

DAFTAR PUSTAKA

- Antony, B, G. Santhakumari, B. Merina, V. Sheeba, and J. Mukkadan, 2006, *Hepatoprotective Effect of Centella asiatica* (L) in Carbon Tetrachloride-Induced Liver Injury in Rats, *Indian Journal of Pharmaceutical Science*, November – December 2006.
- Baratawidjaya, 2002, Kamen Garna. *Imunologi Dasar*. Penerbit Fakultas Kedokteran Universitas Indonesia, Jakarta.
- Bosse JP, Lythgoe B, and Trippett S. D., 1979, Clinical Study of a New Antikeloid Agent, *Annals of plastic surgery*, 1979, 3:13–21.
- Chakrabarty T, Deshmukh S., 1976, *Centella asiatica* in the treatment of leprosy. *Science and culture*, 1976, 42:573.
- Chang Gue Son, Jang Woo Shin, Jung Hyo Cho, Chong Kwan Cho, Cheol-Heui Yun, Wantae Chung, Seung Hyun Han, 2006, Macrophage activation and nitric oxide production by water soluble components of *Hericium erinaceum*, *International Immunopharmacology* 6 (2006) 1363–1369.
- Collins HL, Kaufmann SHE., 2002, *Acquired Immunity against Bacteria*. In: *Immunology of Infectious Diseases*. Eds: SHE Kaufmann, A Sher & R Ahmed. ASM Press, Washington DC.
- Dannenberg AM Jr., 1991, Delayed-type hypersensitivity and cell-mediated immunity in the pathogenesis of tuberculosis. *Immunol Today* 1991; 12: 228-33.
- Davis, Alexander S., Isabelle Vergne, Sharon S. Master, George B. Kyei, Jennifer Chua, Vojo Deretic, 2007, Mechanism of Inducible Nitric Oxide Synthase Exclusion from Mycobacterial Phagosomes, *PLoS Pathogens*, December 2007 Volume 3 Issue 12 e186.
- Departemen Kesehatan Republik Indonesia, 1995, *Farmakope Indonesia*, Edisi IV, Direktorat Jenderal Pengawasan Obat dan Makanan, Jakarta.
- Departemen Kesehatan Republik Indonesia, 2002, *Pedoman Nasional Penanggulangan Tuberkulosis*, Cetakan ke 8, Jakarta.

- Departemen Kesehatan Republik Indonesia, 2005, *pharmaceutical Care untuk Penyakit Tuberkulosis*, Direktorat Bina Farmasi Komunitas dan Klinik, Direktorat Jenderal Bina Kefarmasian dan Alat Kesehatan Departemen kesehatan RI. Jakarta.
- De Valliere S, Abate G, Blazevic A, Heuertz RM, Hoft DF., 2005, Enhancement of innate and cell-mediated immunity by antimycobacterial antibodies. *Infect Immun* 2005; 73: 6711-20.
- Farnsworth NR and Bunyapraphatsara N, 1992, *Thai Medicinal Plants*. Bangkok, Prachachon
- Fatmasari, Ainun Riska dan Maria Immaculata I, 2007, *Efek Immunostimulasi Ekstrak Air Herba Pegagan (Centella asiatica Urb.) dan Daun Beluntas (Pluchea indica Less.) pada Mencit Swiss Webster Betina* (on-line) (<http://bahan-alam.fa.itb.ac.id>, diakses 21 Oktober 2007)
- Fowst C., Cro L., Marozzi A., Marelli C., Tantalo V., Pogliani E.M., *Giorn. It. Angiol.* 7, 145, 1987. Dalam *Centella asiatica* selected triterpenes a highly standardized natural remedy for the maintenance of an healthy venous system, *Indena*
- Goldsby, A. Richard, 2002, *Immunology*, Fifth edition, Third Edition, Humana Press.
- Helgason, Cheryl D. and Cindy L. Miller, 2007, *Basic Cell Culture Protocols*, Third Edition, Humana Press.
- Jayathirtha, M. G. and Mishra, S. H. 2004, Preliminary immunomodulatory activities of metanol extracts of *Eclipta alba* and *Centella asiatica*, *Phytomedicine* 11: 361–365, 2004, on-line (<http://www.elsevier.de/phymed>)
- Jerca, Luminita, Oltita Jerca, Gabriela Mancas, Irina Constantinescu, R. Lupusoru, 2002, Mechanism of Action and Biochemical Effects of Nitric Oxide (NO^{*}), *The Journal of Preventive Medicine*, 2002; 10 (2): 35-45
- Katzung, Bertram G., 2004, *Farmakologi dan dasar Klinik*, Penerjemah dan Editor Bagian Farmakologi Fakultas Kedokteran Universitas Airlangga, Salemba Medika, Surabaya.
- Labro MT., 2000, Interference of Antibacterial Agents with Phagocyte Function; immunomodulation or “immuno-fair tales”?, *Clinical Microbiology Review* 2000; 13(4):615-50

