

BAB VI

RINGKASAN

Penelitian ini untuk mengetahui efek sitotoksik fraksi etil asetat ekstrak etanol umbi sarang semut terhadap sel T47D dan proliferasi limfosit serta melihat pengaruh efek sitotoksik kombinasi fraksi etil asetat ekstrak etanol umbi sarang semut dan doksorubisin terhadap sel T47d dan sel Vero. Sarang semut yang digunakan pada penelitian ini berasal dari Desa Soya, Ambon. Sarang semut diidentifikasi di Laboratorium Biologi Farmasi, Fakultas Farmasi UGM.

Umbi sarang semut dimaserasi dengan pelarut etanol 96%. Kemudian difraksinasi dengan metode ekstraksi cair-cair menggunakan corong pisah, selanjutnya hasil fraksinasi diidentifikasi kandungan flavonoid, fenol dan steroid/terpenoid. Hasil identifikasi menunjukkan hasil yang positif untuk senyawa-senyawa tersebut. Senyawa-senyawa tersebut diduga bertanggung jawab atas aktivitas antiikanker.

Hasil penelitian menunjukkan bahwa fraksi etil asetat ekstrak etanol umbi sarang semut (FEE) bersifat sitotoksik terhadap sel kanker T47D dan meningkatkan proliferasi limfosit. Uji kombinasi FEE dan doksorubisin menunjukkan FEE dapat meningkatkan efek sitotoksik terhadap sel T47D serta menurunkan efek sitotoksik terhadap sel Vero dari doksorubisin.

DAFTAR PUSTAKA

- Abbas A, Lichtman AH, Pober JS. 2005. *Cellular and Molecular Immunology 5th ed.* Philadelphia : Elsevier-Saunders.
- Abcam, 2014. *T47D (Human ductal breast epithelial tumor cell line) Whole Cell Lysate (ab14899)*. <http://www.abcam.com/index.html?datasheet=14899>. [Agustus 2014]
- Abdolmohammadi MH, Fouladdel Sh, Shafiee A, Amin Gh, Ghaffar SM, and Azizi E. 2008. Anticancer Effects and Cell Cycle Analysis on Human Breast Cancer T47D Cells Treated with Extract of *Astrodaucus persicus* (Boiss.) Drude in Comparison to Doksorubisin. *DARU*, 16:2.
- Benzivin, C. Devehat. Tomasi, S. Boustie, J. 2003. Cytotoxic Activities of some Lischen Extracts on Murine and Humasn Cancer Cell Line. *Phytomedicine* 10: 499-503.
- Burdall ES, Hanby MA, Landsdown RJM, Speirs V. 2003. *Breast Cancer Cell Line*. *Breast Cancer Res*5(2): 89-95.
- Brunton L, Laso JS, Parker KL. 2005. *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11 Edition*. McGraw Hill: Lange.
- CCRC. 2012. Prosedur Tetap Uji Sitotoksik Metode MTT. CANCER CHEMOPREVENTION RESEARCH CENTER FAKULTAS FARMASI UGM. <http://www.crc.farmasi.ugm.ac.id/wp-content/uploads/03.010.-Sitotoksik.pdt> (3 maret 2015).
- Chiang LC, Ng LT, Chiang W, ChangMY, Lin CC. 2003. Immunomodulatory activities offlavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenoliccompounds of plantago species. *PlantaMedica*69: 600-604.
- Child AC, Phaneuf SL, Dirks AJ, Phillips T. 2002. Doxorubicin Treatment in Vivo Causse Cytochrome c Release and Cardiomyocyte Apoptosis, As Well As Increased Mithochodrial Efficiense, Superoxide Dismutase Activity and Bcl-2: Bax Ratio. *Cancer Research* 62: 4592-4598.
- Constatinides P. 1994. *General Pathobiologi*. Connecticut: Appleton & Lange.
- Contran RS, Kumar V, Robbins SL. 1994. *Pathologic basis of disease. 5th ed.* Philadelphia: WB Saunders.
- Corwin EJ. 2009. *Buku Saku Patofisiologi Corwin*. Jakarta: Aditya Media.

- Djajanegara I, Wahyudi P. 2010. Uji Sitotoksisitas Ekstrak Etanol Herba Ceplukan(*Physalis angulata* Linn.) terhadap Sel T47D secara In Vitro.*Jurnal Ilmu KefarmasianIndonesia* 8 (1): 41-47
- Depkes RI. 1979. *Farmakope Indonesia Edisi III*. Jakarta : Depertemen Kesehatan Republik Indonesia.
- Depkes RI. 1985. *Cara Pembuatan Simplisia*. Jakarta : Depertemen Kesehatan Republik Indonesia.
- Depkes RI. 1986. *Sedian Galenik*. Jakarta: Depertemen Kesehatan Republik Indonesia.
- Doyle A, andGriffith JB. 2000. *Cell and Tissue Culture for Medical Research*. John Wil, New York.
- Ellis EO, Schnitt SJ, Garau X, Bussolati G, Tavassaoli FA, Eusebi V. 2003. Pathology and Genetic of Tumours of The Breast and Female Genital Organs / WHO Classification of Tumours.*IARC Press P.10* : 34-6.
- Goodman JW. 1998. *The immune response*. In: Stites DP, Terr A I editors. Basic and Clinical Immunologi. 8th ed. USA : Prentice-Hall Int.
- Gunawan D, Mulyani S. 2004. *Ilmu Obat Alam*. Jakarta : Penebar Swadaya.
- Han X, Pan J, Ren D, Cheng Y, Fan P, Lou H. 2008. Naringenin-7-O-glucoside protect againt doxorubicin-induced toxicity in H9c2 cardiomyocytes by induction of endogenous antioxidant enzymes. *Food and Chemical Toxicology* 46 : 3140-3246.
- Handayani L, Suharmiati, Ayuningtyas A. 2012 . *Menaklukan Kanker Serviks dan Kanker Payudara dengan 3 Terapi Alami*. Jakarta : Agro Media Pustaka.
- Hay, Westwood. 2002.*Practical Immunology, 4th edition, 214*. Malden : Blackwell Science
- Harborne JB. 1987. *Metode Fitokimia*.Bandung : ITB.
- Hertiani T, Sasmito E, Sumardi, Ulfah M. 2010. Preliminary Study on Immunomodulator Effect of Sarang-Semut Tubers *Myrmecodia tuberosa* and *Myrmecodia pendens*.*Online Journal of Biological Sciences* 10(3): 136-141.
- Kresno S. 2003. *Ilmu Dasar Onkologi*. Jakarta :PT Quparada Makuda Perkasa.

