Abstract Book of
The International Seminar on Pharmacology and Clinical Pharmacy 2016

ISPCP ITB 2016

Current Trend of Molecular Pharmacology in The Drug Development and Clinical Use

School of Pharmacy
Institut Teknologi Bandung

September 1 - 2, 2016
Harris Hotel and Conventions
Jl. Ciumbuleuit No. 50-58,
Bandung - Indonesia

PROCEEDINGS OF
THE INTERNATIONAL SEMINAR ON PHARMACOLOGY AND CLINICAL PHARMACY 2016
(ISPCP 2016)

EDITORIAL BOARD

GREETING FROM THE DEAN OF SCHOOL OF PHARMACY ITB

Assalamualaikum wr.wb

Dear ISPCP attendees.

First of all, I would like to express my sincere thanks to all of you for participating in this International Seminar on Pharmacology and Clinical Pharmacy (ISPCP ITB 2016) with the theme of "*Current trend of molecular pharmacology in the drug development and clinical use" which is proudly organized by School of Pharmacy Institut Teknology Bandung.*

In recent years, an enormous progress has been made in drug development and uses. Molecular pharmacology is one of the pharmaceutical sciences which has progressed rapidly. It helps scientists to delineate the mechanism of action by which the drug works in the body. This knowledge will also help clinical pharmacists in rational use of drugs.

This seminar is the first International Seminar on Pharmacology and Clinical Pharmacy which is held by School of Pharmacy ITB. On behalf of the faculty, I would like to congratulate members of ISPCP's organizing committee to be able to organize this seminar. All of you have worked hard to make this conference a reality.

This seminar will truly serve as an international forum for researchers*,* students, practitioners and all parties interested in pharmacology and clinical pharmacy to embrace and share a diverse range of basic studies, techniques, and experiences.

Our School of Pharmacy ITB has a vision to be the leading institution in pharmacy education, research and social services, at regional and international level. Therefore, through this seminar, we want to be able to facilitate an event to share the latest knowledge and experience particularly in the fields of pharmacology and clinical pharmacy from around the world. So what we will get through this seminar can be applied for better community services, enhancing profession in pharmacy, and of course to stimulate increased innovative, competitive and continuous research in pharmaceutical fields. Through this event, we also can to create networking in pharmacy fields.

Finally, I wish a pleasant seminar to ISPCP ITB 2016 participant and I hope that this event will continue to be held every year.

Wassalamualaikum wr.wb.

Prof. Dr. Daryono Hadi Tjahjono, M.Sc

GREETING FROM THE CHAIRMAN OF ISPCP ITB 2016

On behalf of ISPCP ITB 2016 committee, I would like to gratitude and welcome to all invited speakers, committee, participants, guests, and all contributing sponsors, and all participants, to the International Seminar on Pharmacology and Clinical Pharmacy (ISPCP ITB 2016) today.

It is a great honor for me to welcome you all in our seminar. Welcome to our beloved city Bandung, and we hope you will enjoy your time here.

This seminar is the first International Seminar on Pharmacology and Clinical Pharmacy conducted by School of Pharmacy Institut Teknologi Bandung to facilitate expert meeting and sharing knowledge and latest development on the subjects among scientists, researchers, hospital and community pharmacists, regulatory officials, members of health professional organizations, representatives from pharmaceutical industries and individuals interested in the fields. Furthermore, the event can serve as a medium to establish networking with colleagues from hospital and industry sectors. International Seminar on Pharmacology and Clinical Pharmacy (ISPCP ITB 2016) will bring up the captivating theme : *"Current trend of molecular pharmacology in the drug development and clinical use", as a respond to the increasing attention for new pharmaceutical product.*

As we know, drug has been an essential part in human life. It is an important element in the prevention, treatment, and management of illness and the preservation of mental and physical well-being. Pharmacology is undoubtedly one of the fundamental knowledge in the development of drug; and molecular pharmacology, in particular, helps scientists to delineate the mechanism of action by which the drug works in the body. Understanding the mechanism of action of drug contributes to the rational use of drug in clinical setting to achieve the intended therapeutic goals.