- Made I, J., 2006, Interaksi antara Antimikroba dengan Sistem Fagosit Neutrofil dan Monosit/Makrofag, *Dexa Media* No. 2, Vol. 19, April - Juni 2006
- Marino, Simeone and Denise E. Kirschner, 2003, The Human Immune Response to *Mycobacterium tuberculosis* in Lung and Lymph Node, *Journal of Theoretical Biology* 227 (2004) 463–486.
- O’Gorman, Maurice R. G. and Albert D. Donnenberg, 2008, *Handbook of Human Immunology*, Second Edition, CRC Press, USA.
- Palomino JC, Martin A, Portaels F., 2007, Rapid drug resistance detection in *Mycobacterium tuberculosis*: a review of colourimetric methods. *Clin Microbiol Infect* 2007.
- Patil, J. S. et. al., 1998, A Study on the Immunostimulant Activity of *Centella asiatica* in Rats. *Ind Drugs* 38: 711–714(1998).
- Rao, K. G. Mohandas, S. Muddanna Rao and S. Gurumadhva Rao, 2006, *Centella asiatica* (L.) Leaf Extract Treatment During the Growth Spurt Period Enhances Hippocampal CA3 Neuronal Dendritic Arborization in Rats, *eCAM* 2006;3(3)349–357
- Rivas-Santiago B, Sada E, Tsutsumi V, Aguilar-Leon D, Contreras JL, Hernandez-Pando R. beta-Defensin gene expression during the course of experimental tuberculosis infection. *J Infect Dis* 2006; 194: 697-701.
- Roy S, Sharma S, Sharma M, Aggarwal R, Bose M., 2004, Induction of nitric oxide release from the human alveolar epithelial cell line A549: an in vitro correlate of innate immune response to *Mycobacterium tuberculosis*. *Immunology* 2004; 112: 471-80.
- Sagrawat, Hemant and Khan, Md. Yaseen, 2007, Immunomodulatory Plants: A Phytopharmacological Review, *Pharmacognosy Reviews* Vol 1, Issue 2, Jul-Dec, 2007
- Saxena R, Weissman D, Saxena Q, Simpson J, Lewis D., 2002, Kinetics of changes in lymphocyte sub-populations in mouse lungs after intrapulmonary infection with *M. bovis* (Bacillus Calmette-Guerin) and identity of cells responsible for IFN-gamma responses. *Clin Exp Immunol* 2002; 128: 405-10.
- Sherwood, Lauralee, 2001, *Fisiologi Manusia dari Sel ke Sistem*, alih bahasa Brahm U. Pendit, Penerbit Buku Kedokteran EGC, Jakarta.

- Shin HS, Rhee JC, and Choi KW, 1982, Clinical Trials of Madecassol (*Centella asiatica*) on Gastrointestinal Ulcer Patients. *Korean journal of gastroenterology*, 1982, 14:49–56.
- Sidhi, Pramono dan D. Ajiastuti, 2004, Standardisasi Ekstrak Herba Pegagan (*Centella asiatica* (L.) Urban) Berdasarkan Kadar Asiatikosida Secara KLTDensitometri, *Majalah Farmasi Indonesia*, 15 (3) 2004: 118-123
- Stevenson, Lesley M., Anita Matthias, Linda Banbury, Kerry G. Penman, Kerry M. Bone, David Leach and Reg P. Lehmann, 2005, *Modulation of Macrophage Immune Responses by Echinacea*, *Molecules* 2005, 10, 1279–1285. (on-line) (<http://www.mdpi.org>, diakses tanggal 24 April 2009)
- Subagyo, Ahmad, dkk., 2006, Pemeriksaan Interferon-gamma Dalam Darah Untuk Deteksi Infeksi Tuberkulosis, *Jurnal Tuberkulosis Indonesia*, Vol. 3 No. 2 September 2006
- Takuji Tanaka, Haruo Sugiura, Ryoichi Inaba, Akiyoshi Nishikawa, Akira Murakami, Koichi Koshimizu, and Hajime Ohigashi, 1999, Immunomodulatory Action of Citrus auraptene on Macrophage Function and Cytokine Production of Lymphocytes in Female BALB/c Mice, *Carcinogenesis* vol. 20 no.8 pp. 1471-1476, 1999.
- Tenni R, Zanaboni G, De Agostini MP, Rossi A, Bendotti C, Cetta G, Effect of the triterpenoid fraction of *Centella asiatica* on macromolecules of the connective matrix in human skin fibroblast cultures, *Ital J Biochem* 1988 Mar-Apr;37(2):69-77
- Titheradge, Michael A., 2007, *Methods in Molecular Biology*, Vol. 100. *Nitric Oxide Protocols*, Humana Press Inc., Japan
- Ulrichs T, Moody DB, Grant E, Kaufmann SE, Porcelli SA., 2003, T-Cell responses to CD1-presented lipid antigens in humans with *Mycobacterium tuberculosis* infection. *Infect Immun* 2003; 71: 3076-87.
- Winfield, A.J. 2004, *Pharmaceutical Practice*, Third Edition, EdinBrug London, Nee York Oxford, Philadelphia, Toronto.
- World Health Organization, 1999, *WHO Monographs on Selected Medicinal Plants*, Volume 1, Geneva.

World Health Organization, 2003, *Tuberculosis control in the South-East Asia Region*. New Delhi, India: WHO Regional Office for South-East Asia.