- Mangan Y. 2009. *Solusi Sehat Mencegah dan Mengatasi Kanker*. Jakarta : Agro Media Pustaka.
- Megaputri PYP. 2012. Uji sitotoksisitas ekstrak etanol sarang semut (*Myrmecodia pendes* Merr & Perry) terhadap karsinoma mammae pada kultur sel MCF-7 [Karya Tulis Ilmiah]. Bandung: Fakultas Kedokteran, Universitas Kristen Maranatha.
- Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. 2004. Anthracyclins: Molecular Advances and Pharmacologic Developments in Antitumor Activity and Cardiotoxicity. *Pharmacol Rev* 56:185-228.
- Mosmann T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *Journal of Immunological Method* 65 : 65-69.
- Muharni, Dachriyanus, Husein HB, Supriyatna. 2011. *Potensi Tumbuhan Manggis Hutan (Garcinia bancana Miq.) sebagai sumber senyawa antikanker*.<http://eprints.unsri.ac.id/201/3/Seminar%2520Solo%25202011.pdf> [22 Januari 2015].
- Mulja HM, Suherman. 1995. Analisis Instrumental. *Airlangga University Press* 145 -146.
- Munasir Z. 2001. Respon Imun Terhadap Infeksi Bakteri. *Sari Pediatri Vol. 2 No. 4 : 193 – 197*.
- Namgoong SY, Son KH, Chang HW, Kang SS, Kim HP. 1994. Effects of Naturally Occurring Flavonoids on Mitogen-Induced Lymphocyte Proliferation and Mixed Lymphocyte Culture. *Life Sci*, 54: 313-320.
- Phonnok, S, Uthaisang T, Thanomsub W. 2010. Anticancer and Apoptosis-Inducing Activities of Microbial Metabolites. *Electronic J. of Biotechnology*, 13(5):1-12.
- Purwoastuti E. 2012. *Kesehatan Masyarakat Kanker Payudara*. Yogyakarta : Kanisius.
- Ren W, Qiao Z, Wang H, Zhu L, Zhang L. 2003. Flavonoids: Promising Anticancer Agents. *Medicinal Research Review*, 23(4): 519-534
- Rihardni MI. 2013. Aktivitas Ekstrak dan Fraksi Tumbuhan Sarang Semut (*Myrmecodiaa tuberosa* Jack.) Terhadap *Quorum Sensing Pseudomonas aeruginosa* PAO1 secara *In Vitro* [Skripsi]. Yogyakarta: Fakultas Farmasi, Universitas Gadjah Mada.

- Roit IM. 1994. *Essential Immunology*. 8th ed. Barcelona : Times Mirror International.
- Saifudin A, Rahayu V, Teruna HY. 2010. *Standarisasi Bahan Obat Alam*. Yogyakarta : Graha Ilmu.
- Sano A, Saraphanchotiwitthaya A, Ingkaninan L, Sripalakit P. 2007. Immunomodulating Activity of Thai Rejuvenating Plants. *Naresuan University Journal* 15 (3) : 149-157
- Sarjadi. 1985. Karsinoma epidermoid serviks uteri (Beberapa aspek epidemiologi serta peran histopatologi dan petanda dalam penentuan prognosis) [Disertasi]. Semarang: Universitas Diponegoro.
- Setiawati A, Susidarti RA, Meiyanto E. 2011. Peningkatan efek sitotoksik doxorubicin oleh hisperidin pada sel T47D. *Bionatura – Jurnal ilmu-ilmu hayati dan fisik*. Vol. 13, No. 2, juli 2011 : 85 – 92.
- Soeksmanto A, Subroto MA, Wijaya H, Simanjuntak P. 2010. Anticancer Activity Test Extracts of Sarang Semut Plant (*Myrmecodya pendens*) to Hela and MCM-B2 Cells. *Pakistan Journal of Biological Sciences* 13 (3): 148-151.
- Sudewo B. 2009. *Buku Pintar Hidup Sehat Cara Mas Dewo*. Jakarta : Agro Media Pustaka.
- Sumardi, Triana H., Ediati S., 2013, Ant Plant (*Myrmecodia tuberosa*) HypocotyExtract Modulates TCD4+ and TCD8+ Cell Profile of DoxorubicinInduced Immune-Suppressed Sparague Dawley Rats In Vivo, *ScientiaPharmaceutica*, 81(4): 1057-1069.
- Staerk D, Lykkeberg AK, Christensen J, Budnik BA, Abe F, Jaroszewski JW. 2002. In Vitro cytotoxic activity of Phenanthroindolizidine alkaloids from *cynanchum vincentoxicum* and *Tylophora tanake* against Drug sensitive and multidrug Resistant Cancerl cells. *Jurnal Nat. Prood* 65: 1299-1302.
- Subroto MA, Saputro H. 2006. *Gempur Penyakit dengan sarang semut*. Depok : Penebar Swadaya.
- Sumorno. 2010. Pengaruh ekstrak sarang semut (*Myrmecodia pendens* Merr. & Perry) terhadap aktifitas proliferasi sel dan indeks apoptosis kanker payudara mencit C3H [Tesis]. Semarang: Ilmu Patologi Anatomi, Universitas Diponegoro.

- Syamsudin, Simanjuntak P. 2012. Cytotoxic activity of triterpenoid fraction of Indonesian propolis on human breast carcinoma cell lines. *Asian journal of pharmaceutical and clinical research*. Vol 5, Suppl 4, 2012, 168-169.
- Tim Cancer Helps. 2010. *Stop Kanker*. Jakarta: PT AgroMedia Pustaka.
- Trihartono. 2009. *The Doctor: Catatan Hati Seorang Dokter*. Yogyakarta: Pustaka Angrek.
- Utomo AB, Suprijono A, Risdianto A. 2011. Uji aktivitas antioksidan kombinasi ekstrak sarang semut (*Myrmecodia pendans*) & ekstrak teh hitam (*Camellia sinensis O.K.var.assamica (mast.)*) DENGAN METODE DPPH. <http://journal.stifar.ac.id/ojs/index.php/js/article/view/6/7> [6 Agustus 2014].
- Wagner H, Bland S. 1996. *Plant Drug Analysis; A Thin Layer Chromatography Atlas. 2nd Edition*. Berlin Heidelberg: Springer.
- Whiteside TL, Haberman RB. 1990. *Characteristics of natural killer cell and lymphokine activated killer cells*. In: Oeitgen H.F editor. *Human cancer immunology*. Philadelphia : WB Saunders Company.
- Wijayakusuma HMH. 2008. *Atasi Kanker dengan Tanaman Obat*. Jakarta : Puspa Swara.
- Willey J, Sons. 2008. Vero cell. *Curr Protoc. Microbiol* 11:A.4E.1-A.4E.7.
- Yayasan Kanker Indonesia. 2006. *Informasi Dasar Tentang Kanker*. Jakarta : Yayasan Kanker Indonesia.
- Zampieri, L, Bianchi, P, Ruff, P, Arbuthnot P, 2002. Differential modulation by estradiol of P-glycoprotein drug resistance protein expression in cultured MCF7 and T47D breast cancer cells. *Anticancer Res* 22(4):2253-9.
- Zhang XY, Li WG, WuYJ and Gao M.T. 2005. Amelioration of Doxorubicin-Induced Myocardial Oxidative Stress and Immunosuppression by Grape Seed Proanthocyanidins in Tumour-Bearing Mice. *J Pharm Pharmacol*, 57(8):1043-52.