In this seminar, we are fortunate to have **Prof. Dr. dr. Nila Djuwita F. Moeloek, SpM (K),** *the Health Minister of Republic of Indonesia, as a keynote speaker. We also invited 9 more experts in various field of pharmacology and clinical pharmacy both form Indonesia or overseas, who will give their inspiring lecturers. Here, among more than 167 participants, there are more than 100 presenter will present their recent research finding either as oral presenter or poster presenter, which are divided into two big topics: pharmacology and clinical pharmacy. Our high appreciation and sincere gratitude are delivered to all speakers and presenters who enthusiastically participate in our seminar.*

The organizing committee deeply acknowledge, The Rector of Institut Teknologi Bandung as well as to all of our sponsors for the invaluable supports to the seminar. As the chairman of the committee, I personally would like to express our high appreciation and gratitude to all team members who has put all the hard work, dedication, and extraordinary efforts for the success of the seminar.

Finally, while the event will serve as a means to showcase the recent development as well as findings in the area of pharmacology and clinical pharmacy, it is expected that results of the seminar will contribute significantly to public welfare. *We also hope that all participants could gain benefit from this event and we wish you an enjoyable moment in Bandung.*

Chairman
Dr. I Ketut Adnyana

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Pharmaceutics : Physical Pharmacy, Pharmaceutical Biotechnology, Pharmaceutical Technology, Biopharmacy and Pharmacokinetics

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SCHEDULE

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08.30 – 09.40 08.40 – 08.50 08.50 – 09.00 09.00 – 09.15	Opening Ceremony : Welcome dance Report of Chairman Rector of Institut Teknologi Bandung Opening Speech by Health Minister, Republic of Indonesia
09.15 – 09.45	Keynote Speaker Health Minister of Republic of Indonesia
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10.00 – 10.30	Plenary lecture 1 **Prof. Ikuo Saiki, PhD** (Toyama University, Japan)
10.30 – 11.00	Plenary lecture 2 **Raymond Tjandrawinata, PhD** (Dexa Medica, Indonesia)
11.00 – 11.30	Plenary lecture 3 Dr. Dan Gubler, Ph.D. (Unicity International, USA)
11.30 – 12.00	Discussion
12.00 – 13.00	Lunch
13.00 – 13.30	Plenary lecture 4 Prof. Dr. Gert Storm (Utrecht University, Netherlands)
13.30 – 14.00	Plenary lecture 5 Dra. Yulia Trisna, M.Pharm, Apt (Dr. Cipto Mangunkusumo National Hospital)
14.00 – 14.15	Discussion
14.15 – 15.15	Poster Presentation Session I
15.15 – 15.30	Coffee break
15.30 – 16.30	Oral Presentation Session I
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08.30 – 09.00	Plenary lecture 2 Dr. Neni Nurainy (PT. Bio Farma, Indonesia)
09.00 – 09.30	Discussion
09.30 – 09.45	Coffee break
09.45 – 10.15	Plenary lecture 3 Prof. Jeff Hughes (Curtin University, Australia)
10.15 – 10.45	Plenary lecture 4 Assoc. Prof. Maria Immaculata Iwo (ITB, Indonesia)
10.45 – 11.15	Discussion
11.15 – 13.00	Lunch
13.00 – 14.00	Oral Presentation Session II
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Cytotoxic Activity of Detam 1 Soybean and Jati Belanda Extracts on SV40 MES 13 Cell

Meilinah Hidayat[1], Sijani Prahatusti[1], Andreanus A Soemardji[2], Dwi Davidson Rihibiha[3], Merry Afni[3], Ervi Afifah[3], Wahyu Widowati[1,3]

[1]Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia
[2]School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia
[3]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia
Email: mellahidayat@yahoo.com

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ABSTRACT

The amount of Diabetes mellitus patients in Indonesia is predicted to be continually increasing. Lifestyle and pancreatic dysfunction in producing insulin, have been known as the main causes. Furthermore, diabetes mellitus type 1 and 2 can progressively develop, resulting in complication known as "diabetic glomerulosclerosis". Natural sources in Indonesia has been reported to contain bioactive compounds that possess medicinal properties. Jati Belanda (*Guazuma ulmifolia* Lamk) and Detam 1 soybean (*Glycine max* L.Merr) has been known to act as antiobesity due to presence of their secondary metabolite. This study aimed to determine cytotoxic activity of ethanol extract of Detam 1 soybean, Jati Belanda, and their combination toward SV40 MES 13 cell (Glomerular Mesangial Kidney, *Mus musculus*). Cytotoxic activity was measured with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Concentrations used were 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. Both Jati Belanda and Detam 1 soybean extract at concentration of 3.125, 6.25, and 12.5 µg/mL showed no toxicity toward SV40 MES 13 cell, as indicated by >90% viable cells, whilst at concentration of 25, 50, and 100 µg/mL showed toxicity, as indicated by <90% viable cells. The combination of extracts showed no toxicity only at low concentration (3.125 µg/mL). Thus, Jati Belanda and Detam 1 soybean extract at concentration of 3.125, 6.25, 12.5 µg/mL and the combination of extracts at 3.125 µg/mL were considered safe toward SV40 MES 13 cell.