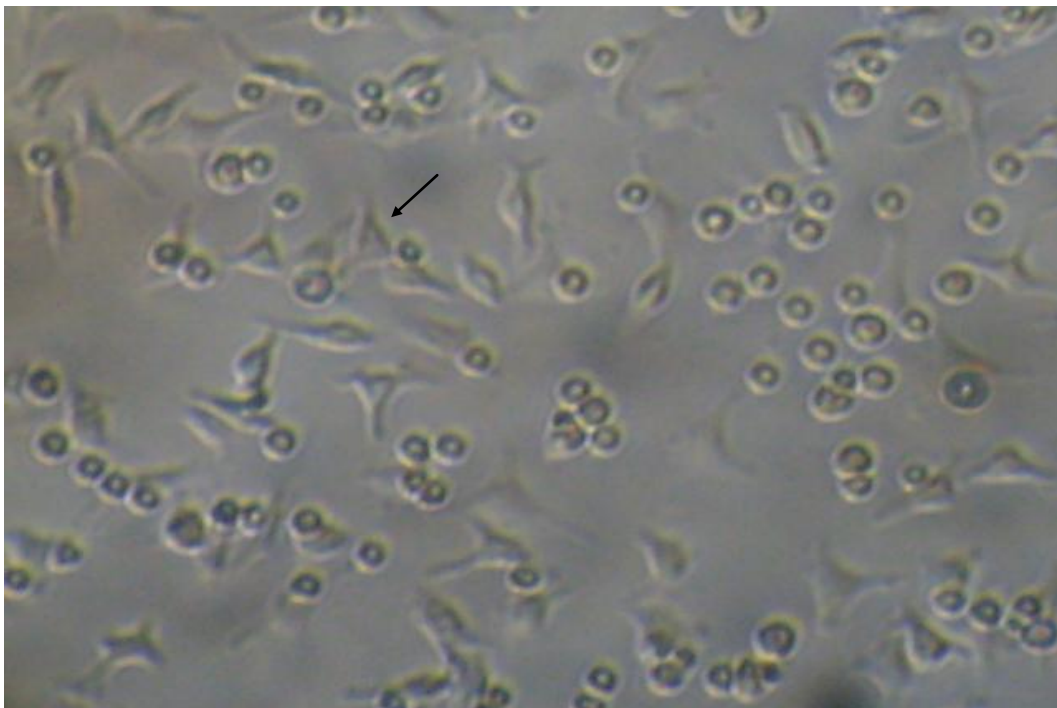
World Health Organization, 2007, *The Global MDR-TB & XDR-TB Response Plan 2007-2008*, WHO-Stop TB Partnership, Geneva.

World Health Organization, 2008, *Implementing the Stop TB Strategy a Handbook for National Tuberculosis Control Programmes*, Geneva.

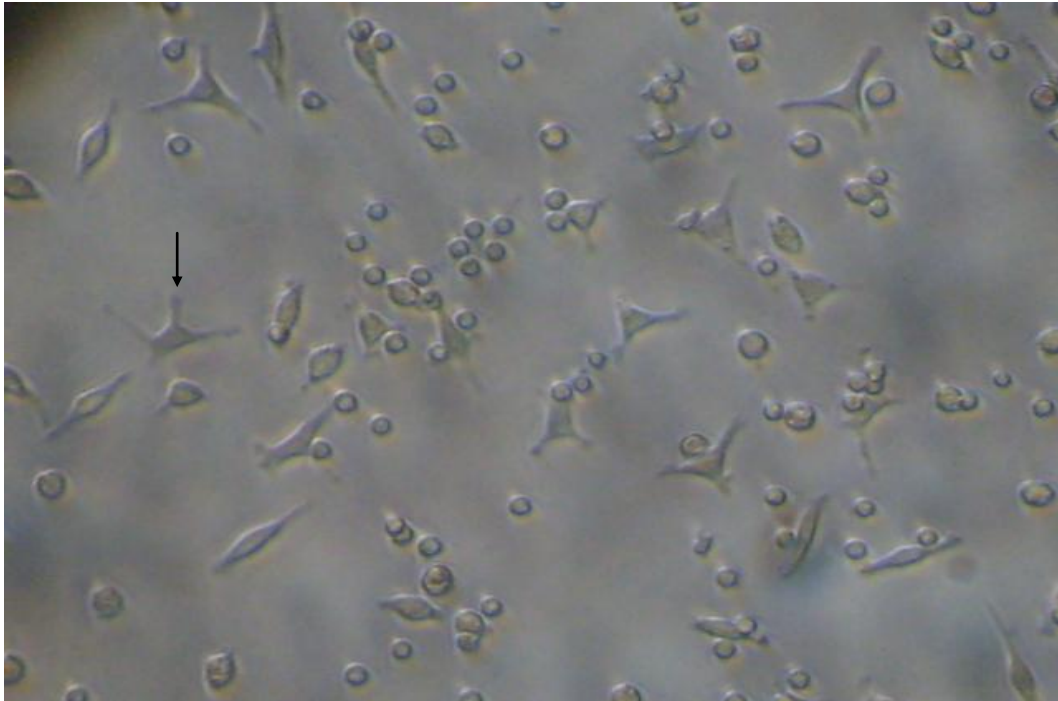
Yang, Chul-Su, Jae-Min Yuk and Eun-Kyeong Jo, 2009, The Role of Nitric Oxide in Mycobacterial Infections, *Immune Network*, P-ISSN 1598-2629, DOI 10.4110/in.2009.9.2.46, (on-line) (<http://www.ksimm.or.kr> diakses tanggal 20 Juni 2009)