LAMPIRAN

Lampiran 1. Hasil identifikasi sarang Semut



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SURAT KETERANGAN

No.: BF/313 / Ident/Det/XIII/2014

Kepada Yth. :
Sdri/Sdr. Karol Giovani B.L.
NIM. SBF 041310049
Fakultas Farmasi USB
Di Surakarta

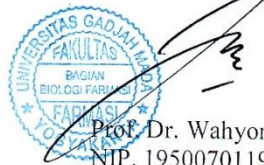
Dengan hormat,

Bersama ini kami sampaikan hasil identifikasi/determinasi sampel yang Saudara kirimkan ke Bagian Biologi Farmasi, Fakultas Farmasi UGM, adalah :

No.Pendaftaran	Jenis	Suku
313	<i>Myrmecodia tuberosa</i> (non Jack) Bl.	Rubiaceae

Demikian, semoga dapat digunakan sebagaimana mestinya.

Yogyakarta, 21 Agustus 2014
Ketua



Prof. Dr. Wahyono, SU., Apt.
NIP. 195007011977021001 *Z*

Lampiran 2. Foto sarang semut



Tumbuhan Sarang semut utuh



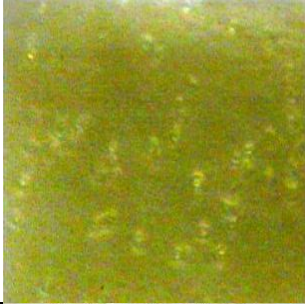
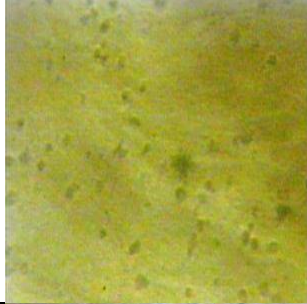


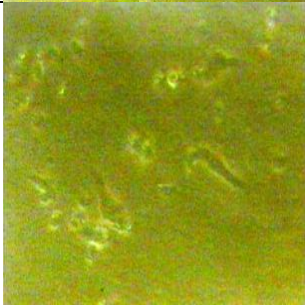
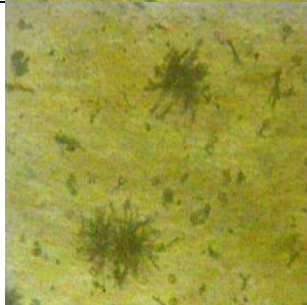
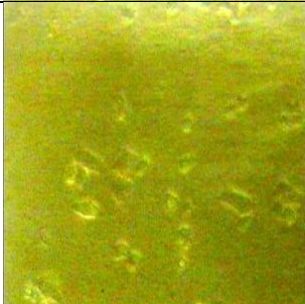

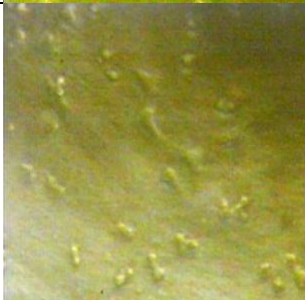
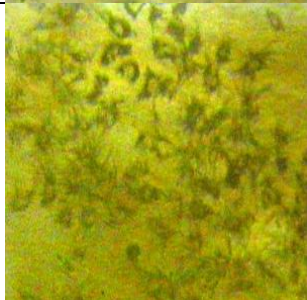
Umbi sarang semut yang telah dipotong

Lampiran 3. Hasil perhitungan Rf

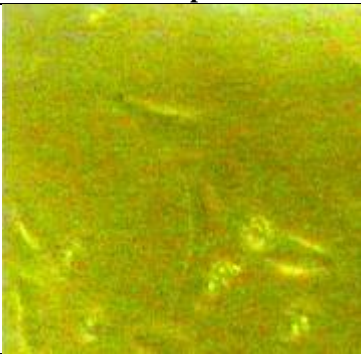
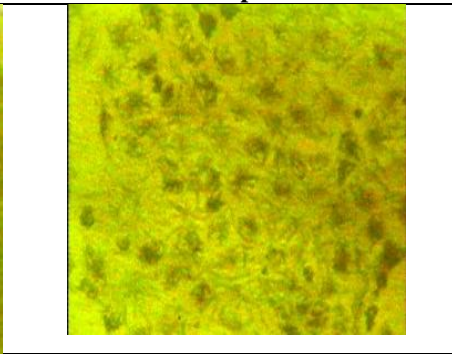
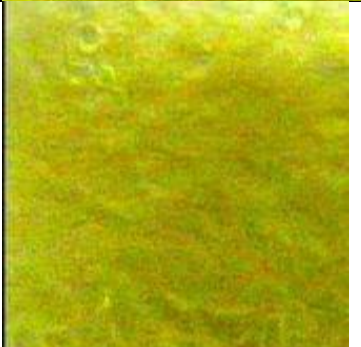
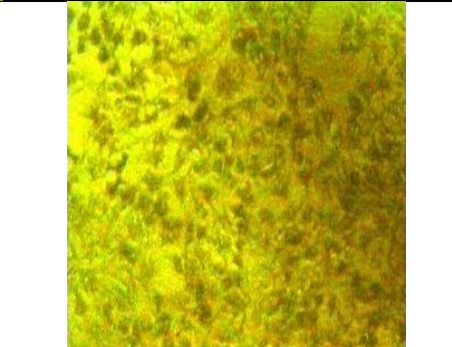
No.	Identifikasi Senyawa	Jarak yang ditempuh oleh senyawa		Jarak yang ditempuh oleh pelarut		Rf	
		FEE	Pembanding	FEE	Pembanding	FEE	Pembanding
1.	Flavonoid	5,2	5,0	8	8	0,65	0,63
2.	Fenolik	7,5	7,7	8	8	0,94	0,96
3.	Steroid	4,2	4,2	8	8	0,53	0,53

$$Rf = \frac{\text{Jarak yang ditempuh oleh senyawa dari titik asal}}{\text{Jarak yang ditempuh oleh pelarut dari titik asal}}$$




