal bahan uji pada pemberian per oral**

**Tabel 13. Daftar volume maksimal bahan uji pada pemberian per oral**

Jenis hewan	Berat rerata (gram)	Volume maksimal (ml)
Mencit	20-30	1,0
Hamster	50	2,5
Tikus putih	100	5,0
Marmot	250	10,0
Kelinci	2500	20,0
Kucing	3000	50,0
Anjing	5000	100,0

(Ngatidjan, 1991)

**Lampiran 16. Luas kandang yang dianjurkan untuk hewan percobaan**

**Tabel 14. Luas kandang yang dianjurkan untuk hewan percobaan**

Jenis hewan	Berat (gram)	Luas lantai (cm <sup>2</sup> )	Tinggi (cm)
Mencit	< 10	38,71	12,70
	10 – 15	51,62	12,70
	15 – 25	77,12	12,70
	> 25	96,78	12,70
Hamster	< 60	45,61	15,24
	60 – 80	61,52	15,24
	80 – 100	83,88	15,24
	> 100	103,23	15,24
Tikus	< 100	96,78	17,78
	100 – 200	109,68	17,78
	200 – 300	118,10	17,78
	300 – 400	187,11	17,78
	400 – 500	256,08	17,78
	> 500	387,12	17,78
Marmut	≤ 350	122,59	17,78
	> 350	387,12	17,78

(Harmita & Maksum Radji 2005)

## Lampiran 17. Brosur HDL precipitant



### HDL Precipitant

Precipitation reagent for in vitro determination of high density lipoprotein cholesterol (HDL-C) according to the CHOD-PAP-method by photometric systems

#### Order Information

Cat. No.	Kit size
1 3540 99 83 885	250 mL Precipitation reagent
1 1350 99 83 021	R 5 x 25 mL + 1 x 3 mL Standard
1 1350 99 83 026	R 6 x 100 mL
1 1350 99 83 023	R 1 x 1000 mL
1 1300 99 83 030	6 x 3 mL Standard

#### Principle

Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to the sample. Centrifugation leaves only the HDL in the supernatant. Their cholesterol content is determined enzymatically using Cholesterol FS.

#### Reagents

##### Concentrations of the reagents

Phosphotungstic acid	1.4 mmol/L
Magnesium chloride	8.6 mmol/L

##### Storage instructions and reagent stability

The reagent is stable up to the end of the indicated month of expiry, if stored at 15 - 25 °C and contamination is avoided.

##### Warnings and precautions

Please refer to the safety data sheet and take the necessary precautions for the use of laboratory reagents.

##### Waste management

Please refer to local legal requirements.

##### Reagent Preparation

The precipitant is ready to use.

##### Material required but not provided

NaCl-Solution 9 g/L  
General laboratory equipment

##### Specimen

Serum, heparin plasma or EDTA plasma

Stability [5]:	7 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	3 months	at	-20 °C

Discard contaminated specimens!

#### Assay procedure

##### Precipitation

Sample/Standard	200 µL
Precipitation reagent	500 µL
Mix and incubate for 15 min. at room temperature, then centrifuge for 20 min. at 2500 g. Within 2 hours after centrifugation transfer 0.1 mL of the clear supernatant to the reaction solution for the determination of cholesterol.	

After centrifugation, the supernatant should be clear. Serum or plasma with triglyceride contents > 1000 mg/dL tends to produce turbid supernatants or floating precipitates. In this case dilute the sample 1 + 1 with NaCl solution (0.9 %) and then perform the precipitation. Multiply the result by 2.

##### Cholesterol determination

Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 - 25 °C, 37 °C
Measurement	Against reagent blank

	Standard	Sample
Supernatant	-	100 µL
Standard	100 µL	-
Cholesterol reagent	1000 µL	1000 µL
Mix and incubate for 10 min at room temperature or 5 min at 37 °C. Then measure the absorbance of the sample or the standard against the reagent blank value within 45 min.		

#### Calculation

##### With Standard

$$\text{HDL - Cholesterol [mg / dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Conc. Standard [mg / dL]}$$

The concentration of the standard is the concentration of the total cholesterol in the cholesterol standard solution.