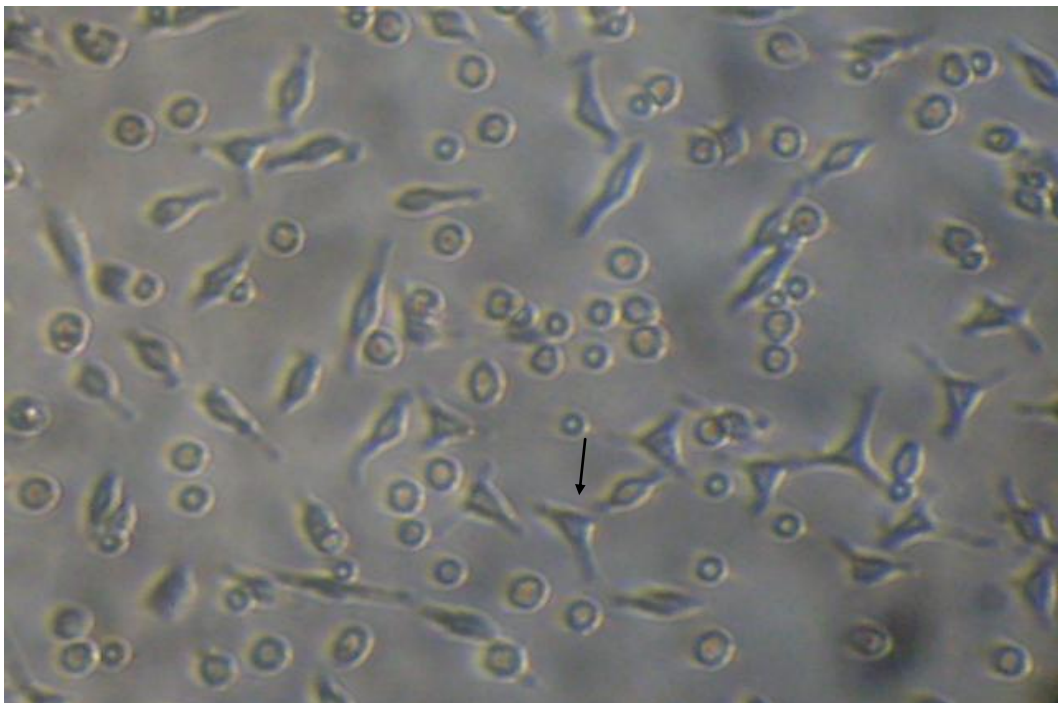
Gambar 2. Mencit yang telah dibuka kulit abdomen dan disuntikkan medium RPMI-1640



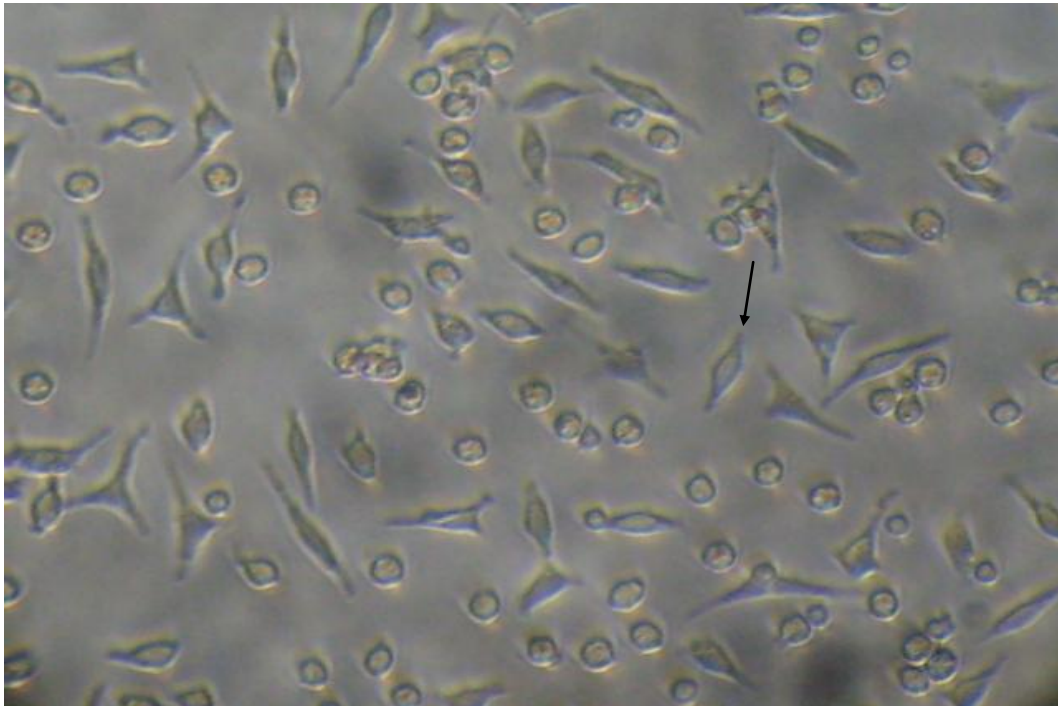
Gambar 3. Makrofag yang telah diinkubasi pada suhu 37°C, 5% CO₂, selama 24 jam. Pembesaran 100 x.



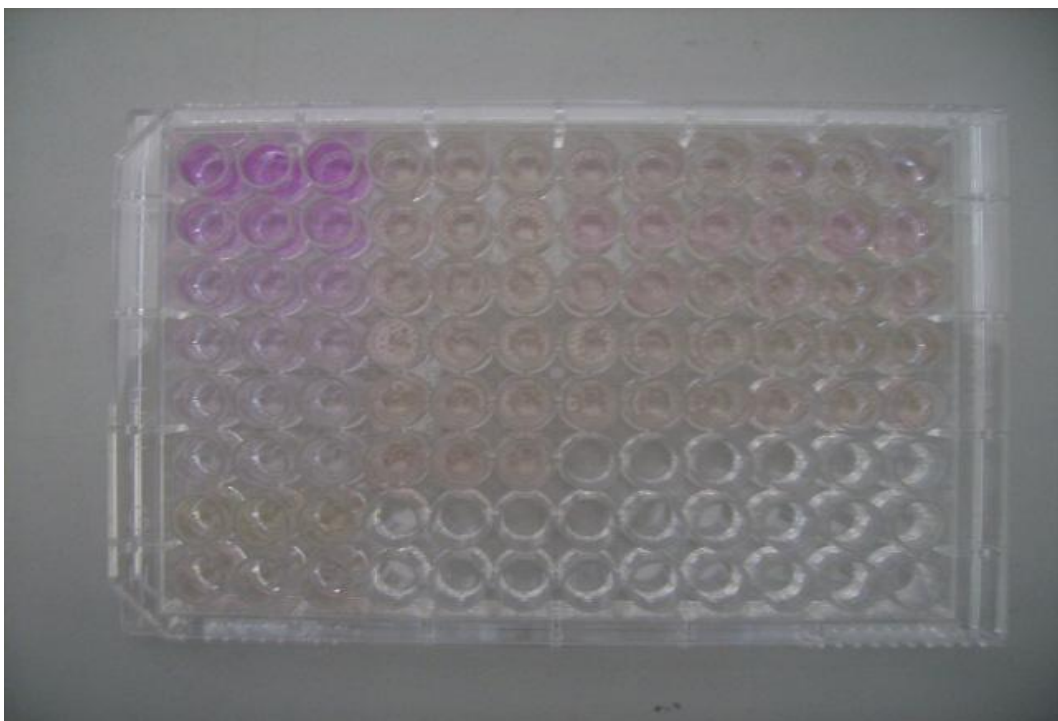
Gambar 4. Makrofag yang telah ditambahkan ekstrak pegagan 5 µg/ml dan diinkubasi pada suhu 37°C, 5% CO₂, selama 48 jam. Pembesaran 100 x.