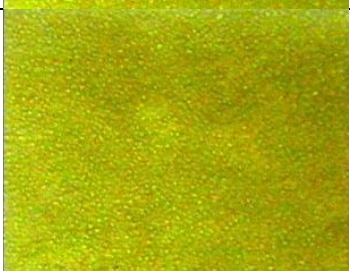

Lampiran 4. Foto uji sitotoksik kombinasi FEE dan doksorubisin terhadap sel T47D

Perlakuan	Setelah perlakuan	Sebelum perlakuan
FEE 14,28 $\mu\text{g/ml}$ + Doksorubisin 0,005 $\mu\text{g/ml}$		
FEE 7,14 $\mu\text{g/ml}$ + Doksorubisin 0,005 $\mu\text{g/ml}$		
FEE 3,57 $\mu\text{g/ml}$ + Doksorubisin 0,005 $\mu\text{g/ml}$		
FEE 1,78 $\mu\text{g/ml}$ + Doksorubisin 0,005 $\mu\text{g/ml}$		
Doksorubisin 0,005 $\mu\text{g/ml}$		

Lampiran 5. Foto Uji sitotoksik kombinasi FEE dan doksorubisin terhadap Vero

Perlakuan	Sebelum perlakuan	Setelah perlakuan
Kombinasi FEE dan doksorubisin	 Micrograph showing a confluent monolayer of Vero cells before treatment. The cells are healthy and exhibit typical epithelial morphology.	 Micrograph showing Vero cells after treatment with the combination of FEE and doxorubicin. The cells appear significantly more rounded and condensed, indicating cytotoxicity.
Doksorubisin	 Micrograph showing a confluent monolayer of Vero cells before treatment with doxorubicin. The cells are healthy and exhibit typical epithelial morphology.	 Micrograph showing Vero cells after treatment with doxorubicin. The cells appear significantly more rounded and condensed, indicating cytotoxicity.

Lampiran 6. Foto Uji proliferasi limfosit

Perlakuan		
FEE 28,56 $\mu\text{g/ml}$		
FEE 14,28 $\mu\text{g/ml}$		
FEE 7,14 $\mu\text{g/ml}$		
FEE 3,57 $\mu\text{g/ml}$		
FEE 1,78 $\mu\text{g/ml}$		

Lampiran 7. Perhitungan IC₅₀FEE

Data Hasil Uji sitotoksik FEE terhadap sel kanker payudara T47D

Kontrol sel dan kontrol media

Absorbansi Kontrol sel	A	Absorbansi kontrol media	B	(A – B)
0,688		0,154		
0,659		0,153		
0,645		0,155		
0,623	0,650	0,158	0,148	0,502
0,689		0,136		
0,658		0,136		
0,632		0,141		
0,609		0,149		

Keterangan :

A : Rata-rata absorbansi kontrol sel

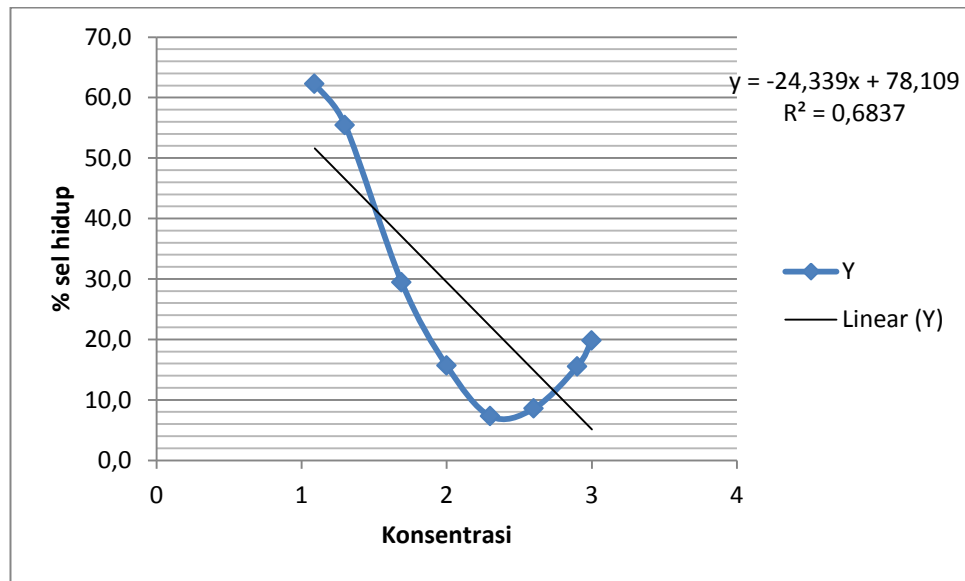
B : Rata-rata absorbansi kontrol media

Absorbansi setelah perlakuan

Konsentrasi	Absorbansi			
1000	0,252	0,248	0,243	0,246
800	0,235	0,227	0,223	0,218
400	0,200	0,197	0,186	0,180
200	0,190	0,189	0,177	0,182
100	0,237	0,229	0,225	0,215
50	0,314	0,301	0,288	0,28
25	0,434	0,419	0,435	0,418
12,5	0,496	0,469	0,451	0,427

Porsentase kehidupan sel T47D

Konsentrasi	% sel hidup				Rata-rata %sel hidup
	$\frac{(\text{Absorbansi perlakuan} - \text{Absorbansi kontrol media}) \times 1}{(\text{Absorbansi kontrol sel} - \text{Absorbansi kontrol media})}$				
1000	20,741	19,945	18,950	19,547	19,796
800	17,358	15,767	14,971	13,976	15,518
400	10,395	9,798	7,6100	6,4163	8,555
200	8,405	8,206	5,8194	6,814	7,311
100	17,756	16,165	15,369	13,379	15,667
50	33,076	30,489	27,903	26,311	29,445
25	56,951	53,966	57,149	53,767	55,458
12,5	69,286	63,914	60,333	55,558	62,273



Grafik persentase sel hidup vs konsentrasi

Grafik log konsentrasi FEE vs %sel hidup

Persamaan $Y = -24,33X + 78,10$

Untuk mencari nilai IC_{50} , maka $Y = 50$

Antilog $X = 14,28$

Maka nilai IC_{50} FEE terhadap sel T47D adalah $14,28 \mu\text{g/ml}$.

