

RINGKASAN

Doksorubisin merupakan salah satu agen kemoterapi yang banyak digunakan dalam terapi kanker payudara. Namun, terapi dengan menggunakan doksorubisin mempunyai keterbatasan karena toksisitas sistemik terutama kardiotoksisitas, penekanan sistem imun (Wattanapitayakul *et al.*, 2005), resistensi obat (Davis *et al.*, 2003) dan kegagalan apoptosis sel kanker (Notarbartolo *et al.*, 2005). Doksorubisin mempengaruhi sistem kekebalan tubuh dengan cara mengurangi ekspresi dari IL- α , produksi IFN- γ , sel NK, proliferasi limfosit dan rasio CD4+ / CD8+ (Zhang *et al.*, 2005). Oleh karena efek samping dari doksorubisin, maka diperlukan suatu senyawa yang bekerja sinergi dengan doksorubisin sehingga dapat meningkatkan efikasi serta mengurangi toksisitas pada jaringan normal.

Pengobatan alternatif yang biasa digunakan masyarakat secara empirik untuk penyakit kanker salah satunya adalah dengan menggunakan tumbuhan sarang semut (*Hydnophytum formicarum*). Di Maluku, sarang semut (*Hydnophytum formicarum*) merupakan salah satu tanaman endemik. Sarang semut (*Hydnophytum formicarum*) suku Rubiaceae termasuk dalam tumbuhan epifit. Secara empiris penggunaan rebusan tumbuhan sarang semut atau kapsulnya telah terbukti dapat menyembuhkan beragam penyakit ringan dan berat seperti kanker dan tumor, asam urat, jantung koroner, wasir, TBC, migren, rematik dan leukimia (Subroto, 2006).

Salah satu model sel kanker payudara yang telah mengalami resistensi terhadap agen kemoterapi doksorubisin adalah sel MCF-7 (Simstein *et al.*, 2003). Sel kanker MCF-7 memiliki karakteristik overekspresi PgP (Davis *et al.*, 2003), overekspresi Bcl-2 dan tidak mengekspresikan caspase-3 sehingga mampu menghindari apoptosis (Simstein *et al.*, 2003). Penelitian ini bertujuan untuk menentukan aktivitas sitotoksik kombinasi fraksi etil asetat dengan agen kemoterapi doksorubisin sehingga dapat mengatasi permasalahan resistensi dan menurunkan dosis efektif doksorubisin dan tidak bersifat toksik terhadap sel vero serta meningkatkan proliferasi limfosit.

Tumbuhan sarang semut (*Hydnophytum formicarum*) diperoleh dari Desa Soya, Ambon-Maluku pada bulan Mei 2014. Bentuk umbi sarang semut adalah bulat tidak beraturan, berwarna coklat dan memiliki rongga pada bagian dalam. Daunnya berbentuk lonjong dengan permukaan yang halus. Determinasi tumbuhan dilakukan di Bagian Biologi Farmasi, Fakultas Farmasi UGM.

Serbuk simplisia sarang semut (*Hydnophytum formicarum*) dimaserasi dalam etanol 96% dan dievaporasi sehingga diperoleh ekstrak kental. Ekstrak etanol sarang semut di fraksinasi dengan kromatografi cair vakum (KCV). Fraksi yang didapat, disederhanakan dengan menggabungkan fraksi berdasarkan nilai R_f pada KLT. Dari 11 fraksi yang terkumpul, dapat dikelompokkan menjadi 2 fraksi yaitu F1 dan F2. Dilakukan uji pendahuluan terhadap F1, F2 dan doksorubisin. Fraksi yang paling baik dilanjutkan untuk uji kombinasi dengan doksorubisin untuk pengujian sitotoksik terhadap sel MCF-7 dan sel vero serta pengujian fraksi tunggal yang paling poten terhadap proliferasi sel limfosit .

Hasil penelitian menunjukkan bahwa, fraksi tunggal yang paling poten terhadap sel MCF-7 adalah fraksi 1 (F1) dengan IC₅₀ sebesar 592 µg/mL. Kombinasi fraksi 1 (F1) dengan doksorubisin tidak bersifat toksik terhadap sel vero bila dibandingkan dengan penggunaan doksorubisin tunggal sedangkan kombinasi F1 dan doksorubisin memberikan efek yang baik terhadap penghambatan proliferasi sel MCF-7 bila dibandingkan dengan kontrol doksorubisin tunggal dan kontrol sel pada konsentrasi (F1) 592 µg/mL + 0,395 µg/mL (doksorubisin). Fraksi etil asetat sarang semut memiliki aktivitas proliferasi limfosit pada konsentrasi 74 µg/mL. Hasil identifikasi menunjukkan bahwa, fraksi etil asetat sarang semut (*Hydnophytum formicarum*) memiliki flavonoid dan fenolik.

DAFTAR PUSTAKA

- Alexander JP *et al.*, 1993, T-cells infiltrating renal cell carcinoma display poor proliferative response even though they can produce interleukin 2 and express interleukin 2 receptors, *Cancer Research* **53** (6): 1380-1387.
- Aouali N, Morjani H, Trussardi A, Soma E, Giroux B, Manfait M, 2003, Enhanced cytotoxicity and nuclear accumulation of doxorubicin-loaded nanospheres in human breast cancer MCF-7 cells expressing MRP1, *International Journal of Oncology*, **23**:1195-1201
- A.S. Levenson, C.V. Jordan, 1997, MCF-7: the first hormoneresponsive breast cancer cell line, *Cancer Research*. **57**: 3071–3078.
- Bustanussalam, 2010, Penentuan struktur molekul dari fraksi air tumbuhan “sarang semut” *Myrmecodia pendens* Merr & Perry yang mempunyai aktivitas sitotoksik dan sebagai antioksidan, *Tesis*, Sekolah Pascasarjana Institut Pertanian Bogor, Bogor.
- Conze D *et al.*, 2001, Autocrine production of interleukin 6 causes multi drug resistance in breast cancer cell, *Cancer Research*, **61**:313-322.
- Crofford LJ, 1997, COX-1 and COX-2 Tissue expression: implication and predictions, *Journal of Rheumatology* 24; Suppl **49**: 15-19.
- Darwis D, Hertiana T, Sasmito E, 2014, The effects of *Hydnophytum formicarum* ethanolic extract towards lymphocyte, vero and T47D cells proliferation *in vitro*, *Journal of Applied Pharmaceutical Science* **4** (05), pp XXX-XXX.
- Davis, JM *et al.*, 2003, Raf-1 and Bcl-2 induce Distinct and common Pathways tha Contribute to Breast Cancer Drug Resistance, *Clinical Cancer Research* **9**: 1161-1170.
- De la Fuente M, V.M Victor, 2000, Antioxidants as modulators of immune function, *Immunology and Cell Biology* **78**: 49-54.
- Dipiro, JT, 2009, *Pharmacoterapy Handbook 7th editioan*, Mc Graw Hill, New York, Hal 679-680.
- Dubois RN *et al*, 1998, Cyclooxygenase in biology and disease. *Faseb Journal* **12**: 1063-1073
- Fimognari C *et al.*, 2006, Sulforaphane Increase the Efficacy of doxorubicin in mouse fibroblasts characterized by p53 mutations, *Mutation Research* **601**: 92-101.

