

# The 3<sup>rd</sup> International Seminar on Chemistry 2014

*Innovation and Advances in Chemistry  
for The 21<sup>st</sup> Century Challenges*

## Program and Abstracts

20 - 21 November 2014

Aula Pusat Studi Bahasa Jepang  
Jatinangor Campus, Universitas Padjadjaran  
Indonesia



organized by

**Department of Chemistry  
Faculty of Mathematics and Natural Sciences  
Universitas Padjadjaran**

in cooperation with

**Indonesia Chemical Society**



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## Schedule of 3<sup>rd</sup> International Seminar on Chemistry 2014 Jatinangor, 20-21 November 2014

### Thursday, 20 November 2014

#### Time

|             |                                                         |
|-------------|---------------------------------------------------------|
| 08.00-08.30 | Registration, poster posting                            |
| 08.30-09.00 | Opening Ceremony                                        |
|             | 1. Chairperson of the Organizing Committee              |
|             | 2. Head of Indonesian Chemical Society                  |
|             | 3. Rector of Universitas Padjadjaran (Official Opening) |
| 09.00-09.40 | Plenary Session I (Chairperson: Juliandri, Ph.D)        |
| 09.00-09.30 | Prof. Dr. Henry F. Schaefer III (PL001)                 |
| 09.30-09.40 | Discussion                                              |
| 09.40-10.00 | Coffee Break                                            |
| 10.00-11.20 | Plenary Session II (Chairperson: Prof. Dr. Husein H. B) |
| 10.00-10.30 | Prof. Dr. Mitsuyasu Kato (PL002)                        |
| 10.30-11.00 | Prof. Dr. Evamarie Hey-Hawkins                          |
| (PL003)     |                                                         |
| 11.00-11.20 | Discussion                                              |
| 11.20-11.40 | Exhibition Presentation (PT. UNITAMA ANALITIKA PERKASA) |
| 11.40-12.00 | Poster Session                                          |
| 12.00-13.00 | Break & Lunch                                           |
| 13.00-14.20 | Plenary Session III (Chairperson: Dr. Desi Natalia)     |
| 13.00-13.30 | Dr. Anke C. Terwisscha Van Scheltinga (PL004)           |
| 13.30-14.00 | Prof. Dr. Toto Subroto (PL005)                          |
| 14.00-14.20 | Discussion                                              |
| 14.20-15.20 | Parallel Session I                                      |
| 15.20-15.30 | Coffee Break                                            |
| 15.30-16.30 | Parallel Session II                                     |

### Friday, 21 November 2014

#### Time

|             |                                                      |
|-------------|------------------------------------------------------|
| 07.30-08.00 | Poster posting                                       |
| 08.00-09.20 | Plenary Session IV (Chairperson: Dr. Iman P. Maksum) |
| 08.00-08.30 | Prof. Dr. Ukun M.S. Soedjanaatmadja (PL006)          |
| 08.30-09.00 | Prof. Dr. Masakazu Anpo (PL007)                      |
| 09.00-09.20 | Discussion                                           |
| 09.20-09.30 | Coffee Break                                         |
| 09.30-10.50 | Plenary Session V (Chairperson: Prof. Dr. Unang S.)  |
| 09.30-10.00 | Prof. Dr. Khalijah Awang (PL008)                     |
| 10.00-10.30 | Hon. Assoc. Prof. Roger W. Read (PL009)              |
| 10.30-10.50 | Discussion                                           |
| 10.50-11.10 | Exhibition Presentation (PT. KROMTEKINDO UTAMA)      |
| 11.10-11.30 | Poster Session                                       |
| 11.30-13.00 | Break & Lunch                                        |
| 13.00-15.00 | Parallel Session III                                 |
| 15.00-15.30 | Coffee Break                                         |
| 15.30-16.30 | Closing Ceremony                                     |