Gambar 5. Makrofag yang telah ditambahkan INH 5 µg/ml dan diinkubasi pada suhu 37°C, 5% CO₂, selama 48 jam. Pembesaran 100x.



Gambar 6. Makrofag yang telah ditambahkan campuran ekstrak pegagan 5 $\mu\text{g/ml}$ + INH 5 $\mu\text{g/ml}$ dan diinkubasi pada suhu 37°C, 5% CO₂, selama 48 jam. Pembesaran 100x.



Gambar 7. Microplate berisi standar NaNO₂, sampel, dan kontrol setelah penambahan reagen Griess.

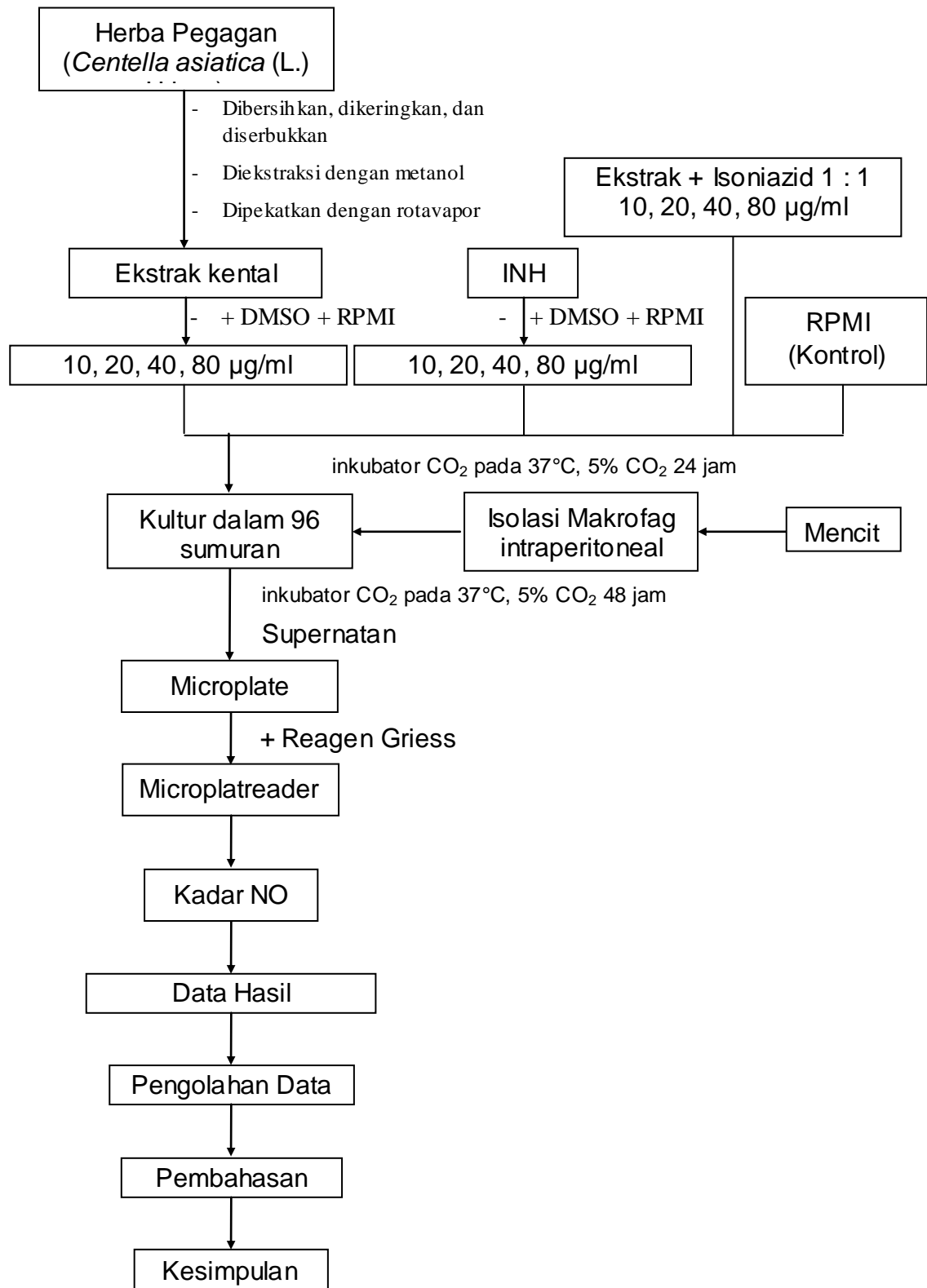


Gambar 8. Microplate Reader



Gambar 9. Tumbuhan Pegagan (*Centella asiatica* (L.)