Keywords: cytotoxicity activity, Detam 1 soybean, Jati Belanda, SV40MES 13 cell culture

Introduction

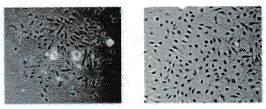
- Diabetes mellitus type 1 and 2 can later develop, resulting in complication to kidney organ, known as "diabetic glomerulosclerosis". The kidney's function becomes worsened if this condition are not well treated.
- The previous study, **subchronic toxicity test of Detam 1 Soybean and jati Belanda leaves** showed that after the administration of combination of both ethanol extracts for 90 days, **the kidney function level (BUN & Creatinine) of Wistar rats is better (lower) than Negative Control** (Hidayat, 2016)
- The best result of kidney function level is the group of combination of both extracts at high dose (600mg/kgBW) (Hidayat, 2016)

Objectives

- to determine cytotoxic activity of ethanol extract of Detam 1 soybean, Jati Belanda, and the combination of both extracts as protective agents in SV40 MES 13 cell as a future treatment for kidney diabetic glomerulosclerosis.

METHODS

Cell: SV40 MES 13 cell (Glomerular Mesangial Kidney, *Mus musculus*)



Sakairi, et al., 2010

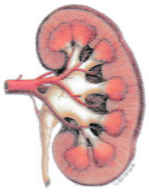
Concentration test:
a. Working solution: 1000; 500; 250; 125; 62.5; 31.25 (µg/mL)
b. Final concentration: 100; 50; 25; 12.5; 6.25; 3.125 (µg/mL)
c. Combination extracts of Detam 1 Soybean and Jati Belanda (2:1)
Kit: 96 cell titer aqueouse one (Abcam, 197010)
Method: MTS

ETHANOL EXTRACTION PROCEDURES *DETAM* 1 Soybean and JATI BELANDA leaves

Detam 1 Soybean (*Glycine max* L.Merr) from Nuts and Tuber Research Institute, Malang Indonesia; high in antioxidants: Fenol, Isoflavones

Jati belanda (*Guazuma ulmifolia* Lamk) Leaves from Bumi Herbal Dago Plantation, North Bandung; contains: Fenol and tannin

COMBINATION



(Hidayat, 2016)

The principle of MTS test

- MTS indirectly measured on **products colored compound** produced by the interaction of **reagents** with **viable cells**.
- MTS is a tetrazolium component [3- (4,5 - dimetiltiazol - 2 – yl) -5- (3 - karboksimetoksifenil) -2- (4 - sulfophenyl) -2H – tetrozolium , salt form , MTS]
- MTS tetrazolium component is reduced by forming cells formazen colored product soluble in the culture medium.
- **Formazen colored total product** measured by spectrophotometer from **absorbance at wavelength of 490 nm**, whereby absorption product is **equivalent to the number of viable cells in culture**.

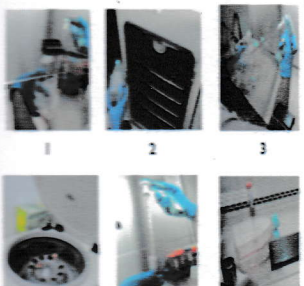
Table 1 Result of BUN level in Wistar Rats After DSJB Combination Toxicity Subchronic Test

		MALE		FEMALE	
GROUP	n	Mean	SD	Mean	SD
Negative Control	10	64.40	9.947	56.30	5.559
Low Dose	10	57.50	4.927	53.10	4.458
Intermediate Dose	10	52.80	4.088	47.00	2.539
High Dose	10	45.20*	2.974	40.10*	1.197
Control Satellite	10	56.40	7.849	49.90	5.626
Satellit High Dose	10	40.90*	2.998	38.00*	2.667
Sig ANOVA	0.000	Tukey *		0.000	Tukey *