- Fisher DE, 1994, Apoptosis in cancer therapy: Crossing The Treshold, *Cell.* **78**: 539-542.
- Gewirtz DA, 1999, Critical evaluation of mechanism of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem, Pharmacol* **57**: 727-741.
- Gu Yeun-Hwa. 2005. Antioxidant activity and anti-tumor immunity by Agaricus, propolis and paffia in mice. Suzuka: University of Medical Science.
- Guyton Arthur C, H. J, 2006, *Textbook Of Medical Physiology Eleventh Edition*, Philadelphia, Pennsylvania: Elsevier.
- Harianto, 2005, "Risiko Penggunaan Pil Kontrasepsi Kombinasi Terhadap Kejadian Kanker Payudara Pada Reseptor Di Perjan RS DR. Cipto Mangunkusumo, *Majalah Ilmu Kefarmasian*, **2** (1), April 2005, 84-99.
- Hay & Westwood, 2002, *Practical Immunology* 4th edition, Blackwell science, Malden.
- Hertiani T, Sasmito E, Sumardi, dan Ulfah M, 2010, Preliminary Study on Immunomodulatory Effect of Sarang-Semut *Tubers Myrmecodia* and *Myrmecodia pendens*, *Online Journal of Biological Sciences* **10** (3): 136-141.
- Ikegawa T *et al.*, 2002, Inhibition P-glycoprotein by flavonoid derivates in adryamycin resistant human myelogenous leukemia (K562/ADM) cell, *Cancer lett* **177**: 89-93.
- Johnson JL, K.R Maddipati, 1998, Paradoxial effect of resveratrol on two prostaglandin H synthases, *Prostaglandin and Other Lipid Mediator* **156**: 131-143.
- Kawamori *et al*, 1999, Chemopreventive effect of curcumin, a naturally anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Research* **59**: 597-601
- Kinoshita T., Y Takahashi, T Sakashita, H Inoue, T Tanabe, T Yoshimoto, 1999, Growth stimulation and induction of epidermal growth factor receptor by over-expression of cyclooxygenase 1 and 2 in human colon carcinoma cells. *Biochemistry and Biophysiscs Acta* **19**: *1438* (1): 120 -130.
- Kitagawa S, 2006, Inhibitory effect of polyphenols on P-glycoprotein-mediated transport, *Biol, Pharm. Bull.* **29**: 1-6.
- Kresno SB, 2001, *Imunologi: Diagnosis dan Prosedur Laboratorium*, edisi IV, Jakarta: Balai Penerbit Fakultas Kedokteran UI.

- Kresno, SB, 2013, Ilmu Dasar Onkologi Diacu Dalam Gangguan Siklus Sel dan Mutasi Gen pada Kanker Payudara Oleh Romadhon Y.A, *CDK-209/40* (10)
- Kristanti AN, 2008, *Buku Ajar Fitokimia*. Surabaya: Airlangga University Press.
- Mechetner E *et al*, 1998, Levels of multidrug resistance (MDR1) P-Glycoprotein expression by human breast cancer correlate with *in vitro* resistance to taxol and doxorubicin, *Clinical Cancer Research*, **4**:389-398
- Meiyanto E, Sismindari, Candra, Moerdiani, 2003, Efek antiproliferatif ekstrak etanol daun dan kulit batang tanaman cangkring (*Erithryma fusca* L.) terhadap sel hela, *Majalah Farmasi Indonesia*. **14** (3): 124-131
- Miksusanti, 2010, Proliferasi Sel Limfosit Secara *In Vitro* oleh Minyak Atsiri Temu Kunci dan Film Edibel Anti Bakteri, *Jurnal Penelitian Sains*, **10**: 06-07.
- Miller AL, 1996, Antioxidant flavonoids: structure, function and clinical usage, *Alt Medical Review* **1** (2): 103-111.
- Miller NJ, C Rice-Evan, 1995. Antioxidant activity of resveratrol in red wine, *Clinical Chemistry* **41**: 1789.
- 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.
- Munasir, Z, 2001, Respons Imun Terhadap Infeksi Bakteri, *Sari Pediatri*, **2** (4): 193-197.
- Neal M.J., E. S, 2005, *At a Glance Farmakologi Medis Edisi Kelima*, Jakarta: Penerbit Erlangga.
- Notobartolo, M., Poma, P., Perri, D., Dusonchet, L., Cervello, M., and Alessandro, N, 2005, Antitumor effects of curcumin, alone or in combination with cisplatin or doxorubicin, on human hepatic cancer cells, analysis of their possible relationship to change in NF- κ B activation levels and in IAP gene expression, *Cancer Letter*. 224, 53-65.
- Pinchuk G, 2002, *Theory and Problems of Immunology*, New York: McGraw-Hill Companies, Inc.
- Prachayasittikul, S., Buraparuangsang P., Worachartcheewan, A., Isarankura-Na-Ayudhya, C., Ruchirawat, S., Prachayasittikul, V, 2008, Antimicrobial and Antioxidative Activities of Bioactive Constituents from *Hydnophytumformicarum* Non Jack BI, *Molecules*, **13**: 904-921.
- Prakash A, 2001, Antioxidant Activity, *Heart of Giant Recource*, **19** (2): 1-4.

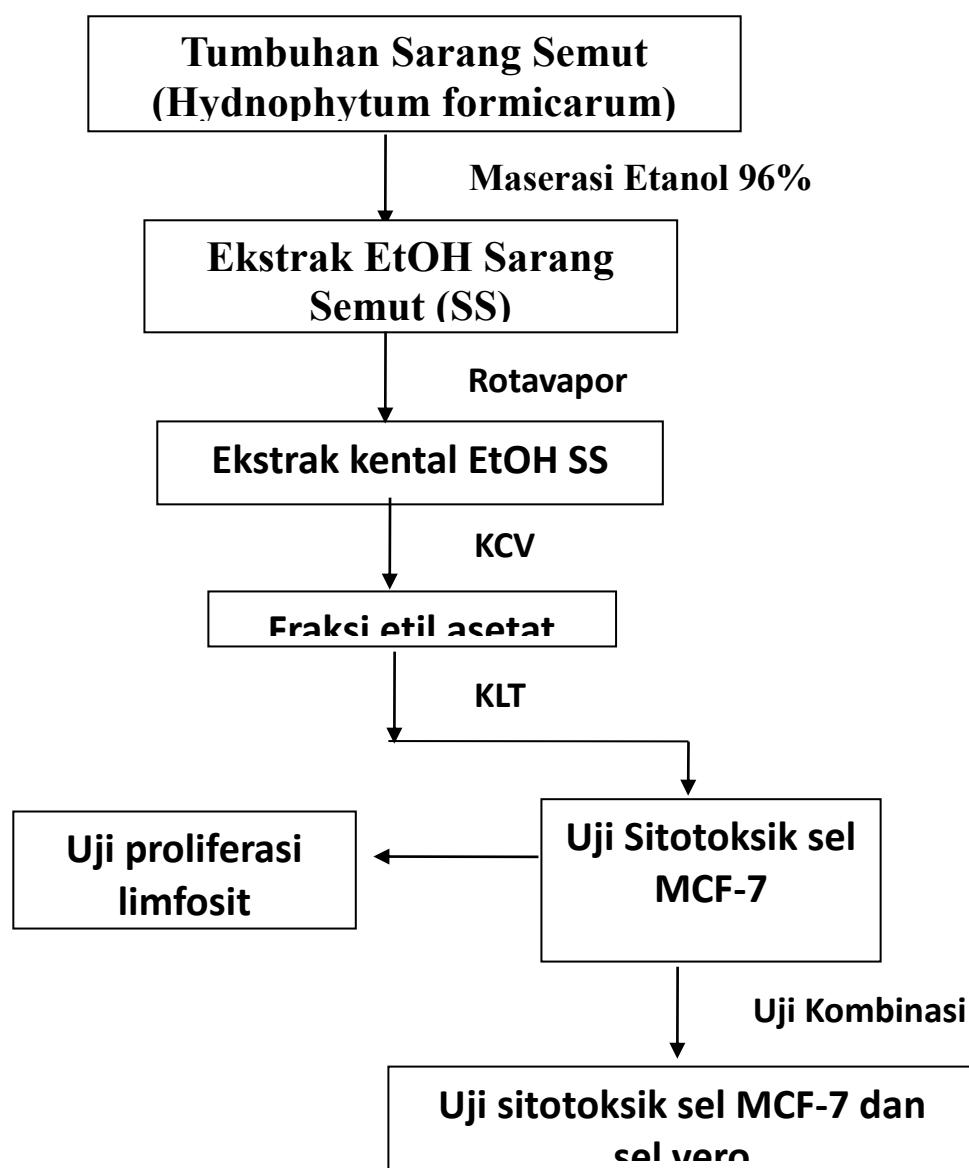
Prosedur Tetap Uji Sitotoksik Metode MTT, Yogyakarta, Indonesia: Fakultas Farmasi UGM.