Lampiran 1. Skema kerja

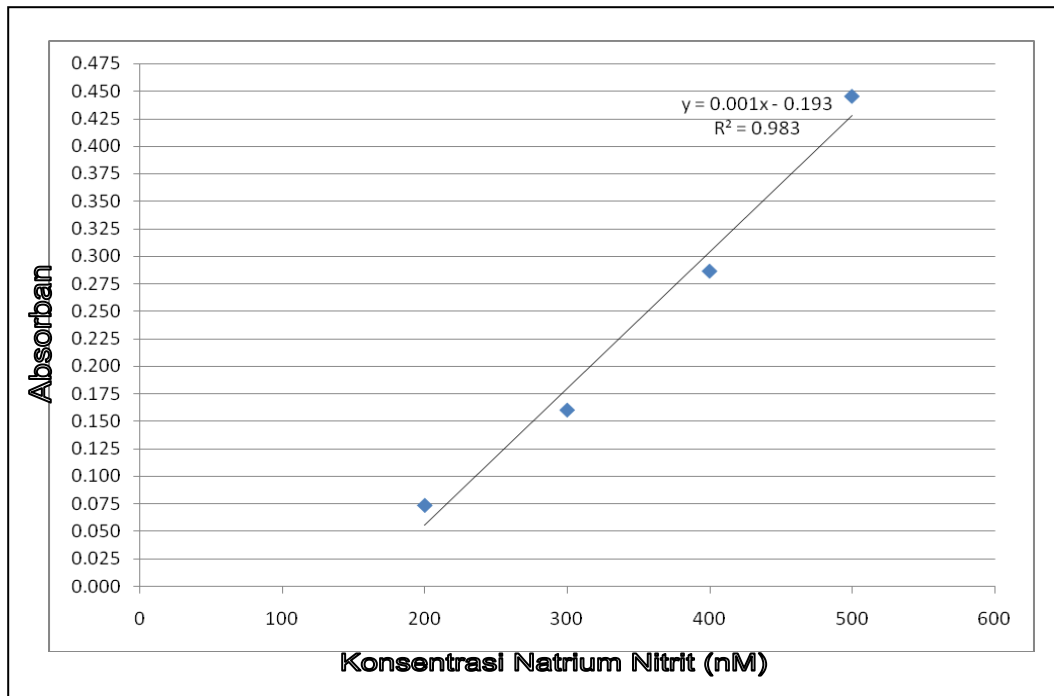


Lampiran 2. Hasil Pengukuran Serapan

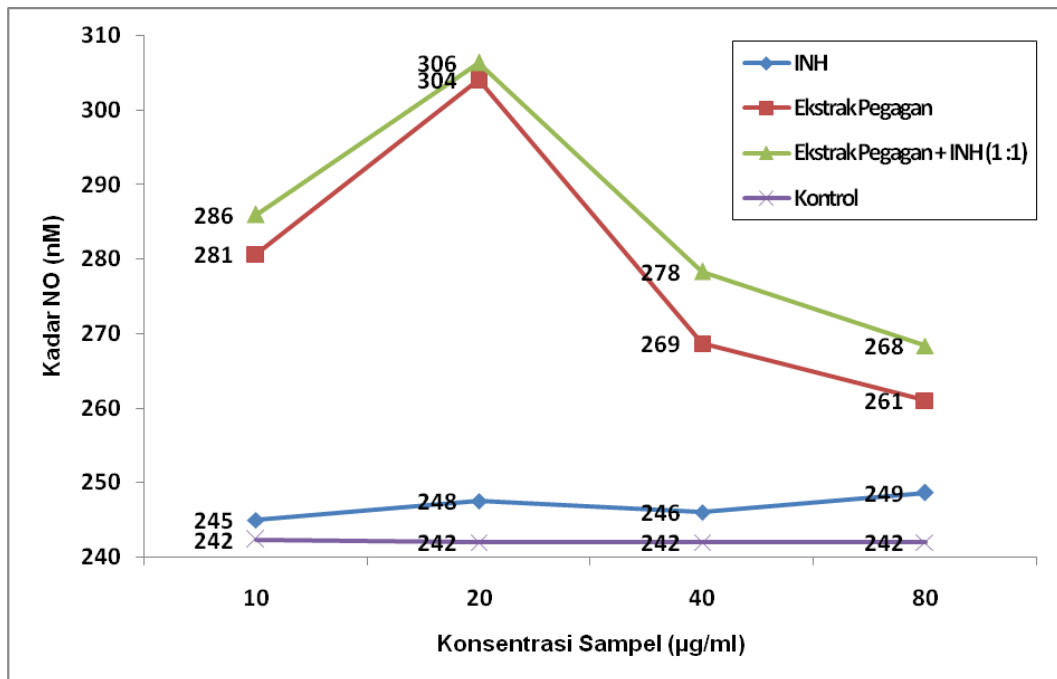
Tabel 2. Hasil pengukuran serapan sampel dengan microplate reader