Table 2 Result of Creatinine level in Wistar Rats After DSJB Combination Toxicity Subchronic Test

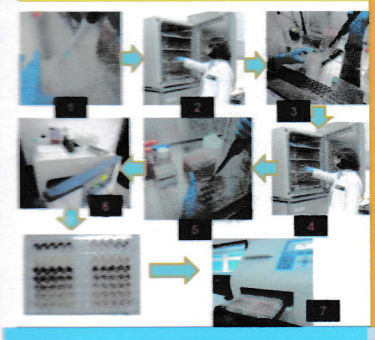
		MALE		FEMALE	
GROUP	n	Mean	SD	Mean	SD
Negative Control	10	0.9910	0.14395	0.9060	0.07121
Low Dose	10	0.8890	0.07631	0.7820*	0.11144
Intermediate Dose	10	0.8640*	0.07604	0.8180	0.07195
High Dose	10	0.9240	0.06518	0.7890	0.07279
Control Satellite	10	0.8790	0.10296	0.7580	0.12347
Satellit High Dose	10	0.8290*	0.05522	0.7610*	0.07078
Sig ANOVA	0.033	Tukey *		0.091	Tukey *

SV40 MES 13 cells culture



1. Macrophage murine cell SV40 MES 13 were grown in DMEM supplemented with 20% FBS, 100U/ml penicillin, 100µg/ml streptomycin.
2. Cells maintained at 37°C humidified atmosphere incubator with 5% CO2 until the cells were confluent.
3. Cells were washed and harvested using Trypsin-EDTA.
4. Cells centrifuged at 2500rpm for 4 minutes.
5. Dispose supernatant and resuspense the pellet.
6. Cells were added to new T flask containing fresh medium (Kang et.al 201; Yoon et.al., 2009)

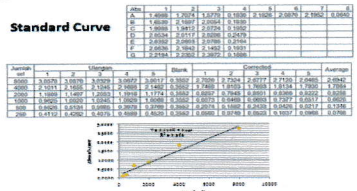
Cytotoxic Activity Test



1. 5000 cell /well SV40 MES 13 (trypan blue stained) DMEM + 10%FBS
2. Incubating 24 h , 37°C, 5% CO2
3. Replacing medium 90 µl (free FBS) and add 10 µl EEDS, EEJB and DSJB at concentration 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL
4. Incubating 24 h, 37°C, 5% CO2
5. Cells viability by add 20 µl MTS each well
6. Incubating 3 h , 37°C, 5% CO2
7. Measuring the absorbance using spectrophotometer at 490 nm.

RESULTS:

Standard Curve



Cytotoxic activity of ethanol extract of Detam 1 soybean (EEDS) toward SV40 MES 13 cell

Samples	No. of cells	Viability cells (%)
Control	10579±1065	100.00±11.93
EEDS 100 µg/mL	9098±351	83.24±3.94
EEDS 50 µg/mL	9522±246	87.22±2.76
EEDS 25 µg/mL	9641±121	89.56±1.35
EEDS 12.5 µg/mL	**9670±259**	**90.23±2.90**
EEDS 6.25 µg/mL	**10045±43**	**94.38±0.48**
EEDS 3.125 µg/mL	**10318±136**	**97.25±1.53**

Cytotoxic activity of ethanol extract of Jati Belanda leaves (EEJB) toward SV40 MES 13 cell

Samples	No. of cells	Viability cells (%)
Control	10579±1065	100.00±11.93
EEJB 100 µg/mL	6666±186	55.93±2.08
EEJB 50 µg/mL	7784±305	67.76±3.41
EEJB 25 µg/mL	9394±538	86.66±6.03
EEJB 12.5 µg/mL	**9708±470**	**90.68±5.27**
EEJB 6.25 µg/mL	**9962±157**	**93.24±1.76**
EEJB 3.125 µg/mL	**10152±74**	**94.82±0.82**

- In concentration of 3.125, 6.25, and 12.5 µg/mL showed no toxicity toward SV40 MES 13 cell. (>90% viable cells).
- In concentration of 25, 50, and 100 µg/mL showed toxicity (<50% viable cells).