- R.A. Campbell, P. Bhat-Nakshatri, N.M. Patel, D. Constantinidou, S. Ali, H. Nakshatri, 2001, Phosphatidylinositol/3-kinase/Akt-mediated activation of estrogen receptor α : a new model for anti-estrogen receptor resistance, *J. Biol. Chem.*, **276**: 9817–9824.
- Ramiro-Puing E, Castell M, 2009, Cocoa: Antioxidant and Immunomodulator, *British J Nut*, **101**:931-940.
- Ren, W.,Qiao, Z., Wang, H., Zhu, L., Zhang, L, 2003, Flavanoids: Promising Anticancer Agents, *Medicinal Research Review*, **23** (4): 519-534.
- Roitt, I. M, 1997, *Roitt's Essential Immunology*, Ninth Edition, 168-178, Blackwell Scientific Publications, London.
- Samik WA, Mardina J, 2002, *Sistem Imun, Imunisasi dan Penyakit Imun*, Widya Medika: Jakarta.
- Schroecksnadel S., Sucher R., Kurs K., Fuchs D., Brandacher G, 2011, Influence of Immunosuppressive Agents on Tryptophan Degradation and Neopterin Production in Human Peripheral Blood Mononuclear Cell's, *Journal of Transplant Immunology*, **25**: 119-123
- Shapiro GI, Harper JW, 1999, Anticancer Drug Target: Cell cycle and checkpoint control, *J Clint Invest*, **104**: 1645-1653.
- Simstein, R., Burow, M., Parker, A., Weldon, C., Beckman B, 2003, Apoptosis, Chemoresistance, and Breast Cancer: Insights From the MCF-7 Cell Model System, *Exp Biol Med*, **228**: 995-1003
- Sinaga E, Suprihatin, Wiryanti I, 2011, Perbandingan daya sitotoksik ekstrak rimpang 3 jenis tumbuhan *Zingiberaceae* terhadap sel kanker MCF-7, *Jurnal Farmasi Indonesia*, **5** (3): 125-133.
- Soeksmanto A, M.A Subroto, H Wijaya, P Simanjuntak, 2010, Anticancer activity for extracts of sarang semut plant (*Myrmecodia pendens*) to hela and MCM-B2 cells, *Pakistan J. Biol.Sci* **13**: 148-151.
- Soeroso A, 2007, Sitokin, *Jurnal Oftalmologi Indonesia*, **5** (3): Hal 171-180.
- Sons W, 2008, *Vero Cell*, Inggris: Curr Protocol Microbiology.
- Subroto, M.A, 2007, *Sarang Semut Penakluk Penyakit Maut*. <http://ilusa.ne/newslettet/berita.com>.

- Subroto, M.A., dan Saputro H, 2006, *Gempur Penyakit dengan Sarang Semut*, Jakarta: Penebar Swadaya, Hal 11-12.
- Thome M, J Tschopp, 2001, Regulation of lymphocyte proliferation and death by FLIP, *Nature* **1**: 42 -57.
- Titus RG, Shery B, Cerami A, 1991, The involvement of TNF, IL-1 and IL-6 in the immune response to protozoan parasites, *Parasitology Today*: A13-A16.
- Tjindarbumi D, Mangunkusumo R, 2002, Cancer in Indonesia, Present and Future, *Japanese Journal of Clinical Oncology*, **32**:S17-S21.
- Tokarska-Schlattner M, Zaugg M, Zuppinger C, Walliman T, Schlattner U, 2006, New insight into doxorubicin-induced cardiotoxicity: The Critical Role of Cellular Energetics, *Journal of Molecular and Cellular Cardiology*, **41**: 389-405.
- Tyastuti EM, Sutarno, Kusmardi, 2006, Efek Imunostimulator Propolis terhadap Proliferasi Limfosit T dan Viabilitas Sel Tumor *Mammae* Mencit secara *in Vitro*, *Bioteknologi* **3** (1): 1-7.
- Ulfah M, Nirmalasari, Sasmito E, 2013, Uji aktivitas imunostimulator fraksi etil asetat ekstrak etanol kelopak bunga rosella (*Hibiscus sabdariffa* L.) terhadap proliferasi sel limfosit mencit galur swiss secara *in vitro* beserta identifikasi kandungan senyawa kimianya, *Journal of Pharmaceutical Science & Clinical Pharmacy* **10** (1): 23-30
- Urban Jl, R.C Burton, JM Holland, ML Kripke, H Schreiber, 1982, Mechanism of allogeneic tumour rejection; susceptibility of host-selected progenitor variants to various immunological effector cells, *Journal of Experience Medicine* **155**: 557-573.
- Wattanapitayakul, S.K., Chularojmontri, L., Herunsalee, A., Charuchongkolwongse, S., Niumsakul, S., and Brauer, JA, 2005, Screening of antioxidants from medicinal plants for cardioprotective effects against doxorubicin toxicity, *Basic & Clinical Pharmacology & Toxicology*, **96** (1) 80-87.
- Wijayanti L, 2005, Aktivitas proliferasi limfosit setelah imunisasi intranasal preotein terlarut Toxoplasma selama infeksi Toxoplasma gondii, *BioSMART* **7** (1): 9-13.
- Zhang XY, Li WG, Wu YJ, Gao MT, 2005, Amelioration of Doxorubicin-Induced Myocardial Oxidative Stress and Immunosuppression by Grape Seed Proanthocyanidins in Tumor-Bearing Mice, *Journal of Pharmacy and Pharmacology*, **57** (8): 1043-1051

Zdanowics, M, (2003), *Essentials Of Pharmacology For Pharmacy*, USA: CRC Press.

LAMPIRAN 1. SKEMA KERJA



LAMPIRAN 2. DETERMINASI TUMBUHAN SARANG SEMUT



**BAGIAN BIOLOGI FARMASI
FAKULTAS FARMASI
UNIVERSITAS GADJAH MADA YOGYAKARTA**

Alamat: Sekip Utara Jl. Kaliurang Km 4, Yogyakarta 55281
Telp., 0274.649.2568 Fax. +274-543120

SURAT KETERANGAN
No.: BF/2014/Ident/Det/V/2014

Kepada Yth. :
Sdri/Sdr. Selfyana Austin Tee
NIM. SBF 041310051
Fakultas Farmasi Universitas Setia Budi
Di Surakarta

Dengan hormat,

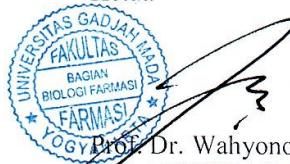
Bersama ini kami sampaikan hasil identifikasi/determinasi sampel yang Saudara kirimkan ke Bagian Biologi Farmasi, Fakultas Farmasi UGM, adalah :

No.Pendaftaran	Jenis	Suku
210	<i>Hydnophytum formicarum</i> Jack	Rubiaceae

Demikian, semoga dapat digunakan sebagaimana mestinya.