Sampel	Konsentrasi	Absorban			Rerata
		I	II	III	
Baku NaNO₂	200 nM	0.07	0.074	0.077	0.074
	300 nM	0.164	0.157	0.16	0.160
	400 nM	0.288	0.284	0.288	0.287
	500 nM	0.448	0.442	0.447	0.446
INH	10 µg/ml	0.047	0.052	0.057	0.052
	20 µg/ml	0.05	0.059	0.084	0.064
	40 µg/ml	0.045	0.056	0.058	0.053
	80 µg/ml	0.048	0.056	0.063	0.056
Ekstrak Pegagan	10 µg/ml	0.082	0.089	0.092	0.088
	20 µg/ml	0.108	0.083	0.114	0.102
	40 µg/ml	0.078	0.078	0.071	0.076
	80 µg/ml	0.061	0.068	0.075	0.068
Ekstrak Pegagan + INH (1 : 1)	10 µg/ml	0.09	0.094	0.095	0.093
	20 µg/ml	0.112	0.118	0.11	0.113
	40 µg/ml	0.084	0.08	0.092	0.085
	80 µg/ml	0.071	0.076	0.079	0.075
Kontrol Sel		0.046	0.052	0.05	0.049

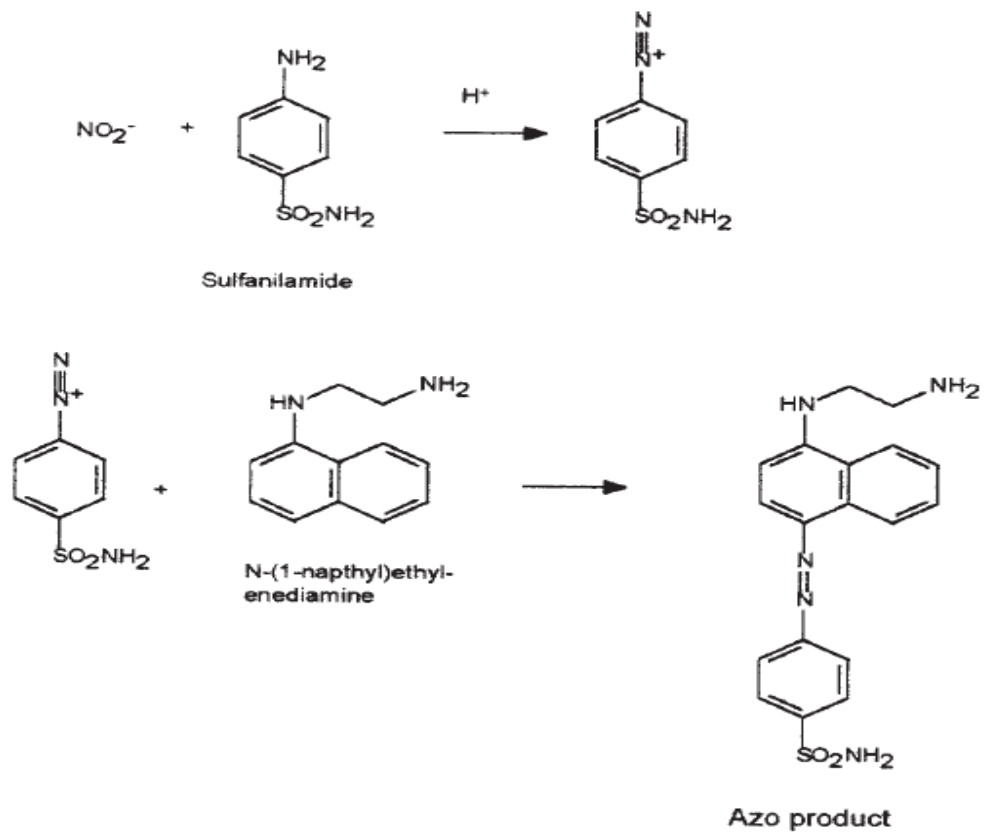
Lampiran 3. Kurva Baku NaNO₂



Lampiran 4. Kurva peningkatan produksi NO oleh sel makrofag dengan beberapa variasi konsentrasi sampel



Lampiran 5. Reaksi antara Natrium Nitrit dengan reagent Griess



Lampiran 6. Hasil Analisis Statistik

Tabel 3. Tabel Anova

ANOVA

Dependent Variable: KadarNO

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6534.375(a)	6	1089.063	10.465	.001
Intercept	1107230.063	1	1107230.063	10640.049	.000
Perlakuan	5629.188	3	1876.396	18.031	.000
Dosis	905.188	3	301.729	2.899	.094
Error	936.563	9	104.063		
Total	1114701.000	16			
Corrected Total	7470.938	15			

a R Squared = .875 (Adjusted R Squared = .791)

Tabel 4. Tabel hasil uji lanjutan LSD dengan variabel sampel

Multiple Comparisons

Dependent Variable: KadarNO

(I) Perlakuan	(J) Perlakuan	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval		
					Lower Bound	Upper Bound	
LSD	Kontrol	INH	-5.0000	7.21327	.506	-21.3175	11.3175
		Ekstrak Pegagan	-36.7500*	7.21327	.001	-53.0675	-20.4325
		Kombinasi Ekstrak Pegagan + INH	-42.5000*	7.21327	.000	-58.8175	-26.1825
	INH	Kontrol	5.0000	7.21327	.506	-11.3175	21.3175
		Ekstrak Pegagan	-31.7500*	7.21327	.002	-48.0675	-15.4325
		Kombinasi Ekstrak Pegagan + INH	-37.5000*	7.21327	.001	-53.8175	-21.1825
	Ekstrak Pegagan	Kontrol	36.7500*	7.21327	.001	20.4325	53.0675
		INH	31.7500*	7.21327	.002	15.4325	48.0675
		Kombinasi Ekstrak Pegagan + INH	-5.7500	7.21327	.446	-22.0675	10.5675
Kombinasi Ekstrak Pegagan + INH	Kontrol	42.5000*	7.21327	.000	26.1825	58.8175	
	INH	37.5000*	7.21327	.001	21.1825	53.8175	
	Ekstrak Pegagan	5.7500	7.21327	.446	-10.5675	22.0675	

Based on observed means.