##### Conversion factor

$$\text{Cholesterol [mg/dL]} \times 0.02586 = \text{Cholesterol [mmol/L]}$$

##### Controls

For internal quality control TruLab N and P or TruLab L controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruLab N	5 9000 99 83 062	20 x 5 mL
	5 9000 99 83 061	6 x 5 mL
TruLab P	5 9050 99 83 062	20 x 5 mL
	5 9050 99 83 061	6 x 5 mL
TruLab L	5 9020 99 83 065	3 x 3 mL

## Performance characteristics

### Measuring range

The test has been developed to determine HDL-Cholesterol concentrations up to 400 mg/dL. When values exceed this range samples should be diluted 1+4 with NaCl-Solution (9 g/L) and the result multiplied by 5.

### Specificity/Interferences

Bilirubin and hemoglobin interfere in low concentrations.

### Limit of detection

The lower limit of detection is 2 mg/dL.

### Precision

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	29	0.60	2.1
Sample 2	61	0.86	1.4
Sample 3	105	1.49	1.4

Inter-assay n = 10	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	27	0.80	3.0
Sample 2	45	0.51	1.1
Sample 3	56	1.34	2.4

### Method comparison

A comparison of Cholesterol FS + HDL Precipitant (y) with a commercially available cholesterol test + HDL precipitant (x) using 60 samples gave following results:  $y = 0.98 \times + 1.65 \text{ mg/dL}$ ;  $r = 0.996$ .

### Reference range [4]

HDL-Cholesterol  $\geq 35 \text{ mg/dL}$  (0.9 mmol/L)

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

### Clinical Interpretation [2]

Epidemiological studies have observed that low HDL-cholesterol concentrations  $< 39 \text{ mg/dL}$  (0.9 mmol/L) in men and  $< 43 \text{ mg/dL}$  (1.0 mmol/L) in women especially if associated with fasting triglycerides  $> 180 \text{ mg/dL}$  (2 mmol/L), predict a high risk of coronary heart disease. The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L).

## Literature

- Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
- Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998;19: 1434-503.
- Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977;23:882-4.
- Schaefer EJ, McNamara J. Overview of the diagnosis and treatment of lipid disorders. In: Rifai N, Warnick GR, Dominicak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press;1997.p.25-48.
- Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: GIT Verlag; 2001. p. 22-3.

## Manufacturer

DiASys Diagnostic Systems GmbH  
Alte Strasse 9 65558 Holzheim Germany  
Distributed by Diagnostika System Indonesia

## Lampiran 18. Brosur LDL precipitant



### LDL Precipitant

**Precipitation reagent for in vitro determination of LDL-Cholesterol with the CHOD-PAP method by photometric systems**

#### Order Information

Cat. No.	Kit size
1 4330 99 83 885	250 mL Precipitation reagent
1 1350 99 83 021	R 5 x 25 mL + 1 x 3 mL Standard
1 1350 99 83 026	R 6 x 100 mL
1 1350 99 83 023	R 1 x 1000 mL
1 1300 99 83 030	6 x 3 mL Standard

#### Principle

Low density lipoproteins (LDL) are precipitated by addition of heparin. High density lipoproteins (HDL) and very low density lipoproteins (VLDL) remain in the supernatant after centrifugation and are measured enzymatically by the CHOD-PAP method. The concentration of LDL cholesterol is calculated as the difference of total cholesterol and cholesterol in the supernatant.

#### Reagents

##### Concentrations of the reagents

Heparin	100 000 U/L
Sodium citrate	64 mmol/L

##### Storage instructions and reagent stability

The precipitant is stable up to the end of the indicated month of expiry, if stored at 2 - 8 °C and contamination is avoided. The standard is stable up to the end of the indicated month of expiry, if stored at 2 - 25 °C.

##### Warnings and precautions

Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

##### Waste management

Please refer to local legal requirements.

##### Reagent Preparation

The precipitant is ready to use.