Yogyakarta, 20 Mei 2014

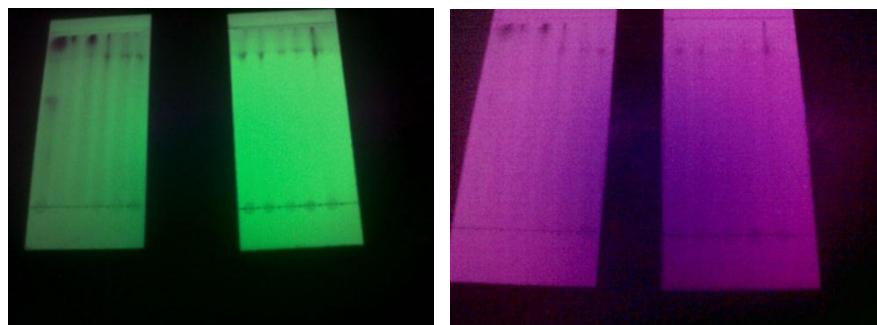
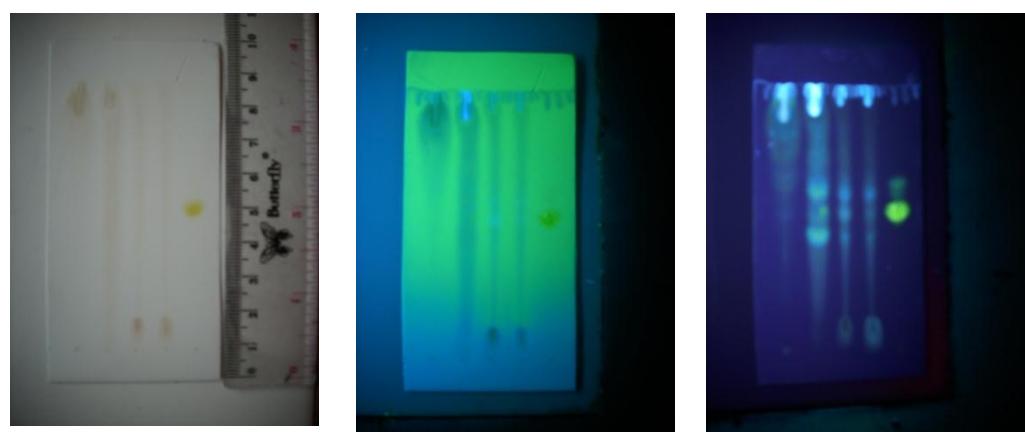
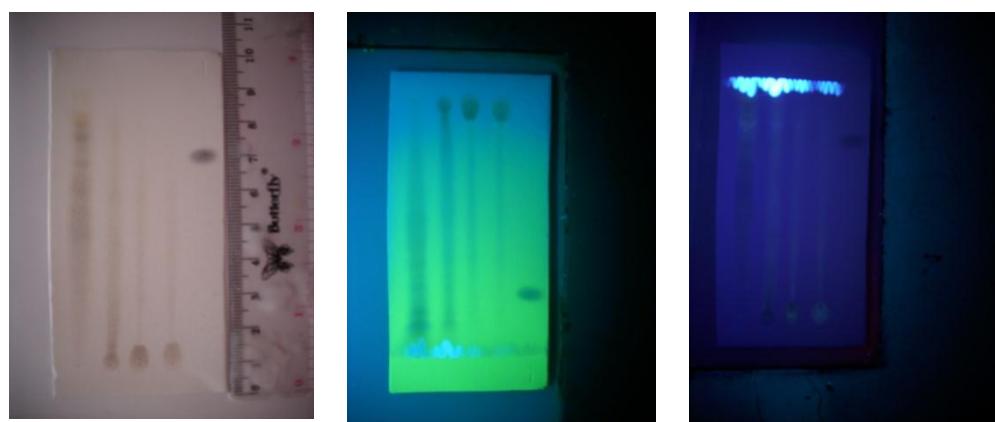
Ketua



Dr. Wahyono, SU., Apt.
NIP. 195007011977021001

LAMPIRAN 3. IDENTIFIKASI SENYAWA

Senyawa Kimia	Fase gerak	Fase diam	Rf	Pembanding	Deteksi
Flavonoid	Etil asetat : asam formiat : asam asetat glasial : air (100 :11:11:27)	Silica gel GF 254	0,5	Rutin 10 mg/1 ml	Sitroborat
Fenolik	Etil asetat : asam formiat : toluen : air (6 :1,5:3:0,5)	Silica gel GF 254	0,7	Asam galat 10 mg/1 ml	FeCl ₃