Lampiran 8. Perhitungan IC₅₀ Dokсорubisin terhadap sel T47D

Data Hasil Uji sitotoksik Dokсорubisin terhadap sel kanker payudara T47D

Kontrol sel dan kontrol media

Absorbansi Kontrol sel	A	Absorbansi kontrol media	B	(A – B)
0,648		0,102		
0,634	0,641	0,102	0,100	0,541
0,643		0,098		

Keterangan :

A : Rata-rata absorbansi kontrol sel

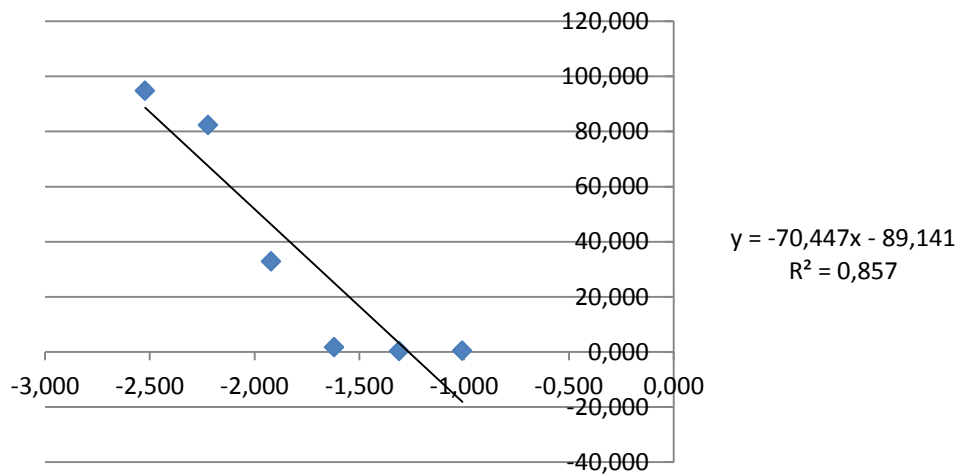
B : Rata-rata absorbansi kontrol media

Absorbansi setelah perlakuan

Konsentrasi	Absorbansi		
0,098	0,105	0,101	0,103
0,049	0,102	0,103	
0,024	0,108	0,113	0,108
0,012	0,274	0,309	0,251
0,006	0,511	0,529	0,597
0,003	0,607	0,636	0,596

Porsentase sel hidup

Konsentrasi	% sel hidup			Rata-rata %sel hidup
	$\frac{(\text{Absorbansi perlakuan} - \text{Absorbansi kontrol media}) \times 100\%}{(\text{Absorbansi kontrol sel} - \text{Absorbansi kontrol media})}$			
0,098	0,801	0,062	0,431	0,431
0,049	0,246	0,431		0,339
0,024	1,356	2,280	1,356	1,664
0,012	32,039	38,509	27,788	32,779
0,006	75,847	79,174	91,744	82,255
0,003	93,592	98,953	91,559	94,701



Grafik log konsentrasi doksorubisin vs % sel hidup

$$Y = -70,44X - 89,14$$

Maka $X = -1,97$

Antilog $X = 0,01$

Maka nilai IC_{50} doksorubisin terhadap sel T47D adalah 0,01

Lampiran 9. Uji sitotoksik kombinasi FEE dan doksorubisin terhadap sel T47D

Data Hasil Uji sitotoksik kombinasi FEE dan doksorubisin terhadap sel kanker payudara T47D

Kontrol sel dan kontrol media

Absorbansi Kontrol sel	A	Absorbansi kontrol media	B	(A – B)
0,783		0,133		
0,783	0,780667	0,136	0,135667	0,645
0,776		0,138		

Keterangan :

A : Rata-rata absorbansi kontrol sel

B : Rata-rata absorbansi kontrol media

Absorbansi setelah perlakuan

Kelompok Uji	Absorbansi*		
A	0,224	0,217	0,227
B	0,290	0,306	0,321
C	0,366	0,382	0,412
D	0,460	0,459	0,467
E	0,472	0,484	0,469
F	0,470	0,477	0,473

Keterangan :

A : Kombinasi 28,56µg/ml FEE dan 0,005 µg/ml doksorubisin

B : Kombinasi 14,28 µg/ml FEE dan 0,005 µg/ml doksorubisin

C : Kombinasi 7,14 µg/ml FEE dan 0,005 µg/ml doksorubisin

D : Kombinasi 3,57µg/ml FEE dan 0,005 µg/ml doksorubisin

E : Kombinasi 1,78µg/ml FEE dan 0,005 µg/ml doksorubisin

F : 0,005 µg/ml doksorubisin

* : data diperoleh dari replikasi sebanyak tiga kali untuk masing-masing kelompok uji

Persentase sel hidup dari sel kanker payudara sel T47D

Kelompok uji	% sel hidup			Rata-rata %sel hidup
	$(\text{Absorbansi perlakuan} - \text{Absorbansi kontrol media}) \times 100\%$ $(\text{Absorbansi kontrol sel} - \text{Absorbansi kontrol media})$			
A	13,695	12,610	14,160	13,488
B	23,928	26,408	28,734	26,357
C	35,711	38,191	42,842	38,915
D	50,284	50,129	51,370	50,594
E	52,145	54,005	51,680	52,610
F	51,835	52,920	52,300	52,351

Uji statistik

1. *One-Sample Kolmogrov-Smirnov Test* (Uji normalitas)

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Persensel	18	3.90526E1	15.280544	12.610	54.005

One-Sample Kolmogorov-Smirnov Test

		persensel
N		18
Normal Parameters ^a	Mean	39.05261
	Std. Deviation	1.528054E1
Most Extreme Differences	Absolute	.266
	Positive	.164
	Negative	-.266
Kolmogorov-Smirnov Z		1.127
Asymp. Sig. (2-tailed)		.157
a. Test distribution is Normal.		

Hasil analisis dengan *One-Sample Kolmogrov-Smirnov Test* diperoleh signifikansi = $0,157 > 0,05$. Berarti data tersebut mengikuti distribusi normal sehingga dapat dilakukan analisis variansi.

2. *One-Way Anova*

ANOVA

Persensel	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	3925.852	5	785.170	216.283	.000
Within Groups	43.563	12	3.630		
Total	3969.416	17			

Hasil Anova diperoleh nilai signifikansi = $0,00 < 0,05$ berarti perbedaan kelompok uji menunjukkan adanya perbedaan persen sel hidup

3. *Tukey test* dan *Bonferroni test*

Multiple Comparisons

Dependent Variable: persensel

	(I)	(J)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	A	B	-12.868333 [*]	1.555697	.000	-18.09380	-7.64287
		C	-25.426333 [*]	1.555697	.000	-30.65180	-20.20087