Cytotoxic activity of DSJB (combination EEDS and EEJB) toward SV40 MES 13 cell

Samples	No. of cells	Viability Cells (%)
Control	7457±328	100.00±4.40
Kontrol DMSO	7038±206	94.8±2.76
DSJB100 µg/Ml	5161±350	69.21±4.69
DSJB 50 µg/Ml	6306±866	84.60±11.61
DSJB 25 µg/Ml	6526±219	87.51±2.94
DSJB 12.5 µg/Ml	6606±73	88.59±0.98
DSJB 6.25 µg/Ml	6704±73	89.90±0..7
DSJB 3.125 µg/mL	**6947±205**	**93.17±2.75**

Combination of EEDS and EEJB showed toxicity in concentration 3.125 µg/mL (>90%)

CONCLUSION

- Ethanol extract of Detam 1 soybean (EEDS), ethanol extract of Jati Belanda leaves (EEJB) in concentration of 3.125, 6.25, and 12.5 µg/mL and
- Combination of both extracts (DSJB) in concentration of 3.125 µg/mL were considered safe toward SV40 MES 13 cell.

Acknowledgements

The authors gratefully acknowledge the financial support from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia (Research Grant No SP3 :1013/K4/KM/2015, DIPA-023.04.1.673453/2015). This research was also supported by Biomolecular & Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia for the laboratory facilities and research methodology.

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PO-PY-013

Cytotoxic Activity of Detam 1 Soybean and Jati Belanda (*Guazuma ulmifolia*) Extracts on SV40 MES 13 Cell

Meilinah Hidayat[1], Sijani Prahatusti[1], Andreanus A Soemardji[2], Dwi Davidson Rihibiha[3], Merry Afni[3], Ervi Afifah[3], Wahyu Widowati[1]

[1]Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

[2]School of Pharmacy, Institut Teknologi Bandung, Indonesia

[3]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, Indonesia

Background: The amount of Diabetes mellitus patients in Indonesia is predicted to be continually increasing. Lifestyle and pancreatic dysfunction in producing insulin, have been known as the main causes. Furthermore, diabetes mellitus type 1 and 2 can progressively develop, resulting in complication known as "diabetic glomerulosclerosis". Diminished glomerular basement membrane causes failure in urine filltration, that causes presence of albumin in urine. Natural sources in Indonesia has been reported to contain bioactive compounds that possess medicinal properties. Jati Belanda (*Guazuma ulmifolia*) and Detam 1 soybean has been known to act as antiobesity and antidiabetes due to presence of its secondary metabolite. **Objectives:** This study aimed to determine cytotoxic activity of extract of Detam 1 soybean, Jati Belanda, and combination of extracts, toward SV40 MES 13 cell (Glomerular Mesangial Kidney, *Mus musculus*). **Methods:** Cytotoxic activity was measured with 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS) assay. Concentrations used were 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. **Results:** Both Jati Belanda and Detam 1 soybean extract at concentration of 3.125, 6.25, and 12.5 µg/mL showed no toxicity toward SV40 MES 13 cell, as indicated by >90% viable cells, whilst at concentration of 25, 50, and 100 µg/mL showed toxicity, as indicated by <90% viable cells. The combination of extracts showed no toxicity only at low concentration (3.125 µg/mL). **Conclusion:** Thus, Jati Belanda and Detam 1 soybean extract at concentration of 3.125, 6.25, 12.5 µg/mL and the combination of extracts at 3.125 µg/mL were considered safe toward SV40 MES 13 cell.

Keywords: Diabetic glomerulosclerosis, Jati Belanda, Detam 1 Soybean, SV40 MES 13 cell.

Background

Diabetes mellitus type 1 and 2 can later develop, resulting in complication known as "diabetic glomerulosclerosis". The kidney function becomes worsened if this condition are not well treated. Diminished glomerular basement membrane causes failure in urine filltration, that causes presence of albumin in urine. The previous study, subchronic toxicity test of combination extract of Detam 1 soybean and jati Belanda leaves, showed that after treatment the administration of combination of both extract s, the kidney function level (Blood Urea Nitrogen and creatinine level) of Wistar Rats are better (lower) than the negative control group. The best result of kidney function level was the high dose group (600 mg/kgBW).

