



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

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



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

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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 wellbeing. 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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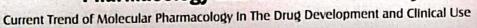
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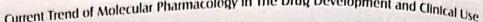
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SCHEDULE

Day 1: Thursday	(September 1, 2016)
08.00 - 08.30	Registration
08.00	Opening Ceremony:
08.30 - 09.40 08.40 - 08.50 08.50 - 09.00 09.00 - 09.15	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
09.45 - 10.00	Coffee break (Pharmacoustic)
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
	Gala Dinner (optional)

The International Seminar on Pharmacology and Clinical Pharmacy 2016 Current Trend of Molecular Pharmacology In The Drug Development and Clinical Use



Day 2 : Friday (Se	ptember 2, 2016)
08.00 - 08.30	Plenary lecture 1
	Assoc. Prof. Kenichi Ogawara (Okayama
	University, Japan)
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
14.00 – 15.00	Oral Presentation Session III
15.00 - 15.15	Coffee break
15.15 – 16.15	Oral Presentation Session IV
16.15 – 16.30	Closing Ceremony and Award Presentation





The International Seminar on Pharmacology and Clinical Pharmacy 2016 Current Trend of Molecular Pharmacology in The Drug Development and Clinical Use



12.	PO-PY-012	Acute Toxicity of Liman	Meti Widiya Lestari, Andreanus A
		(Elephantopus scaber L.) Leaves	Soemardji, Irda Fidrianny, Ayda T
		Extract and Coriander (Coriandrum	Yusuf
L NO.		sativum L.) Seed Extract	,
13.	PO-PY-013	Cytotoxic Activity of Detam Soybean	Meilinah Hidayat, Sijani Prahatusti,
-		and Jati Belanda (Guazuma ulmifolia)	Andreanus A Soemardji, Dwi
		Extracts on SV40 MES 13 Cell	Davidson Rihibiha, Merry Afni, Ervi
			Afifah, Wahyu Widowati
14.	PO-PY-014	Anti-obesity effect Solanum	Dytha Andri Deswati, Sri Maryam,
	•	betaceum Cav extract in mice	Erliana Fatmawati3
15.	PO-PY-015	Hepatoprotective Activity Of Water	Puspa Sari Dewi, Soraya Riyanti
		Extract Of Mexican Sunflower Against	
		Paracetamol Induced Hepatotoxicity	
		In Rats	<u> </u>
16.	PO-PY-016	Effect Of Oral Administration Of	Wahyuningsih S, Sukandar EY,
		Ethanol Extract Of Roselle Calyx	Sukrasno
		(Hibiscus sabdariffa L) On Kidney And	
		Liver Function On Wistar Rats	1398 IX
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٠,,	,	Combination of k-Carrageenan and	Sasongko, I Ketut Adnyana, Sundani
		Glucomannan as Soft Capsule Shell	Nurono Soewandhi





Cytotoxic Activity of Detam 1 Soybean and Jati Belanda Extracts on SV40 MES 13 Cell

Meilinah Hidayat¹, Sijani Prahatusti¹, Andreanus A Soemardji², Dwi Davidson Rihibiha³, Merry Afni³, Ervi Afifah³, Wahyu Widowati^{1,3}

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ABSTRACT

The amount of Diabetes melitius 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 melitius type and 2 can progressively develop, resulting in complication known as "diabetic glomerulosclerosis". Natural sources in Indonesia has been reported to contain bloactive compounds that possess medicinal properties. Jati Belanda Guezuma 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 Detarn 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-dimethythiazol-2-yf)-5-(3-carboxyme-thoxyphenyl)-2-(4sufformeny/-2+-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 inward 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 Belands 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: cytotoxicy activity, Detam 1 soybean, Jati Belanda, SV40MES 13 cell culture

Introduction



- Diabetes mellitus type 1 and 2 can later develop, resulting in complication to kildney organ, known as "diabetic glomerulos clerosis". The kildney's function becomes worsened if this condition are not well treated.
- 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 (Market 2016).
- The best result of kidney function level is the group of combination of both extracts at high dose (600mg/kgBW) (Hobyat.2018)

METHODS

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





Concentration test:

- 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)
- Combination extracts of Detam 1 Soybean and Jati Belanda (2:1) Kit: 96 cell titer aqueouse one (Abcam, 197010)

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 -

MTS tetrazolium component is reduced by forming cells

formazen colored product soluble in the culture medium.

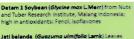
Formazen colored total product measured by spectrophotometer

from absorbance at wavelength of 490 nm, whereby absorption

product is equivalent to the number of viable cells in culture

ETHANOL EXTRACTION PROCEDURES

DETAM: Soybean and JATI BELANDA leaves





from Bumi Herbal Dago Plantation, N Bandung: contains: Fenol and tannin



		MALE		FEMALE	
GROUP	n	Mean	50	Mean	50
Negative Control	10	64.40	9.947	56.30	5.359
Low Dose	10	57.50	4.927	52.10	4.458
Intermediate Dose	10	52.60	4.088	47.00	2.589
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	50	Mean	50			
Negative Control	10	0.9910	0.14395	0.9060	0.07121			
Low Dose	10	0.8890	0.07651	0.7820*	0.11144			
Intermediate Dose	10	0.8640°	9.97504	0.8180	0.07193			
High Dose	10	0.9240	0.06518	0.7890	0.07279			
Control Satellite	10	0.8790	0.10256	0.7590	0.12547			
Satellit High Dose	10	0.8290°	0.05322	0.7510°	0.07078			
Sie ANOVA	0.088	Tukey *		0.091	Tukey*			