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

Tabel 5. Tabel hasil uji lanjutan LSD dengan variabel konsentrasi

Multiple Comparisons

Dependent Variable: KadarNO

	(I) Dosis	(J) Dosis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	10 ug/ml	20 ug/ml	-11.5000	7.21327	.145	-27.8175	4.8175
		40 ug/ml	4.7500	7.21327	.527	-11.5675	21.0675
		80 ug/ml	8.5000	7.21327	.269	-7.8175	24.8175
	20 ug/ml	10 ug/ml	11.5000	7.21327	.145	-4.8175	27.8175
		40 ug/ml	16.2500	7.21327	.051	-.0675	32.5675
		80 ug/ml	20.0000*	7.21327	.022	3.6825	36.3175
	40 ug/ml	10 ug/ml	-4.7500	7.21327	.527	-21.0675	11.5675
		20 ug/ml	-16.2500	7.21327	.051	-32.5675	.0675
		80 ug/ml	3.7500	7.21327	.616	-12.5675	20.0675
	80 ug/ml	10 ug/ml	-8.5000	7.21327	.269	-24.8175	7.8175
		20 ug/ml	-20.0000*	7.21327	.022	-36.3175	-3.6825
		40 ug/ml	-3.7500	7.21327	.616	-20.0675	12.5675

Based on observed means.

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

Lampiran 7. Hasil Ekstraksi

Sampel Kering : 500 gram

Ekstrak metanol kental : 22,15 gram

Rendemen : 4,43 %

Lampiran 8. Komposisi medium RPMI 1640

Komponen	Berat Molekul	Konsentrasi (mg/L)	mM
Asam Amino			
Glycin	75	10	0.133
L-Arginin	174	200	1.15
L-Asparagin	132	50	0.379
L-Asam Aspartat	133	20	0.15
L-Sistin 2HCl	313	65	0.208
L-Asam Glutamat	147	20	0.136
L-Glutamin	146	300	2.05
L-Histidin	155	15	0.0968
L-Hidroksiprolin	131	20	0.153
L-Isoleusin	131	50	0.382
L-Leusin	131	50	0.382
L-Lisin HCl	146	40	0.274
L-Metionin	149	15	0.101
L-Fenilalanin	165	15	0.0909
L-Prolin	115	20	0.174
L-Serin	105	30	0.286
L-Treonin	119	20	0.168
L-Tryptofan	204	5	0.0245
L-Dinatrium Tirosin dihidrat	261	29	0.111
L-Valin	117	20	0.171
Vitamin			
Biotin	244	0.2	0.00082
Kolin Klorida	140	3	0.0214
D-Kalsium pantotenat	477	0.25	0.000524
Asam Folat	441	1	0.00227
Niasinamida	122	1	0.0082
Asam Para-Aminobenzoat	137	1	0.0073

Piridoksin HCl	206	1	0.00485
Riboflavin	376	0.2	0.000532
Thiamin HCl	337	1	0.00297
Vitamin B12	1355	0.005	0.0000037
i-Inositol	180	35	0.194
Garam anorganik			
Kalsium nitrat ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$)	236	100	0.424
Magnesium Sulfat (MgSO_4) (anhidrat.)	120	48.84	0.407
Magnesium Sulfat ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	246	100	0.407
Kalium Klorida (KCl)	75	400	5.33
Natrium Klorida (NaCl)	58	5850	100.86
Dinatrium Hidrogen Fosfat (Na_2HPO_4) Anhidrat	142	800	5.63
Komponen Lain			
D-Glukosa (Dekstrosa)	180	2000	11.11
Glutathion (tereduksi)	307	1	0.00326
HEPES	238	5958	25.03
Fenol merah	376.4	5	0.0133

Lampiran 9. Daftar Singkatan

Singkatan	Kepanjangan
AcpM	Acyl protein M
BD	Beta defensin
BCG	Basil Calmette Geurin
BTA	Basil Tahan Asam.
CD 4+	Cluster of Differentiation Four
CD 8+	Cluster of Differentiation Eight
cGMP	cyclic Guanosine Monophosphate
Cfu	Coloni forming unit
DMSO	Dimetil Sulfoksida
DNA	Deoxyribonucleic Acid
β -defensin	Beta defensin
Fc	Fragmen crystalible
G-6-PD	Glucose-6-phosphate dehydrogenase
μ g	micro gram
IFN- α	Interferon alfa.
Ig	Immunoglobulin : antibody
INH	Isonikotin Hidrazida, Isoniazida
HBD	Human Beta Defensin
HIV	Human Immunodeficiency Virus
IL	Interleukin
KasA	Ketoacyl Synthases
MI	Milli liter
MHC	Major Histocompatibility Complex
nNOS	neuronal Nitric Oxide Synthase
iNOS	Inductible Nitric Oxide Synthase
eNOS	endothelial Nitric Oxide Synthase
nM	nano Molar

NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NO	Nitric Oxide
NK	Natural Killer
pH	power of Hydrogen / potenz of Hydrogen
PMNs	Polymorphonuclear Neutrophils
RPMI-1640	Roswell Park Memorial Institute -1640
RNI	Reactive Nitrogen Intermediate
ROI	Reactive Oxygen Intermediate
SPS	Sewaktu Pagi Sewaktu
SOD	Super Oxide Dismutans
TBC	Tuberculosis
Tc	T cytotoxic
Th	T helper
Ts	T suppressor
TNF- α	Tumor Necrosis Factor alfa
TLRs	Toll Like Receptors
WBC	White blood cells