## Distribution for Poster Presentation

### Day 1 (Thursday, 20 November 2014)

|         |                      |         |                     |
|---------|----------------------|---------|---------------------|
| PP-A001 | Anita Oktari         | PP-C009 | Akhmad Darmawan     |
| PP-A002 | Diana Hendrati       | PP-C010 | Tatang Shabur       |
| PP-A003 | Euis Yuliani         | PP-C011 | Sook Yee Liew       |
| PP-A004 | Korry Novitriani     | PP-C012 | Tati Herlina        |
| PP-A005 | Ratna Nurmalasari    | PP-C013 | Tresna L            |
| PP-A006 | Shanty Wyantuti      | PP-C014 | Tri Mayanti         |
| PP-B001 | Akhmad Zainal Abidin | PP-D001 | Abdul muis          |
| PP-B002 | Atiek Rostika        | PP-D002 | Adithya Sukma       |
| PP-B003 | Dani Permana         | PP-D003 | Ayra Ulpiyana       |
| PP-B004 | Dolih Gozali         | PP-D004 | Isti Daruwati       |
| PP-B005 | E. Evy Ernawati      | PP-D005 | Martalena Ramli     |
| PP-B006 | Fitri Khoerunnisa    | PP-D006 | Meilinah Hidayat    |
| PP-B007 | Iwan Hastiawan       | PP-D007 | Nenden Indrayati    |
| PP-B008 | Muryeti              | PP-D008 | Rina Budi S         |
| PP-C001 | Charlena             | PP-D009 | Ruswanto            |
| PP-C002 | Chong Soon Lim       | PP-D010 | Riski Dwimalida P   |
| PP-C003 | Adawiyah             | PP-D011 | Saronom Silaban     |
| PP-C004 | Desnelli             | PP-D012 | Shabarni Gaffar     |
| PP-C005 | Hadi Kuncoro         | PP-F001 | Christi L. Natanael |
| PP-C006 | Leny Heliawati       | PP-F002 | Diana Widiastuti    |
| PP-C007 | Lilis Siti Aisyah    | PP-F003 | Eti Rohaeti         |
| PP-C008 | Meti Kusmiati        |         |                     |



# The G6PDH Inhibition Activity Effects of Ethanol Extract of Detam 1 soybean, Jati Belanda leaves and Their Combinations in 3T3-L1 Culture Cells

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ISC-2014  
International Seminar on Chemistry

## ABSTRACTS

The prevalence of obesity is definitely increasing in all over the world. In obese subjects it was found that serum concentration of Glucose-6-phosphate dehydrogenase (G6PDH) increased significantly with increasing body weight. There are many Indonesian herbs have good effects in weight loss, for example, Detam 1 soybean from Balisakel Malang and Jati Belanda from BHO plantation. In this study, we examined the ethanol extract of Detam 1 soybean seed (EEDS), Jati Belanda leaves (EEJB) and their combinations (EESJB). The aim of this study are to determine the effects of EEDS, EEJB and G6PDH toward cytotoxicity & inhibition activity in 3T3-L1 culture cells. **Methodology:** the effects of 3 concentrations (100µg/ml, 50 µg/ml, 10 µg/ml) of each samples to viability 3T3-L1 cells were measured triplo using cell titer 96 MTS Assay and G6PDH inhibition activity affects to 3T3-L1 cells using G6PDH kit, Abnova Cat KA 0880 with calorimetric method, counted at minutes 0 and 30. **Results:** average cell viability which treated with EEDS 100µg/ml: 85.39; 50 µg/ml: 95.79; 10 µg/ml: 98.59. With EEJB: 81.77; 90.79; 95.52. With EESJB: 76.66; 81.64; 88.60. The samples which viability > 90.0 is proved to be safe, so we only continued EEDS and EEJB 50 µg/ml, 10 µg/ml. Results of average G6PDH inhibition activity: EEDS 50 µg/ml: 19.52; 10 µg/ml: 11.91. EEJB 50 µg/ml: 26.12. **Conclusion:** The EEDS and EEJB 50 µg/ml, 10 µg/ml is proved to be safe to cells and EEJB 50 µg/ml has the best effect in G6PDH inhibition activity.

**Key words:** Detam 1 soybean - Jati Belanda - G6PDH - 3T3-L1 culture cells

## Research Objective

to determine effects of Ethanol Extract of Detam 1 Soybean (EEDS), Jati Belanda (EEJB) and Extract combination (EESJB) toward 3T3-L1 cells, in:

1. Cytotoxicity test toward Viability of cells
2. GPDH inhibition activity



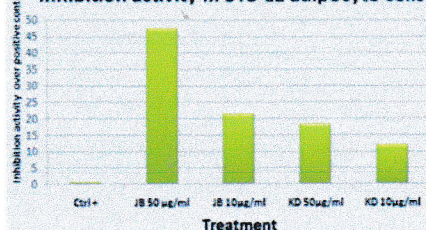
1. Standard Curve Plate that has treated were added MTS and incubated for 3 hours
2. Absorbance were read at 490 nm wave length using microplate reader