COMBINATION

(Hidavat, 2016)

Objectives

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

5V40 MES 13 cells culture























- maintained at 37°C Cells umidified atmosphere incubator with 5% CO2 until the cells were
- Cells were washed and harvested
- using Trypsin-EDTA. Cells centrifuged at 2500rpm for 4
- resuspense the pellet.
- Cells were added to new I flask containing fresh medium (Kang et.al.201;Yoon et.al., 2009)

5000 cell /well SV40

MES 13 (trypan blue stained) DMEM + 10%FBS Incubating 24 h 37°C, 5% CO2 Replacing medium 90 µl (free FBS)

and add 10 µl EEDS.

concentration 3.125, 6.25, 12.5, 25, 50, and

Cells viability by add 20 µl MTS each well Incubating 3 h , 37°C, 5% CO2 Measuring the absorbance using spectrophotometer at 490 nm.

EEJB and DSJB at

100 µg/mL Incubating 24 h, 37°C, 5% CO2

RESULTS:

tetrozolium, salt form, MTS]



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

Samples	No. of cells	Viability cells (%)	In concentration of 3.125, 6.
Control	10579±1065	100.00±11.93	25, and 12.5 µg/mL showed
EEJB 100 µg/mL	6666±186	55.93±2.08	no toxicity toward 5V40 MES 19 cell. (>90% yiable cells).
EEJB 50 µg/mL	7784±305	67.76±3.41	In concentration of 25, 50, a
EEJB 25 µg/mL	9394±538	86.66±6.03	nd 100 µg/m), showed toxici ty (<90% viable cells).
EEJB 12.5 µg/mL	9708±470	90.6815.27	4(
EEJB 6.25 µg/mL	9962±157	93.24±1.76	
EEJB 3.125 µg/mL	10152±74	94.82±0.82	

otoxic Activity Test



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.

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

COLUMN TO SERVICE SERV		
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
EEDE 2 125 ug/ml	10318+136	97,25+1,53

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

Samples	No. of cells	Viability Cells (%)	Combination of EEDS and
Control	7457±328	100.00±4.40	EEJB showed toxicity in
Kontrol DMSO	7038±206	94.8±2.76	concentration 3.125 µg/mL
DS JB100 µg/MI	5161±350	69.21±4.69	(same)
DSJB 50 µg/MI	6309±866	84.60±11.61	
DSJB 25 µg/MI	6526±219	87.51±2.94	
DSJB 12.5 µg/Ml	6606±73	88.59±0.98	
DSJB 6.25 µg/MI	6704±73	89.90±0,.7	
DSJB 3.125 µg/mL	6947±205	93.1722.75	

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

Meilinah Hidayat¹, Sijani Prahatusti¹, Andreanus A Soemardji², Dwi Davidson Rihibiha³, Merry Afni³, Ervi Afifah³, Wahyu Widowati¹

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

²School of Pharmacy, Institut Teknologi Bandung, Indonesia

³Biomolecular 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 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme-thoxyphenyl)-2-(4measured 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 μ L), Yellow tip (10-100 μ L), White tip (2-10 μ L), 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® CRL1927TM) 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 μ L), Yellow tip (10-100 μ L), White tip (2-10 μ L), 96 well plate, Refrigerated Centrifuge, Microscope Inverted

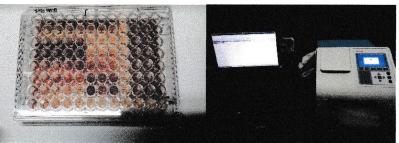
Concentration test

- 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 μ g/mL.Incubating for 24 hours, and checked the cells viability using MTS, and measured the absorbance at 490 nm.



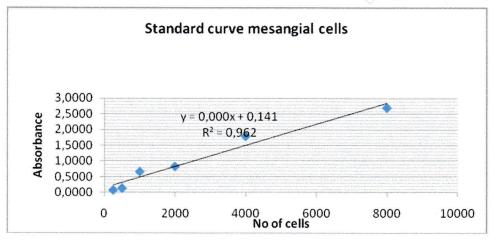


Results:

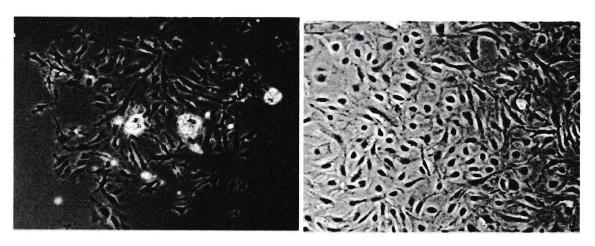
First we must make standard curve, which result is fulfill the requirement. The result R^2 is almost nearly 1.000, that is 0.962.

Abs	1	2	3	4	5	6	7	8
Α	1,4998	1,7074	1,5779	0,1830	2,1926	2,0870	2,1952	0,0640
В	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			Ulangan			Blank		4	Corrected	i		Augraga
sel	1	2	3	4	5	DIGITA	1	2	3	4	5	Average
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 $3,125 \mu g/mL$	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	
EEJB 12,5 μg/mL EEJB 6,25 μg/mL	9708±470 9962±157	90,68±5,27 93,24±1,76	

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

References

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