##### Material required but not provided

NaCl-Solution 9 g/L  
General laboratory equipment

##### Specimen

###### Serum

Stability [5]:	7 days	at	20 - 25 °C
	7 days	at	4 - 8 °C
	3 months	at	-20 °C

Discard contaminated specimens!

#### Assay procedure

##### Precipitation

<b>Sample</b>	100 µL
<b>Precipitating reagent</b>	1000 µL
Mix and incubate for 15 min. at room temperature, then centrifuge for 20 min. at 2500 g. Within one hour after centrifugation transfer of 100 µL of the clear supernatant to the reaction solution for the determination of cholesterol.	

The cholesterol standard has to be diluted 1 + 10 with NaCl (9 g/L). After dilution the standard is treated like the supernatant.

##### Cholesterol determination

Wavelength	500 nm, Hg 546 nm
Optical path	1 cm
Temperature	20 - 25 °C, 37 °C
Measurement	Against reagent blank

	Standard	Sample
<b>Supernatant</b>	-	100 µL
<b>Standard</b>	100 µL	-
<b>Cholesterol reagent</b>	1000 µL	1000 µL
Mix and incubate 10 min. at room temperature or 5 min at 37 °C, read absorbance of the sample for the standard within 45 min. against reagent blank.		

#### Calculation

##### Cholesterol in supernatant

$$\text{Cholesterol in supernatant [mg/dL]} = \frac{\Delta E_{\text{Sample}}}{\Delta E_{\text{Standard}}} \times \text{Conc. Standard [mg/dL]}$$

The standard concentration is the concentration of the total cholesterol in the cholesterol standard solution.

##### LDL Cholesterol

$$\text{LDL-Cholesterol [mg/dL]} = \text{total cholesterol [mg/dL]} - \text{Cholesterol in the supernatant [mg/dL]}$$

##### Controls

For internal quality control TruLab N and P or TruLab L controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruLab N	5 9000 99 83 062	20 x 5 mL
	5 9000 99 83 061	6 x 5 mL
TruLab P	5 9050 99 83 062	20 x 5 mL
	5 9050 99 83 061	6 x 5 mL
TruLab L	5 9020 99 83 065	3 x 3 mL

## Performance characteristics

### Measuring range

The test has been developed to determine LDL-Cholesterol concentration until 400 mg/dL. When values exceed this range samples should be diluted 1+4 with NaCl-Solution (9 g/L) and the result multiplied by 5.

### Specificity/Interferences

No interference was observed by bilirubin up to 30 mg/dL and hemoglobin up to 800 mg/dL.

### Limit of detection

The lower limit of detection is 2 mg/dL.

### Precision

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	20	0.81	4.1
Sample 2	57	2.47	4.3
Sample 3	141	1.39	1.0

Inter-assay n = 10	Mean [mg/dL]	SD [mg/dL]	VK [%]
Sample 1	62	1.90	3.0
Sample 2	131	2.80	2.1
Sample 3	283	2.09	0.7

### Method comparison

A comparison of LDL-Cholesterol values obtained with the LDL precipitant (y) with the Friedewald calculation (x) using 49 samples gave following results:  
 $y = 1.121 x - 9.62 \text{ mg/dL}$ ;  $r = 0.947$ .

### Reference range [4]

#### LDL cholesterol

Desirable	$\leq 130 \text{ mg/dL}$ (3.4 mmol/L)
Borderline high risk	$130\text{--}160 \text{ mg/dL}$ (3.4 – 4.1 mmol/L)
High risk	$> 160 \text{ mg/dL}$ ( $> 4.1 \text{ mmol/L}$ )

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

### Clinical Interpretation [2]

Epidemiological studies have observed that low HDL-cholesterol concentrations  $< 39 \text{ mg/dL}$  (0.9 mmol/L) in men and  $< 43 \text{ mg/dL}$  (1.0 mmol/L) in women especially if associated with fasting triglycerides  $> 180 \text{ mg/dL}$  (2 mmol/L), predict a high risk of coronary heart disease. The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL-cholesterol to less than 115 mg/dL (3.0 mmol/L).

## Literature

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