## RESULTS:

### 1. Cytotoxicity Effects of EEDS, EEJB and EESJB to Viability of 3T3-L1 cells

| Cate   | Sample | Replication | 1      | 2      | 3      | Average | Replication | 1      | 2    | 3 | Average | STD |
|--------|--------|-------------|--------|--------|--------|---------|-------------|--------|------|---|---------|-----|
| Cell   | 1.4245 | 1.1407      | 1.3768 | 1.3479 | 105.64 | 92.05   | 102.24      | 100.00 | 7.09 |   |         |     |
| JB 100 | 1.1037 | 1.1197      | 1.0830 | 1.1021 | 81.88  | 83.07   | 80.35       | 81.77  | 1.37 |   |         |     |
| JB 50  | 1.2311 | 1.2050      | 1.2355 | 1.2237 | 91.33  | 89.40   | 91.63       | 90.79  | 1.21 |   |         |     |
| JB 10  | 1.2707 | 1.2527      | 1.2582 | 1.2606 | 94.27  | 92.94   | 93.35       | 93.52  | 0.64 |   |         |     |
| KD 100 | 1.1155 | 1.1243      | 1.1636 | 1.1510 | 89.69  | 84.15   | 86.33       | 85.39  | 1.12 |   |         |     |
| KD 50  | 1.2097 | 1.2647      | 1.2580 | 1.2641 | 94.20  | 95.83   | 93.35       | 93.79  | 0.44 |   |         |     |
| KD 10  | 1.2993 | 1.2874      | 1.3593 | 1.3010 | 96.39  | 95.10   | 97.84       | 96.59  | 1.20 |   |         |     |
| SD 100 | 1.0524 | 1.0232      | 1.0244 | 1.0333 | 78.08  | 75.55   | 76.00       | 76.46  | 1.21 |   |         |     |
| SD 50  | 1.2309 | 1.0968      | 1.0983 | 1.1004 | 84.27  | 79.89   | 80.76       | 81.64  | 2.30 |   |         |     |
| SD 10  | 1.1582 | 1.1848      | 1.2126 | 1.1952 | 87.93  | 87.90   | 89.95       | 88.60  | 1.18 |   |         |     |

### Effects of EEDS, EEJB toward G6PDH inhibition activity in 3T3-L1 adipocyte cells



## INTRODUCTION

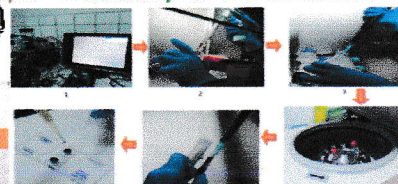
The prevalence of obesity is definitely increasing in all over the world. In obese subjects, serum concentration of Glucose-6-phosphate dehydrogenase (GPDH) increased significantly with increasing body weight.



Glucose-6-phosphate dehydrogenase (G6PDH) is a key enzyme that regulates cellular redox potential.

The G6PDH levels in macrophages in the adipose tissue of obese animals were elevated (Ham, 2013).

## Cytotoxicity tests toward Viability of 3T3-L1 cells



## METHODOLOGY

### Viability test

1. Cytotoxicity tests toward Viability of 3T3-L1 cells:

1. EEDS 100µg/ml, 50 µg/ml, 10 µg/ml
  2. EEJB 100µg/ml, 50 µg/ml, 10 µg/ml
  3. Combination (EESJB) 100µg/ml, 50 µg/ml, 10 µg/ml
- samples were measured triplo using cell titer 96 MTS Assay

## METHODOLOGY

### G6PDH inhibition activity

- Using G6PDH kit, Abnova Cat KA-0880 with calorimetric method, counted at minutes 0 and 30
- The samples treatment which caused viability of cells > 90.0 is proved to be safe:

| CYTOTOXIC | AVERAGE |
|-----------|---------|
| KD 50     | 93.79   |
| KD 10     | 96.59   |
| JB 50     | 90.79   |
| JB 10     | 93.52   |

so we only continued EEDS and EEJB 50 µg/ml, 10 µg/ml. Each samples were measured triplo/ three times