	D	-37.106000	1.555697	.000	-42.33146	-31.88054
	E	-39.121667	1.555697	.000	-44.34713	-33.89620
	F	-38.863333	1.555697	.000	-44.08880	-33.63787
B	A	12.868333	1.555697	.000	7.64287	18.09380
	C	-12.558000	1.555697	.000	-17.78346	-7.33254
	D	-24.237667	1.555697	.000	-29.46313	-19.01220
	E	-26.253333	1.555697	.000	-31.47880	-21.02787
	F	-25.995000	1.555697	.000	-31.22046	-20.76954
C	A	25.426333	1.555697	.000	20.20087	30.65180
	B	12.558000	1.555697	.000	7.33254	17.78346
	D	-11.679667	1.555697	.000	-16.90513	-6.45420
	E	-13.695333	1.555697	.000	-18.92080	-8.46987
	F	-13.437000	1.555697	.000	-18.66246	-8.21154
D	A	37.106000	1.555697	.000	31.88054	42.33146
	B	24.237667	1.555697	.000	19.01220	29.46313
	C	11.679667	1.555697	.000	6.45420	16.90513
	E	-2.015667	1.555697	.782	-7.24113	3.20980
	F	-1.757333	1.555697	.860	-6.98280	3.46813
E	A	39.121667	1.555697	.000	33.89620	44.34713
	B	26.253333	1.555697	.000	21.02787	31.47880
	C	13.695333	1.555697	.000	8.46987	18.92080
	D	2.015667	1.555697	.782	-3.20980	7.24113
	F	.258333	1.555697	1.000	-4.96713	5.48380
F	A	38.863333	1.555697	.000	33.63787	44.08880
	B	25.995000	1.555697	.000	20.76954	31.22046
	C	13.437000	1.555697	.000	8.21154	18.66246
	D	1.757333	1.555697	.860	-3.46813	6.98280
	E	-.258333	1.555697	1.000	-5.48380	4.96713
Bonferroni	A					
	B	-12.868333	1.555697	.000	-18.54490	-7.19177
	C	-25.426333	1.555697	.000	-31.10290	-19.74977
	D	-37.106000	1.555697	.000	-42.78257	-31.42943
	E	-39.121667	1.555697	.000	-44.79823	-33.44510
	F	-38.863333	1.555697	.000	-44.53990	-33.18677
B	A	12.868333	1.555697	.000	7.19177	18.54490
	C	-12.558000	1.555697	.000	-18.23457	-6.88143
	D	-24.237667	1.555697	.000	-29.91423	-18.56110
	E	-26.253333	1.555697	.000	-31.92990	-20.57677
	F	-25.995000	1.555697	.000	-31.67157	-20.31843
C	A	25.426333	1.555697	.000	19.74977	31.10290
	B	12.558000	1.555697	.000	6.88143	18.23457
	D	-11.679667	1.555697	.000	-17.35623	-6.00310
	E	-13.695333	1.555697	.000	-19.37190	-8.01877

	F	-13.437000 [*]	1.555697	.000	-19.11357	-7.76043
D	A	37.106000 [*]	1.555697	.000	31.42943	42.78257
	B	24.237667 [*]	1.555697	.000	18.56110	29.91423
	C	11.679667 [*]	1.555697	.000	6.00310	17.35623
	E	-2.015667	1.555697	1.000	-7.69223	3.66090
	F	-1.757333	1.555697	1.000	-7.43390	3.91923
E	A	39.121667 [*]	1.555697	.000	33.44510	44.79823
	B	26.253333 [*]	1.555697	.000	20.57677	31.92990
	C	13.695333 [*]	1.555697	.000	8.01877	19.37190
	D	2.015667	1.555697	1.000	-3.66090	7.69223
	F	.258333	1.555697	1.000	-5.41823	5.93490
F	A	38.863333 [*]	1.555697	.000	33.18677	44.53990
	B	25.995000 [*]	1.555697	.000	20.31843	31.67157
	C	13.437000 [*]	1.555697	.000	7.76043	19.11357
	D	1.757333	1.555697	1.000	-3.91923	7.43390
	E	-.258333	1.555697	1.000	-5.93490	5.41823

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Persensel

Sediaan jia	N	Subset for alpha = 0.05				
		1	2	3	4	
Tukey HSD ^a	A	3	1.34883E1			
	B	3		2.63567E1		
	C	3			3.89147E1	
	D	3				5.05943E1
	F	3				5.23517E1
	E	3				5.26100E1
	Sig.			1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Terlihat keenam kelompok uji terbagi dalam empat subset, yang menunjukkan :

1. Kelompok uji D, E dan F tidak mempunyai perbedaan yang nyata, karena berada dalam satu subset.
2. Kelompok A, B, C, D/E/F mempunyai perbedaan yang nyata, karena tidak berada dalam satu subset.

Keterangan :

Lampiran 10. Uji sitotoksik kombinasi FEE dan doksorubisin terhadap sel Vero

Data Hasil Uji sitotoksik kombinasi FEE dan doksorubisin terhadap Sel Vero

Kontrol sel dan kontrol media

Absorbansi Kontrol sel	A	Absorbansi kontrol media	B	(A – B)
0,658		0,275		
0,640	0,640667	0,271	0,276	0,364667
0,624		0,282		

Keterangan :

A : Rata-rata absorbansi kontrol sel

B : Rata-rata absorbansi kontrol media

Absorbansi setelah perlakuan

Kelompok Uji	Absorbansi*		
A	0,581	0,583	0,599
B	0,612	0,604	0,576
C	0,603	0,586	0,591
D	0,568	0,577	0,584
E	0,594	0,566	0,613
F	0,282	0,282	0,281

Keterangan :

A : Kombinasi 28,56µg/ml FEE dan 0,005 µg/ml doksorubisin

B : Kombinasi 14,28 µg/ml FEE dan 0,005 µg/ml doksorubisin

C : Kombinasi 7,14 µg/ml FEE dan 0,005 µg/ml doksorubisin

D : Kombinasi 3,57µg/ml FEE dan 0,005 µg/ml doksorubisin

E : Kombinasi 1,78µg/ml FEE dan 0,005 µg/ml doksorubisin

F : 0,005 µg/ml doksorubisin

* : data diperoleh dari replikasi sebanyak tiga kali untuk masing-masing kelompok uji

Persentase sel hidup dari sel normal sel Vero

Kelompok uji	% sel hidup			Rata-rata %sel hidup
	$(\text{Absorbansi perlakuan} - \text{Absorbansi kontrol media}) \times 100\%$ $(\text{Absorbansi kontrol sel} - \text{Absorbansi kontrol media})$			
A	47,607	47,919	50,416	48,647
B	52,445	51,197	46,826	50,156
C	51,041	48,387	49,168	49,532
D	45,578	46,982	48,075	46,878
E	49,636	45,265	52,601	49,168
F	0,937	0,937	0,780	0,884

Uji statistik

1. *One-Sample Kolmogrov-Smirnov Test*(Uji normalitas)

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Persensel	18	42.6555	20.65206	.78	79.92

One-Sample Kolmogorov-Smirnov Test

		Persensel
N		18
Normal Parameters ^a	Mean	42.6555
	Std. Deviation	20.65206
Most Extreme Differences	Absolute	.384
	Positive	.259
	Negative	-.384
Kolmogorov-Smirnov Z		1.628
Asymp. Sig. (2-tailed)		.010

a. Test distribution is Normal.

Hasil analisis dengan *One-Sample Kolmogrov-Smirnov Test* diperoleh signifikansi = $0,10 > 0,05$. Berarti data tersebut mengikuti distribusi normal sehingga dapat dilakukan analisis variansi.