Natural sources in Indonesia has been reported to contain bioactive compounds that possess medicinal properties. Jati Belanda (*Guazuma ulmifolia*) and Detam 1 soybean (*Glycine max*) has been known to act as antiobesity and antilipidemia due to presence of its secondary metabolite.

The aim of this study is to determine cytotoxic activity of extract of Detam 1 soybean, Jati Belanda, and combination of both extracts as a protective agent in SV40 MES 13 cell (Glomerular Mesangial Kidney, *Mus musculus*) induced kidney glucose as an in vitro diabetic glomerulosclerosis.

Methodology

Cytotoxic Activity of ethano extrcat of Detam 1 soybean (EEDS) and ethanol extract of Jati Belanda leaves (EEJB) toward SV40 MES 13 cell (Glomerular Mesangial Kidney, *Mus musculus*) using MTS test.

The principal of MTS test

MTS indirectly measures on products of coloured compound produced by the interaction of reagen and viable cells. MTS is a tetrazolium component [3-(4,5 - dimetiltiazol - 2 - yl) -5- (3 - karboksimtoksifenil) - 2 - (sulfophenyl) - 2 H - tetrzolium, saltform, MTS]. MTS tetrazolium component is rduced by forming cells formazen colored product soluble in the culture medium. Total formazen colored product is measured by spectrophotometer from absorbance at wavelength 0f 490 nm, whereby absorption product is equivalent to the number of viable cells in the culture.

Step 1. SV40 MES 13 cells culture

Sample:

Growth Medium: DMEM : F-12K Mix Nutrient +14Mm HEPES (1:3), 5% FBS, 1% PenStrep

Tools:

Centrifuge Tube, CentriStar Cap15 ml, TC Flask 25 cm Vent Cap, Blue tip (100-1000 µL), Yellow tip (10-100 µL), White tip (2-10 µL), Serological Pippet 10 mL, Serological Pippet 5 mL, Refrigerated Centrifuge, Microscope Inverted

Procedures:

Macrophage murine cell SV40 MES 13 were grown in DMEM supplemented with 20% FBS, 100U/ml penicillin, 100µg/ml streptomycin. Cells were maintained at 37°C humidified atmosphere incubator with 5% CO2 until the cells were confluent. Cells were washed and harvested using Trypsin-EDTA. Cells centrifuged at 2500rpm for 4 minutes. Dispose supernatant and resuspense the pellet then added to new T flask containing fresh medium (Kang *et.al.*201;Yoon *et.al.*, 2009).

Step 2. Cytotoxic Activity

Sample:

Sel SV40 MES 13 (ATCC® CRL1927™) Aretha Medika Utama BBRC, DMEM (Gibco), F-12 K Mix Nutrient (Gibco), 14 Mm HEPES (Sigma), 5% FBS (Gibco), 1% PenStrep (BioWest), Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (Promega, G3581)

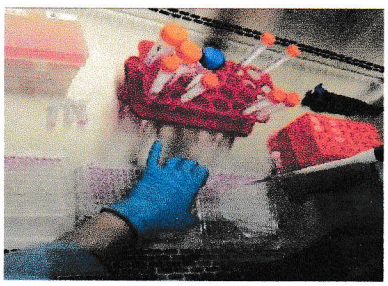
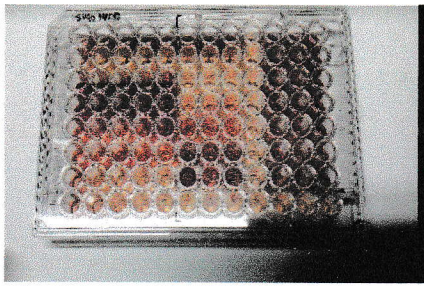
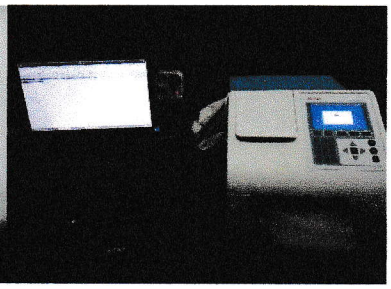
Tools:

Centrifuge Tube, CentriStar Cap, 15 ml, TC Flask 25 cm Vent Cap, Blue tip (100-1000 µL), Yellow tip (10-100 µL), White tip (2-10 µL), 96 well plate, Refrigerated Centrifuge, Microscope Inverted