LAMPIRAN 4. GAMBAR KLT**A. Uji Pendahuluan****B. Flavonoid****C. Fenolik**

**LAMPIRAN 5. GAMBAR HASIL PENGUJIAN SEL MCF-7, SEL VERO,
PROLIFERASI LIMFOSIT**

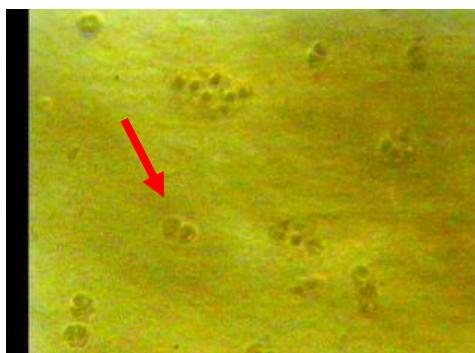
A. Sel MCF-7



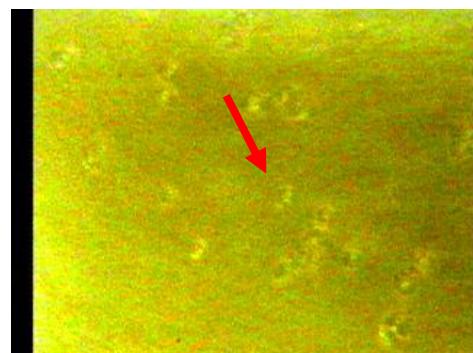
0,395 µg/mL Dokso + DMSO 1%



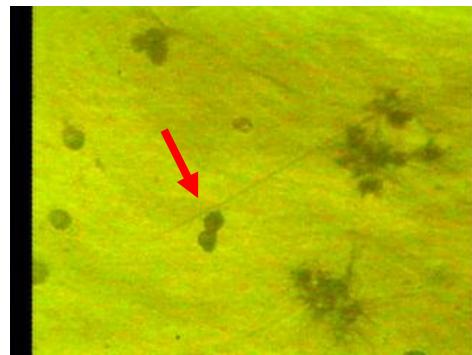
Kontrol Sel



1.184 µg/mL F1 + 0,395 µg/mL
Dokso



592 µg/mL F1 + 0,395 µg/mL
Dokso

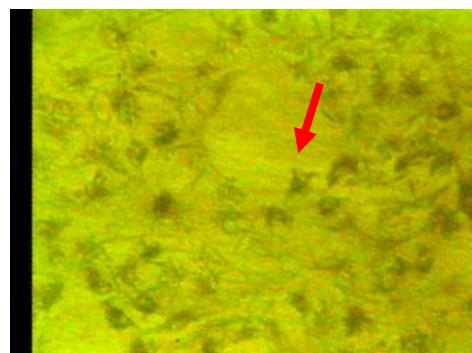


296 µg/mL F1 + 0,395 µg/mL
Dokso

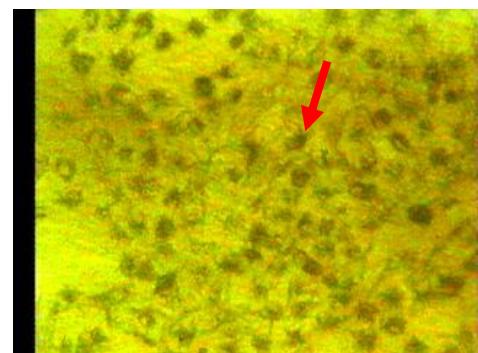


148 µg/mL F1 + 0,395 µg/mL
Dokso

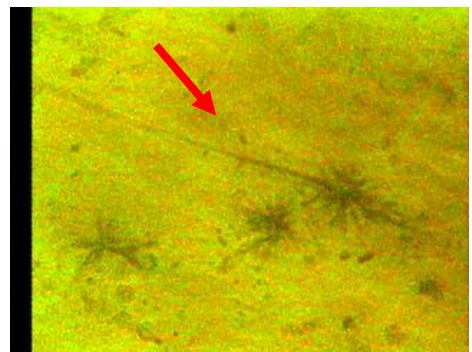
B. Sel Vero



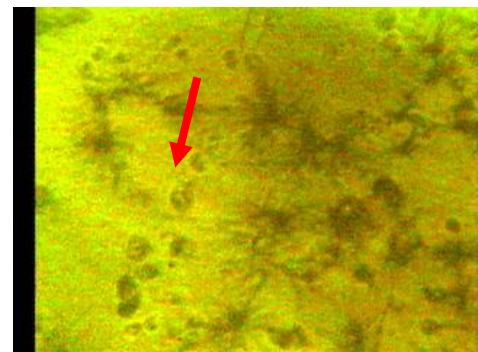
0,395 µg/mL Dokso + DMSO 1%



Kontrol sel

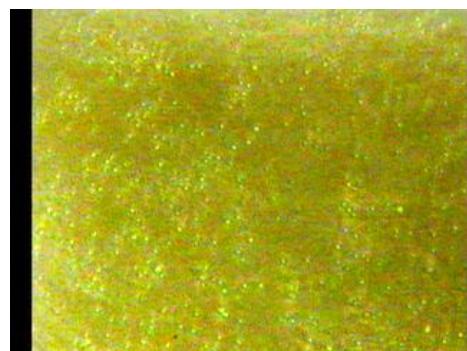


1.184 µg/mL F1 + 0,395 µg/mL
Dokso



592 µg/mL F1 + 0,395 µg/mL
Dokso

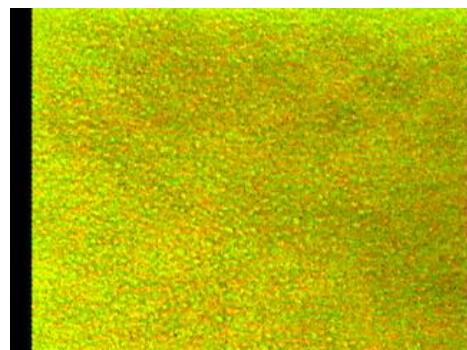
C. Proliferasi Limfosit



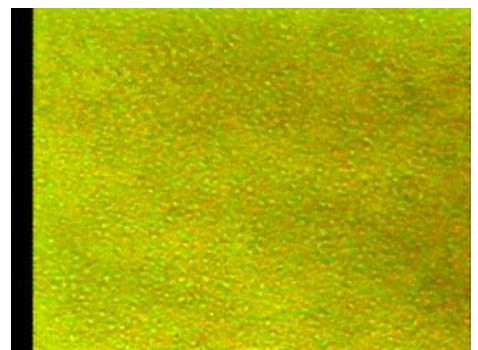
Kontrol Sel



PHA



592 µg/mL



296 µg/mL



148 µg/mL



74 µg/mL

LAMPIRAN 6. OPTICAL DENSITY

A. Uji Pendahuluan

Perlakuan	RI	RII	RIII	RIV	Rata-rata	% viabilitas sel ±SD	% kematian sel
F1 2000	0,449	0,447	0,420	0,445	0,440	17,51±1,88	82,49
F1 1500	0,401	0,419	0,395	0,429	0,411	13,47±5,78	86,53
F1 1000	0,416	0,424	0,390	0,418	0,412	13,60±2,08	86,4
F1 750	0,406	0,410	0,405	0,390	0,403	12,33±1,21	87,67
F1 500	0,773	0,826	0,831	0,797	0,807	68,13±3,73	31,87
F1 375	0,893	0,974	0,878	0,900	0,911	82,56±5,92	17,44
F1 250	0,984	1,015	0,987	0,991	0,994	94,03±1,95	5,97
F1 187,5	0,883	0,971	1,021	0,940	1,058	88,43±7,98	11,57
F2 2000	0,369	0,380	0,381	0,616	0,437	16,99±16,54	83,01
F2 1500	0,576	0,378	0,665	0,683	0,576	36,19±19,30	63,81
F2 1000	0,608	0,480	0,697	0,715	0,625	43,02±14,83	56,98
F2 750	0,679	0,645	0,762	0,796	0,721	56,22±9,71	43,78
F2 500	0,752	0,765	0,849	0,924	0,823	70,30±11,07	29,7
F2 375	0,755	0,724	0,828	0,868	0,794	66,33±9,02	33,67
F2 250	0,737	0,766	0,843	0,967	0,828	71,10±14,19	28,9
F2 187,5	0,828	0,796	0,952	0,973	0,887	79,25±12,20	20,75
A	1,040	1,058	1,001	1,051	1,038	100±3,51	0
B	0,313	0,307	0,313	0,321	0,314		

Keterangan:

F1 = Fraksi etil asetat 1

F2 = Fraksi etil asetat 2

A = Kontrol sel

B = Kontrol media

B. Uji sitotoksik doksorubisin tunggal terhadap sel MCF-7

Perlakuan	RI	RII	RIII	RIV	RV	RVI	Rata-rata	% viabilitas sel ±SD
100	0,19	0,196	0,161	-	-	-	0,182	7,57±7,09
50	0,176	0,163	0,14	-	-	-	0,160	-0,75±6,9
25	0,17	0,147	0,176	-	-	-	0,164	0,75±5,8
12,5	0,175	0,197	0,207	-	-	-	0,193	11,74±6,21
6,25	0,267	0,258	0,246	-	-	-	0,267	35,98±3,99
3,125	0,255	0,256	0,266	-	-	-	0,259	36,74±2,3
1,563	0,259	0,289	0,276	-	-	-	0,274	43,18±5,7
Kontrol sel	0,459	0,438	0,423	0,418	0,359	0,423	0,426	100±12,66
Kontrol media	0,148	0,185	0,168	0,144	0,166	0,161	0,162	

C. Uji sitotoksik kombinasi F1 dan doksorubisin terhadap sel MCF-7

Perlakuan	RI	RII	RIII	RIV	Rata-rata	% viabilitas sel ±SD
A	0,457	0,438	0,450	0,459	0,451	72,92±3,42
B	0,372	0,369	0,366	0,375	0,371	43,86±1,4
C	0,461	0,455	0,470	0,481	0,467	78,70±4,09
D	0,446	0,412	0,432	0,440	0,433	66,42±5,35
E	0,428	0,415	0,394	0,433	0,418	61,01±6,28
F	0,410	0,403	0,401	0,398	0,403	55,59±1,84
G	0,526	0,559	0,507	0,511	0,526	100±8,53
H	0,250	0,244	0,252	0,251	0,249	

Keterangan:

- A 1.184 µg/mL (F1) + 0,395 µg/mL (doksorubisin)
- B 592 µg/mL (F1) + 0,395 µg/mL (doksorubisin)
- C 296 µg/mL (F1) + 0,395 µg/mL (doksorubisin)
- D 148 µg/mL (F1) + 0,395 µg/mL (doksorubisin)
- E 74 µg/mL (F1) + 0,395 µg/mL (doksorubisin)
- F Doksorubisin 0,395 µg/mL
- G Kontrol Sel
- H Kontrol Medium

D. Uji sitotoksik kombinasi F1 dan doktorubisin terhadap sel vero

Perlakuan	RI	RII	RIII	Rata-rata	% viabilitas sel ±SD
A	0,716	0,756	0,689	0,720	131,56±10,53
B	0,774	0,749	0,748	0,757	143,12±4,6
C	0,759	0,778	0,743	0,760	144,06±5,47
D	0,694	0,696	0,693	0,694	123,44±0,47
E	0,606	0,603	0,607	0,605	95,62±0,65
F	0,540	0,537	0,533	0,536	74±1,09
G	0,612	0,622	0,624	0,619	100±2,01
H	0,293	0,300	0,304	0,299	

Keterangan:

- A 1.184 µg/mL (F1) + 0,395 µg/mL (doktorubisin)
- B 592 µg/mL (F1) + 0,395 µg/mL (doktorubisin)
- C 296 µg/mL (F1) + 0,395 µg/mL (doktorubisin)
- D 148 µg/mL (F1) + 0,395 µg/mL (doktorubisin)
- E 74 µg/mL (F1) + 0,395 µg/mL (doktorubisin)
- F Doktorubisin 0,395 µg/mL
- G Kontrol Sel
- H Kontrol Medium

E. Proliferasi Limfosit

	Replikasi OD						
	A	B	C	D	E	F	G
1	0,790	0,761	0,859	1,150	0,294	0,373	0,399
2	0,935	0,754	0,888	1,109	0,279	0,358	0,402
3	0,706	0,666	0,839	1,086	0,284	0,332	0,397
4	0,665	0,608	0,788	1,110	-	0,336	0,388
Rata-rata	0,744*	0,697*	0,844*	1,114*	0,286	0,349	0,396
SD	0,119	0,073	0,042	0,026	0,007	0,019	0,006

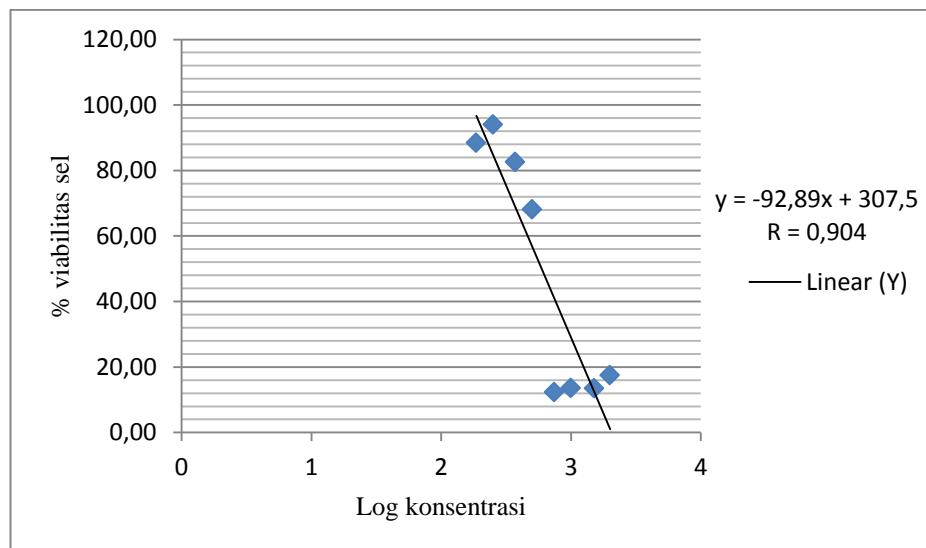
Keterangan:

- A 592 µg/mL
- B 296 µg/mL
- C 148 µg/mL
- D 74 µg/mL
- E Kontrol Media
- F Kontrol Negatif : Sel + Vaksin
- G Kontrol Positif : PHA

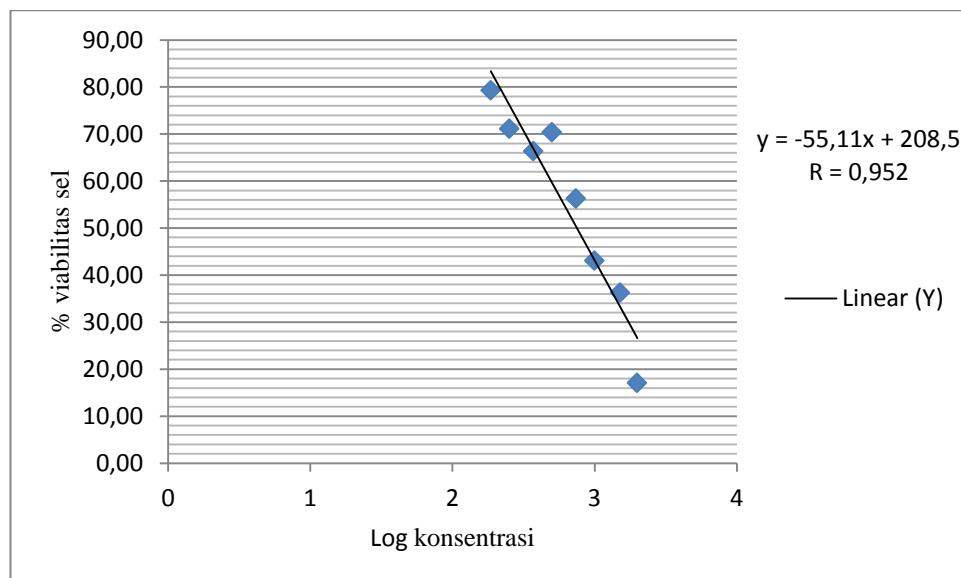
* = berbeda bermakna dengan kontrol negatif, p<0,05

LAMPIRAN 7. GRAFIK REGRESI LINEAR

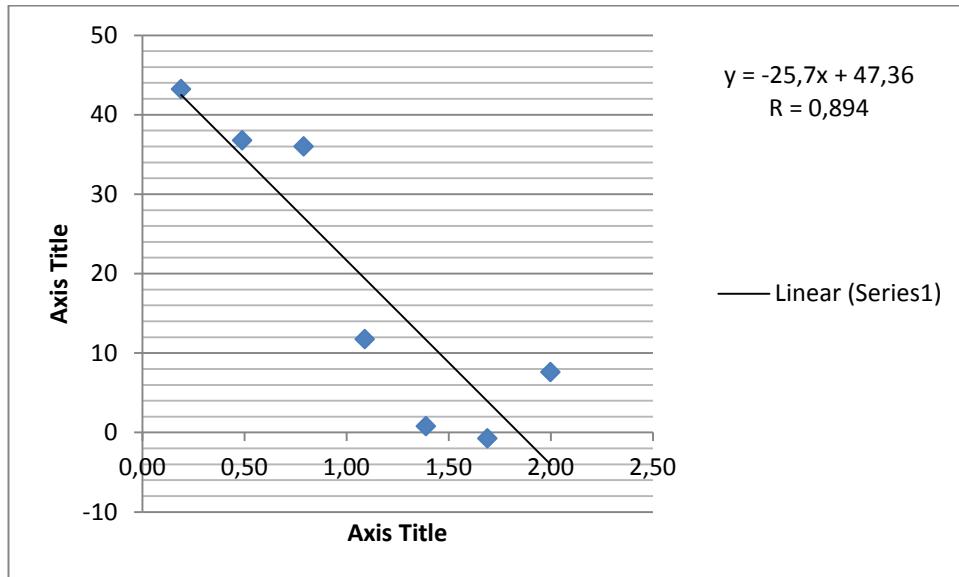
Grafik Regresi Linear Fraksi Etil Asetat 1 (F1)



Grafik Regresi Linear Fraksi Etil Asetat 2 (F2)



Grafik Regresi Linear Doksurubisin



LAMPIRAN 8. ANALISIS STATISTIK

1. Sel MCF-7

NPar Tests

One-Sample Kolmogorov-Smirnov Test

	%ViabilitasSel
N	28
Normal Parameters ^{a,,b}	
Mean	68.2743
Std. Deviation	17.53985
Most Extreme Differences	
Absolute	.105
Positive	.105
Negative	-.069
Kolmogorov-Smirnov Z	.554
Asymp. Sig. (2-tailed)	.919

a. Test distribution is Normal.

b. Calculated from data.