2. *Anova*

ANOVA

Persensel	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	6558.305	5	1311.661	22.735	.000
Within Groups	692.321	12	57.693		
Total	7250.626	17			

Hasil Anova diperoleh nilai signifikansi = $0,00 < 0,05$ berarti perbedaan kelompok uji menunjukkan adanya perbedaan persen sel hidup.

3. *Tukey*

Multiple Comparisons

Dependent Variable: Persensel

(I) Kel_uj i	(J) Kel_uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Tukey HSD	B	9.15710	6.20179	.684	-11.6742	29.9884
	C	9.78200	6.20179	.627	-11.0493	30.6133
	D	12.43567	6.20179	.393	-8.3957	33.2670
	E	10.14667	6.20179	.593	-10.6847	30.9780
	F	58.42933	6.20179	.000	37.5980	79.2607
B	A	-9.15710	6.20179	.684	-29.9884	11.6742
	C	.62490	6.20179	1.000	-20.2064	21.4562

	D	3.27857	6.20179	.994	-17.5528	24.1099
	E	.98957	6.20179	1.000	-19.8418	21.8209
	F	49.27223	6.20179	.000	28.4409	70.1036
C	A	-9.78200	6.20179	.627	-30.6133	11.0493
	B	-.62490	6.20179	1.000	-21.4562	20.2064
	D	2.65367	6.20179	.998	-18.1777	23.4850
	E	.36467	6.20179	1.000	-20.4667	21.1960
	F	48.64733	6.20179	.000	27.8160	69.4787
D	A	-12.43567	6.20179	.393	-33.2670	8.3957
	B	-3.27857	6.20179	.994	-24.1099	17.5528
	C	-2.65367	6.20179	.998	-23.4850	18.1777
	E	-2.28900	6.20179	.999	-23.1203	18.5423
	F	45.99367	6.20179	.000	25.1623	66.8250
E	A	-10.14667	6.20179	.593	-30.9780	10.6847
	B	-.98957	6.20179	1.000	-21.8209	19.8418
	C	-.36467	6.20179	1.000	-21.1960	20.4667
	D	2.28900	6.20179	.999	-18.5423	23.1203
	F	48.28267	6.20179	.000	27.4513	69.1140
F	A	-58.42933	6.20179	.000	-79.2607	-37.5980
	B	-49.27223	6.20179	.000	-70.1036	-28.4409
	C	-48.64733	6.20179	.000	-69.4787	-27.8160
	D	-45.99367	6.20179	.000	-66.8250	-25.1623
	E	-48.28267	6.20179	.000	-69.1140	-27.4513
Bonfe A rroni	B	9.15710	6.20179	1.000	-13.4726	31.7868
	C	9.78200	6.20179	1.000	-12.8477	32.4117
	D	12.43567	6.20179	1.000	-10.1940	35.0653
	E	10.14667	6.20179	1.000	-12.4830	32.7763
	F	58.42933	6.20179	.000	35.7997	81.0590
B	A	-9.15710	6.20179	1.000	-31.7868	13.4726
	C	.62490	6.20179	1.000	-22.0048	23.2546
	D	3.27857	6.20179	1.000	-19.3511	25.9082
	E	.98957	6.20179	1.000	-21.6401	23.6192
	F	49.27223	6.20179	.000	26.6426	71.9019
C	A	-9.78200	6.20179	1.000	-32.4117	12.8477
	B	-.62490	6.20179	1.000	-23.2546	22.0048
	D	2.65367	6.20179	1.000	-19.9760	25.2833
	E	.36467	6.20179	1.000	-22.2650	22.9943
	F	48.64733	6.20179	.000	26.0177	71.2770
D	A	-12.43567	6.20179	1.000	-35.0653	10.1940
	B	-3.27857	6.20179	1.000	-25.9082	19.3511
	C	-2.65367	6.20179	1.000	-25.2833	19.9760
	E	-2.28900	6.20179	1.000	-24.9187	20.3407
	F	45.99367	6.20179	.000	23.3640	68.6233

E	A	-10.14667	6.20179	1.000	-32.7763	12.4830
	B	-.98957	6.20179	1.000	-23.6192	21.6401
	C	-.36467	6.20179	1.000	-22.9943	22.2650
	D	2.28900	6.20179	1.000	-20.3407	24.9187
	F	48.28267	6.20179	.000	25.6530	70.9123
F	A	-58.42933	6.20179	.000	-81.0590	-35.7997
	B	-49.27223	6.20179	.000	-71.9019	-26.6426
	C	-48.64733	6.20179	.000	-71.2770	-26.0177
	D	-45.99367	6.20179	.000	-68.6233	-23.3640
	E	-48.28267	6.20179	.000	-70.9123	-25.6530

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Persensel

	Kel_uji	N	Subset for alpha = 0.05	
			1	2
Tukey HSD ^a	F	3	.8847	
	D	3		46.8783
	E	3		49.1673
	C	3		49.5320
	B	3		50.1569
	A	3		59.3140
	Sig.			1.000

Means for groups in homogeneous subsets are displayed.

Terlihat keenam kelompok uji terbagi dalam empat subset, yang menunjukkan :

1. Kelompok uji A, B, C, D, dan E tidak mempunyai perbedaan yang nyata, karena berada dalam satu subset.
2. Kelompok F dan A/B/C/D/E mempunyai perbedaan yang nyata, karena tidak berada dalam satu subset.

Lampiran 11. Uji efek proliferasi limfosit dari FEE

Absorbansi

Kelompok Uji	Replikasi			Rata-rata \pm SD
A	0,799	0,774	0,788	0,720 \pm 0,064
B	1,150	1,109	1,110	0,727 \pm 0,053
C	0,859	0,888	0,839	0,862 \pm 0,025
D	0,761	0,754	0,666	1,123 \pm 0,023
E	0,790	0,706	0,665	0,787 \pm 0,029
F	0,651	0,444	0,553	0,549 \pm 0,103
G	0,358	0,367	0,364	0,363 \pm 0,005

Keterangan :

- A : Kombinasi 28,56 μ g/ml FEE
- B : Kombinasi 14,28 μ g/ml FEE
- C : Kombinasi 7,14 μ g/ml FEE
- D : Kombinasi 3,57 μ g/ml FEE
- E : Kombinasi 1,78 μ g/ml FEE
- F : PHA (kontrol positif)
- G : Havric (Kontrol negatif)

Idenks Stimulasi

Kelompok uji	Indeks stimulasi			Rata-rata \pm SD	
A	219,707	212,832	216,682	216,407	\pm 3,445
B	316,224	304,950	305,225	308,799	\pm 6,431
C	236,205	244,180	216,682	232,356	\pm 14,147
D	316,224	304,950	305,225	186,526	\pm 21,240
E	219,707	212,832	216,682	198,075	\pm 17,522
F	183,685	179,010	152,062	171,586	\pm 17,069

Uji statistik

1. *One-Sample Kolmogorov-Smirnov Test* (Uji normalitas)

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
IS	12	2.1808E2	47.18404	167.19	305.22

One-Sample Kolmogorov-Smirnov Test

		IS
N		12
Normal Parameters ^a	Mean	2.1808E2
	Std. Deviation	4.71840E1
Most Extreme Differences	Absolute	.194
	Positive	.194
	Negative	-.140
Kolmogorov-Smirnov Z		.672
Asymp. Sig. (2-tailed)		.757

a. Test distribution is Normal.