Concentration test
a. working solution : 1000; 500; 250; 125; 62,5; 31,25 (ug/ml)
b. final concentration : 100; 50; 25; 12,5; 6,25; 3,125 (ug/ml)
c. kombinasi ekstrak Jati Belanda dan Kedelai Detam (2:1)

Procedures

Amount of confluent SV40 MES 13 cell: $5x10^3$ cell /well were put with in 96 well plate which has contained EEJB and EEDS, DMEM+10%FBS, then plate are incubated at 37°C humidified atmosphere incubator with 5% CO2. After that replaced the medium (free FBS). Incubating for 24 hours, after that the SV40 MES 13 cell were treated with EEJB and EEDS in 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL.Incubating for 24 hours, and checked the cells viability using MTS, and measured the absorbance at 490 nm.

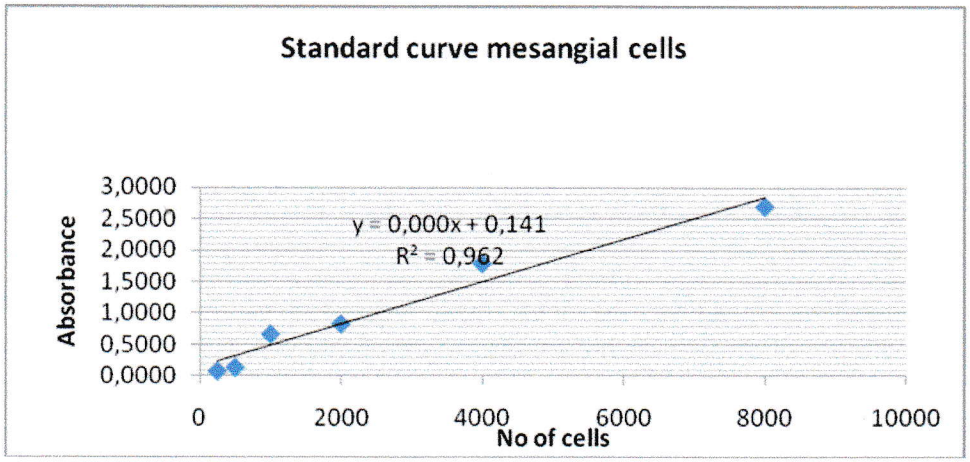




Results:

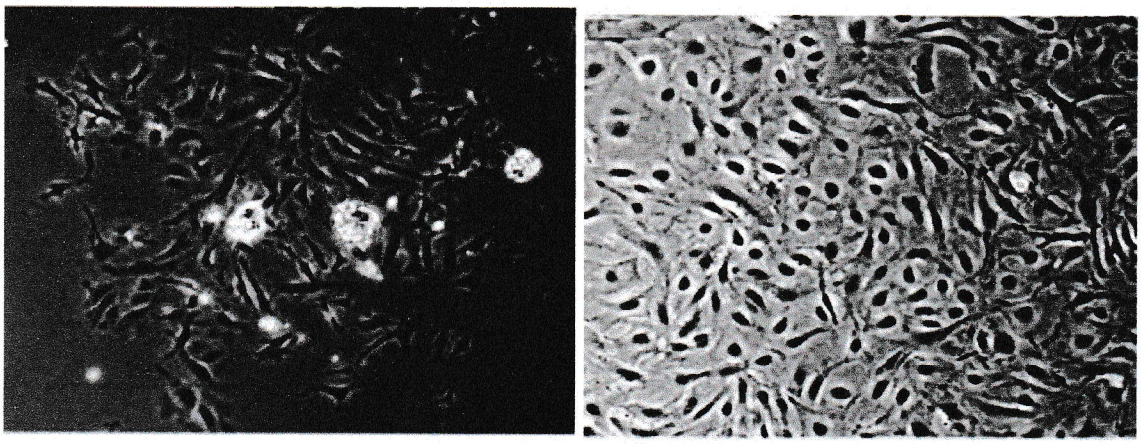
First we must make standard curve, which result is fulfill the requirement. The result R^2 is almost nearly 1.000 , that is 0.962.