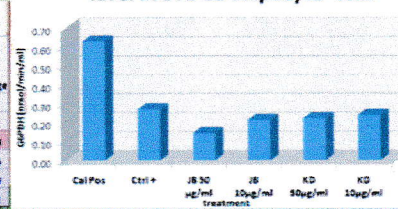
## Results of G6PDH test

| Sample   | G6PDH activity (nmol/min/ml) |      |      |         | G6PDH inhibition activity (%) |       |       |         |
|----------|------------------------------|------|------|---------|-------------------------------|-------|-------|---------|
|          | 1                            | 2    | 3    | Average | 1                             | 2     | 3     | Average |
| Ctrl Pos | 0.63                         | 0.57 | 0.68 | 0.63    |                               |       |       |         |
| Ctrl+    | 0.25                         | 0.28 | 0.28 | 0.27    | 7.41                          | -3.77 | -2.04 | 0.53    |
| JB 50    | 0.15                         | 0.14 | 0.13 | 0.14    | 43.89                         | 46.54 | 51.49 | 47.30   |
| JB 10    | 0.20                         | 0.22 | 0.22 | 0.21    | 24.75                         | 19.20 | 20.12 | 21.36   |
| KD 50    | 0.22                         | 0.22 | 0.22 | 0.22    | 10.98                         | 18.27 | 19.32 | 18.19   |
| KD 10    | 0.24                         | 0.23 | 0.24 | 0.24    | 10.49                         | 13.02 | 11.91 | 11.81   |

## G6PDH test [Abnova KA0880]



## Effects of EEDS, EEJB toward G6PDH level in 3T3-L1 adipocyte cells



## CONCLUSION

- The EEDS and EEJB concentration of 50 µg/ml and 10 µg/ml is proved to be safe toward viability of 3T3-L1 cells
- EEJB 50 µg/ml has the best effect in GPDH inhibition activity.

## ACKNOWLEDGEMENT

We gratefully acknowledge the financial support of Higher Education Ministry of Republic Indonesia for research grant SP DIPA-023.04.2.189789/2014

## References:

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- Ham et al. Macrophage Glucose-6-Phosphate Dehydrogenase Stimulates proinflammatory responses with Oxidative Stress. Mol Cell Biol. 2013; 33 (12): 2423-2435
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- Hidayat M, Soeng S, Prahastuti S. Pengujian Aktivitas Lipase Inhibitor Ekstrak Etanol dan Isolat Tradisional Sari Kerdas Detam 1 dan Daun Jati Belanda. Jurnal Chemia et Natura Acta. 2014; 2(1): 76-82
- Hidayat M, Soeng S, Prahastuti S. 2014. The Highest Activity of Lipase in Combination of Water Fraction of Detam 1 Soybean and Jati Belanda Leaves. Poster in Seminar Nasional Bidang Kimia 2014. MIPA UNPAD, Bandung



## ARTIKEL LENGKAP SEMINAR KIMIA INTERNASIONAL 2014

### **The G6PDH Inhibition Activity Effects of Ethanol Extract of *Detam 1* soybean, Jati Belanda leaves and Their Combinations in 3T3-L1 Culture Cells**

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The prevalence of obesity is definitely increasing in all over the world. In obese subjects it was found that serum concentration of Glucose -6-phosphate dehydrogenase (G6PDH) increased significantly with increasing body weight. There are many Indonesian herbs have good effects in weight loss, for examples, *Detam 1* soybean from Balitkabi Malang and Jati Belanda from BHD plantation. In this study, we examined the ethanol extract of *Detam 1* soybean seed (EEDS), Jati Belanda leaves (EEJB) and their combinations (EESJB). The purposes of this study are to determine the safety of these extracts, and their effects to GPDH inhibition activity in 3T3-L1 culture cells. Method: the effects of 3 concentration (100ug/ml, 50 µg/ml, 10 µg/ml) of each samples to viability 3T3-L1 cells were measured triplo using cell titer 96 MTS Assay and GPDH inhibition activity effects to 3T3-L1 cells using G6PDH kit, Abnova Cat KA 0880 with calorimetric method, counted at minutes 0 and 30. Average results of viability cells which treated with EEDS 100ug/ml: 85.39; 50 µg/ml: 93.79; 10 µg/ml: 96.59. With EEJB 81.77; 90.79; 93.52. With EESJB 76.66; 81.64; 88.60. The samples which viability > 90.0 was proved to be safe, so we only continued EEDS and EEJB 50 µg/ml, 10 µg/ml. Average results of G6PDH inhibition activity: EEDS 50 µg/ml: 19.32, 10 µg/ml: 11.91. EEJB 51.48; 20.12. Conclusion: The EEDS and EEJB 50 µg/ml, 10 µg/ml is proved to be safe to cells and EEJB 50 µg/ml has the best effect in G6PDH inhibition activity.