Hasil analisis dengan *One-Sample Kolmogorov-Smirnov Test* diperoleh signifikansi = 0,757 > 0,05 berarti data tersebut mengikuti distribusi normal sehingga dapat dilakukan analisis variansi.

2. *One Way Anova*

ANOVA

IS	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24251.589	5	4850.318	122.237	.000
Within Groups	238.078	6	39.680		
Total	24489.668	11			

Hasil Anova diperoleh nilai signifikansi = 0,00 < 0,05 berarti perbedaan kelompok uji menunjukkan adanya perbedaan indeks stimulasi.

3. Tukey test dan Benferroni test

Multiple Comparisons

Dependent Variable:IS

	(I) Kel_uji	(J) Kel_uji	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	E	D	13.33650	6.29918	.386	-11.7333	38.4063
		C	-51.69550 [*]	6.29918	.001	-76.7653	-26.6257
		B	-116.59050 [*]	6.29918	.000	-141.6603	-91.5207
		A	-29.69750 [*]	6.29918	.024	-54.7673	-4.6277
		F	7.14950	6.29918	.851	-17.9203	32.2193
	D	E	-13.33650	6.29918	.386	-38.4063	11.7333
		C	-65.03200 [*]	6.29918	.000	-90.1018	-39.9622
		B	-129.92700 [*]	6.29918	.000	-154.9968	-104.857
		A	-43.03400 [*]	6.29918	.004	-68.1038	-17.9642
		F	-6.18700	6.29918	.908	-31.2568	18.8828
	C	E	51.69550 [*]	6.29918	.001	26.6257	76.7653
		D	65.03200 [*]	6.29918	.000	39.9622	90.1018
		B	-64.89500 [*]	6.29918	.000	-89.9648	-39.8252
		A	21.99800	6.29918	.085	-3.0718	47.0678
		F	58.84500 [*]	6.29918	.001	33.7752	83.9148
	B	E	116.59050 [*]	6.29918	.000	91.5207	141.6603
		D	129.92700 [*]	6.29918	.000	104.8572	154.9968
		C	64.89500 [*]	6.29918	.000	39.8252	89.9648
		A	86.89300 [*]	6.29918	.000	61.8232	111.9628
		F	123.74000 [*]	6.29918	.000	98.6702	148.8098
A	E	29.69750 [*]	6.29918	.024	4.6277	54.7673	
	D	43.03400 [*]	6.29918	.004	17.9642	68.1038	
	C	-21.99800	6.29918	.085	-47.0678	3.0718	

		B	-86.89300 [*]	6.29918	.000	-111.9628	-61.8232
		F	36.84700 [*]	6.29918	.008	11.7772	61.9168
	F	E	-7.14950	6.29918	.851	-32.2193	17.9203
		D	6.18700	6.29918	.908	-18.8828	31.2568
		C	-58.84500 [*]	6.29918	.001	-83.9148	-33.7752
		B	-123.74000 [*]	6.29918	.000	-148.8098	-98.6702
		A	-36.84700 [*]	6.29918	.008	-61.9168	-11.7772
Bonferroni	E	D	13.33650	6.29918	1.000	-16.2566	42.9296
		C	-51.69550 [*]	6.29918	.003	-81.2886	-22.1024
		B	-116.59050 [*]	6.29918	.000	-146.1836	-86.9974
		A	-29.69750 [*]	6.29918	.049	-59.2906	-.1044
		F	7.14950	6.29918	1.000	-22.4436	36.7426
	D	E	-13.33650	6.29918	1.000	-42.9296	16.2566
		C	-65.03200 [*]	6.29918	.001	-94.6251	-35.4389
		B	-129.92700 [*]	6.29918	.000	-159.5201	-100.334
		A	-43.03400 [*]	6.29918	.007	-72.6271	-13.4409
		F	-6.18700	6.29918	1.000	-35.7801	23.4061
	C	E	51.69550 [*]	6.29918	.003	22.1024	81.2886
		D	65.03200 [*]	6.29918	.001	35.4389	94.6251
		B	-64.89500 [*]	6.29918	.001	-94.4881	-35.3019
		A	21.99800	6.29918	.194	-7.5951	51.5911
		F	58.84500 [*]	6.29918	.001	29.2519	88.4381
	B	E	116.59050 [*]	6.29918	.000	86.9974	146.1836
		D	129.92700 [*]	6.29918	.000	100.3339	159.5201
		C	64.89500 [*]	6.29918	.001	35.3019	94.4881
		A	86.89300 [*]	6.29918	.000	57.2999	116.4861
		F	123.74000 [*]	6.29918	.000	94.1469	153.3331
	A	E	29.69750 [*]	6.29918	.049	.1044	59.2906
		D	43.03400 [*]	6.29918	.007	13.4409	72.6271
		C	-21.99800	6.29918	.194	-51.5911	7.5951
		B	-86.89300 [*]	6.29918	.000	-116.4861	-57.2999

	F	36.84700*	6.29918	.017	7.2539	66.4401
F	E	-7.14950	6.29918	1.000	-36.7426	22.4436
	D	6.18700	6.29918	1.000	-23.4061	35.7801
	C	-58.84500*	6.29918	.001	-88.4381	-29.2519
	B	-123.74000*	6.29918	.000	-153.3331	-94.1469
	A	-36.84700*	6.29918	.017	-66.4401	-7.2539

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

IS

Kel_uji	N	Subset for alpha = 0.05		
		1	2	3
Tukey HSD ^a				
D	2	1.7516E2		
F	2	1.8135E2		
E	2	1.8850E2		
A	2		2.1819E2	
C	2		2.4019E2	
B	2			3.0509E2
Sig.		.386	.085	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2,000.

Terlihat keenam kelompok uji terbagi dalam empat subset, yang menunjukkan:

1. Kelompok uji D, F, dan E tidak mempunyai perbedaan yang nyata, karena berada dalam satu subset (subset 1).
2. Kelompok A dan C tidak mempunyai perbedaan yang nyata, karena berada dalam satu subset (subset 2).
3. Kelompok D/F/E, A/C, B mempunyai perbedaan yang nyata, karena tidak berada dalam satu subset.