Abs	1	2	3	4	5	6	7	8
A	1,4998	1,7074	1,5779	0,1830	2,1926	2,0870	2,1952	0,0640
B	1,6530	2,1597	2,0054	0,1830				
C	1,9998	1,9412	2,0724	0,1952				
D	2,0534	2,0117	2,0208	0,2479				
E	2,0352	2,0603	2,0786	0,2164				
F	2,0636	2,1842	2,1452	0,1931				
G	2,2194	2,2352	2,3972	0,1886				

Jumlah sel	Ulangan					Blank	Corrected					Average
	1	2	3	4	5		1	2	3	4	5	
8000	3,0578	3,0876	3,0329	3,0672	3,0017	0,3552	2,7026	2,7324	2,6777	2,7120	2,6465	2,6942
4000	2,1011	2,1655	2,1245	2,1686	2,1482	0,3552	1,7459	1,8103	1,7693	1,8134	1,7930	1,7864
2000	1,1809	1,1497	1,2053	1,1918	1,1774	0,3552	0,8257	0,7945	0,8501	0,8366	0,8222	0,8258
1000	0,9625	1,0020	1,0245	1,0929	1,0069	0,3552	0,6073	0,6468	0,6693	0,7377	0,6517	0,6626
500	0,5626	0,5134	0,5985	0,3978	0,3769	0,3552	0,2074	0,1582	0,2433	0,0426	0,0217	0,1346
250	0,4112	0,4292	0,4075	0,4589	0,4520	0,3552	0,0560	0,0740	0,0523	0,1037	0,0968	0,0766



Morphology SV40 MES 13



Sakairi, et al., 2010

Tabel 1. Cytotoxic activity of ethanol extract of Detam 1 soybean (EEDS) toward SV40 MES 13 cell

Samples	No. of cells	Viability cells (%)
Control	10579±1065	100,00±11,93
EEDS 100 µg/mL	9098±351	83,24±3,94
EEDS 50 µg/mL	9522±246	87,22±2,76
EEDS 25 µg/mL	9641±121	89,56±1,35
EEDS 12,5 µg/mL	9670±259	90,23±2,90
EEDS 6,25 µg/mL	10045±43	94,38±0,48
EEDS 3,125 µg/mL	10318±136	97,25±1,53

Tabel 2. Cytotoxic activity of ethanol extract of Jati Belanda leaves (EEJB) toward SV40 MES 13 cell

Samples	No. of cells	Viability cells (%)
Control	10579±1065	100,00±11,93
EEJB 100 µg/mL	6666±186	55,93±2,08
EEJB 50 µg/mL	7784±305	67,76±3,41
EEJB 25 µg/mL	9394±538	86,66±6,03
EEJB 12,5 µg/mL	9708±470	90,68±5,27
EEJB 6,25 µg/mL	9962±157	93,24±1,76
EEJB 3,125 µg/mL	10152±74	94,82±0,82

In concentration of 3.125, 6.25, and 12.5 µg/mL showed no toxicity toward SV40 MES 13 cell, (>90% viable cells). In concentration of 25, 50, and 100 µg/mL showed toxicity (<90% viable cells).

Table 3. Cytotoxic activity of combination ethanol extract of Detam 1 soybean (EEDS) and ethanol extract of Jati Belanda leaves (EEJB) toward SV40 MES 13 cell

Samples	No. of cells	Viability Cells (%)
Control	7457±328	100,00±4,40
Kontrol DMSO	7038±206	94,38±2,76
JBDS 100 µg/Ml	5161±350	69,21±4,69
JBDS 50 µg/Ml	6309±866	84,60±11,61
JBKDS 25 µg/Ml	6526±219	87,51±2,94
JBDS 12,5 µg/Ml	6606±73	88,59±0,98
JBDS 6,25 µg/Ml	6704±73	89,90±0,97
JBDS 3,125 µg/mL	6947±205	93,17±2,75

Combination ethanol extract of Detam 1 soybean (EEDS) and ethanol extract of Jati Belanda leaves (EEJB)In concentration of 3.125 µg/mL showed no toxicity toward SV40 MES 13 cell, (>90% viable cells).

Conclusion:

- Ethanol extract of Detam 1 soybean (EEDS), ethanol extract of Jati Belanda leaves (EEJB) in concentration of 3.125, 6.25, and 12.5 µg/mL and
- Combination of both extracts in concentration of 3.125 µg/mL were considered safe toward SV40 MES 13 cell.

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