**Key words:** *Detam 1* soybean - Jati Belanda - Glucose -6-phosphate dehydrogenase - 3T3-L1 culture cells

#### **Introduction**

The prevalence of obesity is definitely increasing in all over the world. In obese subjects, serum concentration of Glucose -6-phosphate dehydrogenase (GPDH) increased significantly with increasing body weight. Glucose-6-phosphate dehydrogenase (G6PDH) is a



key enzyme that regulates cellular redox potential. The G6PDH levels in macrophages in the adipose tissue of obese animals were elevated (Ham, 2013).

Research Objective of this study is to determine effects of Ethanol Extract of Detam 1 Soybean (EEDS), Jati Belanda (EEJB) and Extract combination (EESJB) toward 3T3-L1 cells, in Cytotoxicity test toward Viability of cells and GPDH inhibition activity .

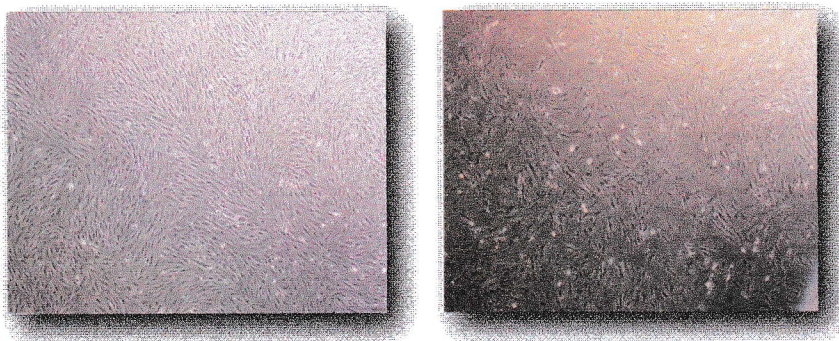
## **METHODOLOGY**

Tool: Centrifuge Tube, Centri Star Cap 15 ml, TC Flask 25 cm Vent Cap, Blue tip (100-1000 mL), Yellow tip (10-100 mL), White tip (2-10 mL), Serological Pippet 10 mL, Serological Pippet5 mL, Refrigerated Centrifuge, Serological Pippet 10 Ml, Inverted Microscope