Oneway

Descriptives

%ViabilitasSel

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	72.9225	3.42475	1.71237	67.4730	78.3720	68.23	75.81
2	4	43.8625	1.39815	.69908	41.6377	46.0873	42.24	45.49
3	4	78.6075	4.08626	2.04313	72.1054	85.1096	74.37	83.75
4	4	66.2425	5.35260	2.67630	57.7253	74.7597	58.84	71.12
5	4	60.7825	6.24392	3.12196	50.8470	70.7180	52.35	66.43
6	4	55.5950	1.84023	.92011	52.6668	58.5232	53.79	58.12
7	4	99.9075	8.52936	4.26468	86.3354	113.4796	93.14	111.91
Total	28	68.2743	17.53985	3.31472	61.4730	75.0755	42.24	111.91

Test of Homogeneity of Variances

%ViabilitasSel

Levene Statistic	df1	df2	Sig.
1.726	6	21	.164

ANOVA

%ViabilitasSel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7783.984	6	1297.331	52.145	.000
Within Groups	522.463	21	24.879		
Total	8306.447	27			

Post Hoc Tests

Multiple Comparisons

%ViabilitasSel

Tukey HSD

(I)	(J)	Konsent rasif1do kso	Konsent rasif1do kso	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
1	2			29.06000*	3.52698	.000	17.5945	40.5255
	3			-5.68500	3.52698	.677	-17.1505	5.7805
	4			6.68000	3.52698	.505	-4.7855	18.1455
	5			12.14000*	3.52698	.033	.6745	23.6055
	6			17.32750*	3.52698	.001	5.8620	28.7930
	7			-26.98500*	3.52698	.000	-38.4505	-15.5195
	2			-29.06000*	3.52698	.000	-40.5255	-17.5945
2	1			-34.74500*	3.52698	.000	-46.2105	-23.2795
	3			-22.38000*	3.52698	.000	-33.8455	-10.9145
	4			-16.92000*	3.52698	.002	-28.3855	-5.4545
	5			-11.73250*	3.52698	.043	-23.1980	-.2670
	6			-56.04500*	3.52698	.000	-67.5105	-44.5795
	7							

3	1	5.68500*	3.52698	.677	-5.7805	17.1505
	2	34.74500*	3.52698	.000	23.2795	46.2105
	4	12.36500*	3.52698	.029	.8995	23.8305
	5	17.82500*	3.52698	.001	6.3595	29.2905
	6	23.01250*	3.52698	.000	11.5470	34.4780
	7	-21.30000*	3.52698	.000	-32.7655	-9.8345
4	1	-6.68000	3.52698	.505	-18.1455	4.7855
	2	22.38000*	3.52698	.000	10.9145	33.8455
	3	-12.36500*	3.52698	.029	-23.8305	-.8995
	5	5.46000	3.52698	.714	-6.0055	16.9255
	6	10.64750	3.52698	.080	-.8180	22.1130
	7	-33.66500*	3.52698	.000	-45.1305	-22.1995
5	1	-12.14000*	3.52698	.033	-23.6055	-.6745
	2	16.92000*	3.52698	.002	5.4545	28.3855
	3	-17.82500*	3.52698	.001	-29.2905	-6.3595
	4	-5.46000	3.52698	.714	-16.9255	6.0055
	6	5.18750	3.52698	.758	-6.2780	16.6530
	7	-39.12500*	3.52698	.000	-50.5905	-27.6595
6	1	-17.32750*	3.52698	.001	-28.7930	-5.8620
	2	11.73250*	3.52698	.043	.2670	23.1980
	3	-23.01250*	3.52698	.000	-34.4780	-11.5470
	4	-10.64750	3.52698	.080	-22.1130	.8180
	5	-5.18750	3.52698	.758	-16.6530	6.2780
	7	-44.31250*	3.52698	.000	-55.7780	-32.8470
7	1	26.98500*	3.52698	.000	15.5195	38.4505
	2	56.04500*	3.52698	.000	44.5795	67.5105
	3	21.30000*	3.52698	.000	9.8345	32.7655
	4	33.66500*	3.52698	.000	22.1995	45.1305
	5	39.12500*	3.52698	.000	27.6595	50.5905
	6	44.31250*	3.52698	.000	32.8470	55.7780

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

Homogeneous Subsets

%ViabilitasSel

Tukey HSD^a

Konsent rasif1do kso	N	Subset for alpha = 0.05				
		1	2	3	4	5
2	4	43.8625				
6	4		55.5950			
5	4			60.7825		
4	4				66.2425	
1	4					72.9225
3	4					78.6075
7	4					99.9075
Sig.		1.000	.080	.505	.677	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4,000.

2. Sel Vero

NPar Tests

One-Sample Kolmogorov-Smirnov Test

		%ViabilitasSel
N		21
Normal Parameters ^{a,b}	Mean	116.0719
	Std. Deviation	25.52107
Most Extreme Differences	Absolute	.161
	Positive	.144
	Negative	-.161
Kolmogorov-Smirnov Z		.740
Asymp. Sig. (2-tailed)		.644

a. Test distribution is Normal.

b. Calculated from data.

Oneway

Descriptives

%ViabilitasSel

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	131.6667	10.53075	6.07993	105.5068	157.8265	121.88	142.81
2	3	143.1267	4.60426	2.65827	131.6890	154.5643	140.31	148.44
3	3	144.0600	5.47159	3.15902	130.4678	157.6522	138.75	149.68
4	3	123.5433	.47353	.27339	122.3670	124.7197	123.13	124.06
5	3	95.7300	.65092	.37581	94.1130	97.3470	95.00	96.25
6	3	74.2733	1.09391	.63157	71.5559	76.9907	73.13	75.31
7	3	100.1033	2.01013	1.16055	95.1099	105.0968	97.81	101.56
Total	21	116.0719	25.52107	5.56915	104.4549	127.6890	73.13	149.68

Test of Homogeneity of Variances

%ViabilitasSel

Levene Statistic	df1	df2	Sig.
3.433	6	14	.027

ANOVA

%ViabilitasSel

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12690.664	6	2115.111	88.172	.000
Within Groups	335.839	14	23.988		
Total	13026.503	20			

Post Hoc Tests

Multiple Comparisons

%ViabilitasSel

Tukey HSD

(I) konsentr asi f1dokso	(J) konsentr asi f1dokso	95% Confidence Interval			
		Mean Difference (I-J)		Sig.	Lower Bound
		Std. Error			
1	2	-11.46000	3.99904	.129	-25.1151
	3	-12.39333	3.99904	.087	-26.0484
	4	8.12333	3.99904	.438	-5.5317
	5	35.93667*	3.99904	.000	22.2816
	6	57.39333*	3.99904	.000	43.7383
	7	31.56333*	3.99904	.000	17.9083

2	1	11.46000	3.99904	.129	-2.1951	25.1151
	3	-.93333	3.99904	1.000	-14.5884	12.7217
	4	19.58333*	3.99904	.003	5.9283	33.2384
	5	47.39667*	3.99904	.000	33.7416	61.0517
	6	68.85333*	3.99904	.000	55.1983	82.5084
	7	43.02333*	3.99904	.000	29.3683	56.6784
3	1	12.39333	3.99904	.087	-1.2617	26.0484
	2	.93333	3.99904	1.000	-12.7217	14.5884
	4	20.51667*	3.99904	.002	6.8616	34.1717
	5	48.33000*	3.99904	.000	34.6749	61.9851
	6	69.78667*	3.99904	.000	56.1316	83.4417
	7	43.95667*	3.99904	.000	30.3016	57.6117
4	1	-8.12333	3.99904	.438	-21.7784	5.5317
	2	-19.58333*	3.99904	.003	-33.2384	-5.9283
	3	-20.51667*	3.99904	.002	-34.1717	-6.8616
	5	27.81333*	3.99904	.000	14.1583	41.4684
	6	49.27000*	3.99904	.000	35.6149	62.9251
	7	23.44000*	3.99904	.001	9.7849	37.0951
5	1	-35.93667*	3.99904	.000	-49.5917	-22.2816
	2	-47.39667*	3.99904	.000	-61.0517	-33.7416
	3	-48.33000*	3.99904	.000	-61.9851	-34.6749

	4	-27.81333*	3.99904	.000	-41.4684	-14.1583
	6	21.45667*	3.99904	.001	7.8016	35.1117
	7	-4.37333	3.99904	.920	-18.0284	9.2817
6	1	-57.39333*	3.99904	.000	-71.0484	-43.7383
	2	-68.85333*	3.99904	.000	-82.5084	-55.1983
	3	-69.78667*	3.99904	.000	-83.4417	-56.1316
	4	-49.27000*	3.99904	.000	-62.9251	-35.6149
	5	-21.45667*	3.99904	.001	-35.1117	-7.8016
	7	-25.83000*	3.99904	.000	-39.4851	-12.1749
7	1	-31.56333*	3.99904	.000	-45.2184	-17.9083
	2	-43.02333*	3.99904	.000	-56.6784	-29.3683
	3	-43.95667*	3.99904	.000	-57.6117	-30.3016
	4	-23.44000*	3.99904	.001	-37.0951	-9.7849
	5	4.37333	3.99904	.920	-9.2817	18.0284
	6	25.83000*	3.99904	.000	12.1749	39.4851

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

Homogeneous Subsets

%ViabilitasSel

Tukey HSD^a

konsentr asi f1dokso	N	Subset for alpha = 0.05			
		1	2	3	4
6	3	74.2733			
5	3		95.7300		
7	3			100.1033	
4	3				123.5433
1	3				131.6667
2	3				143.1267
3	3				144.0600
Sig.		1.000	.920	.438	.087

Means for groups in homogeneous subsets are displayed.

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

3. Proliferasi Limfosit

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Absorbansi	27	.65022	.288498	.279	1.150

One-Sample Kolmogorov-Smirnov Test

		Absorbansi
N		27
Normal Parameters ^{a,,b}	Mean	.65022
	Std. Deviation	.288498
Most Extreme Differences	Absolute	.213
	Positive	.213
	Negative	-.099
Kolmogorov-Smirnov Z		1.105
Asymp. Sig. (2-tailed)		.174

a. Test distribution is Normal.

b. Calculated from data.

Oneway

Descriptives

Absorbansi

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	4	.77400	.119278	.059639	.58420	.96380	.665	.935
2	4	.69725	.073545	.036773	.58022	.81428	.608	.761
3	4	.84350	.042115	.021057	.77649	.91051	.788	.888
4	4	1.11375	.026588	.013294	1.07144	1.15606	1.086	1.150
5	3	.28567	.007638	.004410	.26669	.30464	.279	.294
6	4	.34975	.019259	.009630	.31910	.38040	.332	.373
7	4	.39650	.006028	.003014	.38691	.40609	.388	.402
Total	27	.65022	.288498	.055522	.53610	.76435	.279	1.150

Test of Homogeneity of Variances

Absorbansi

Levene Statistic	df1	df2	Sig.
4.855	6	20	.003

ANOVA

Absorbansi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.096	6	.349	103.233	.000
Within Groups	.068	20	.003		
Total	2.164	26			

Post Hoc Tests

Multiple Comparisons

Absorbansi

Scheffe

(I) Konsent rasi	(J) Konsent rasi	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	.076750	.041137	.742	-.08569	.23919
	3	-.069500	.041137	.818	-.23194	.09294
	4	-.339750*	.041137	.000	-.50219	-.17731
	5	.488333*	.044433	.000	.31287	.66379
	6	.424250*	.041137	.000	.26181	.58669
	7	.377500*	.041137	.000	.21506	.53994
2	1	-.076750	.041137	.742	-.23919	.08569
	3	-.146250	.041137	.098	-.30869	.01619
	4	-.416500*	.041137	.000	-.57894	-.25406
	5	.411583*	.044433	.000	.23612	.58704
	6	.347500*	.041137	.000	.18506	.50994
	7	.300750*	.041137	.000	.13831	.46319
3	1	.069500	.041137	.818	-.09294	.23194
	2	.146250	.041137	.098	-.01619	.30869
	4	-.270250*	.041137	.000	-.43269	-.10781
	5	.557833*	.044433	.000	.38237	.73329
	6	.493750*	.041137	.000	.33131	.65619
	7	.447000*	.041137	.000	.28456	.60944
4	1	.339750*	.041137	.000	.17731	.50219
	2	.416500*	.041137	.000	.25406	.57894
	3	.270250*	.041137	.000	.10781	.43269
	5	.828083*	.044433	.000	.65262	1.00354
	6	.764000*	.041137	.000	.60156	.92644

	7	.717250*	.041137	.000	.55481	.87969
5	1	-.488333*	.044433	.000	-.66379	-.31287
	2	-.411583*	.044433	.000	-.58704	-.23612
	3	-.557833*	.044433	.000	-.73329	-.38237
	4	-.828083*	.044433	.000	-1.00354	-.65262
	6	-.064083	.044433	.904	-.23954	.11138
	7	-.110833	.044433	.431	-.28629	.06463
6	1	-.424250*	.041137	.000	-.58669	-.26181
	2	-.347500*	.041137	.000	-.50994	-.18506
	3	-.493750*	.041137	.000	-.65619	-.33131
	4	-.764000*	.041137	.000	-.92644	-.60156
	5	.064083	.044433	.904	-.11138	.23954
	7	-.046750	.041137	.968	-.20919	.11569
7	1	-.377500*	.041137	.000	-.53994	-.21506
	2	-.300750*	.041137	.000	-.46319	-.13831
	3	-.447000*	.041137	.000	-.60944	-.28456
	4	-.717250*	.041137	.000	-.87969	-.55481
	5	.110833	.044433	.431	-.06463	.28629
	6	.046750	.041137	.968	-.11569	.20919

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

Homogeneous Subsets

Absorbansi

Scheffe^{a,b}

Konsent rasi	N	Subset for alpha = 0.05		
		1	2	3
5	3	.28567		
6	4	.34975		
7	4	.39650		
2	4		.69725	
1	4		.77400	
3	4		.84350	
4	4			1.11375
Sig.		.368	.112	1.000

Means for groups in homogeneous subsets are displayed.

- a. Uses Harmonic Mean Sample Size = 3,818.
- b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.