### **1. Viability test**

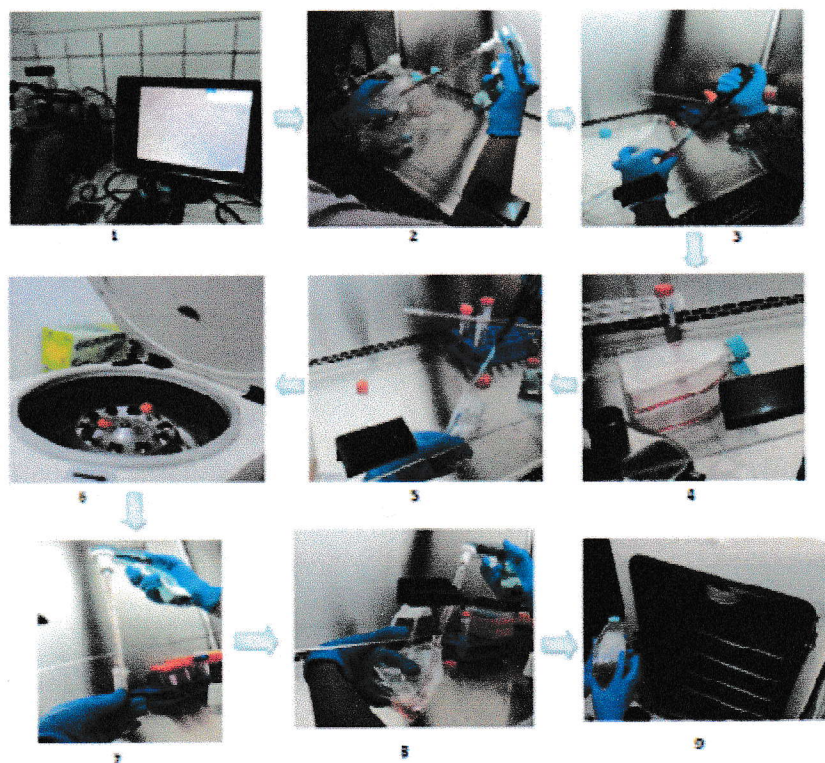
#### **Procedures:**

First, we have to checked the condition of the cells (80-90% confluent), then previously medium cells removed and cells were rinsed with PBS 3x. The cells were rinsed with 0.02% and 0.25% trypsin EDTA 3 ml. After that cells were incubated at 36,5<sup>0</sup>C for 2 minutes then added the growth medium to the cells. Cells and growth media was centrifuged at  $500 \times g$  for 5 minutes, the supernatant was discarded and the pellet resuspended in 4-5 growth medium. Suspension was divided on T flasks which had previously been filled with medium with a density of 8000 cells / cm<sup>2</sup>. Then the suspension in the T flasks were incubated at 37<sup>0</sup>C, 5% CO<sub>2</sub>. The medium was changed every 2 days for maintenance.



**Picture 1. Viable Cells of 3T3-L1**





## 2. Plating 3T3 L1 cells to Prepare for Treatments

### Ingredients of Growth Medium:

DMEM (Biowest), 10% FBS (Biowest), 1% antibiotic-antimycotic (Biowest), PBS (Biowest), trypsin EDTA (Biowest)

Such procedures are listed below:

First, standard curve plate that has treated were added MTS and incubated for 3 hours. The absorbance were read at 490 nm wave length using microplate reader using G6PDH kit, Abnova Cat KA 0880 with calorimetric method, counted at minutes 0 and 30. The samples treatment which caused viability of cells  $> 90.0$  was proved to be safe, so we only continued EEDS and EEJB 50  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ . The results of the combination treatment caused viability of  $< 90.0$  cells, so we assumed that this treatment were not safe enough to be continued. Each samples were measured triplo/ three times.

### Samples to be tested are:

1. Ethanol Extract of Detam 1 Soybean (EEDS) 100 $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$
2. Ethanol Extract of Jati belanda (EEJB) 100 $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$
3. Combination (EESJB) 100 $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$

Each samples were measured triplo using cell titer 96 MTS Assay using cell titer and



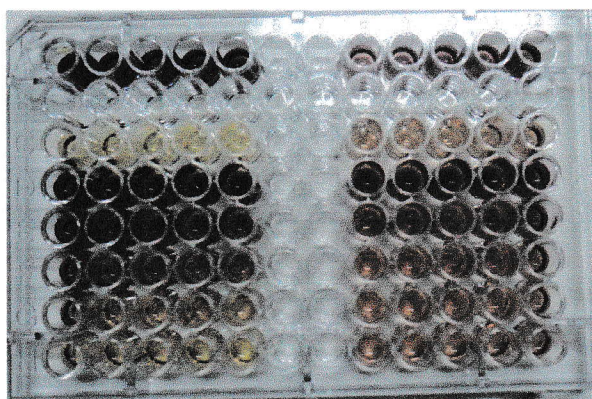
GPDH inhibition activity effects to 3T3-L1 cells using G6PDH kit, Abnova Cat KA 0880 with calorimetric method, counted at minutes 0 and 30.

## RESULTS

**Tabel 1. Viability of 3T3 L1 cells in 96 well plates**

| Abs | 1      | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      | 10     | 11     | 12     |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| A   | 1.36   | 1.6846 | 1.4935 | 1.6331 |        |        |        |        |        |        |        |        |
| B   | 0.2611 | 0.2602 | 0.2528 | 0.2545 |        |        |        |        |        |        |        |        |
| C   | 1.5038 | 1.5198 | 1.4831 | 0.4001 | 1.4043 | 1.3836 | 1.4129 | 0.2493 | 1.3549 | 1.3257 | 1.3269 | 0.3025 |
| D   | 1.5316 | 1.5055 | 1.5356 | 0.3005 | 1.4808 | 1.4758 | 1.4691 | 0.2111 | 1.3986 | 1.3395 | 1.3512 | 0.2627 |
| E   | 1.5239 | 1.5059 | 1.5114 | 0.2532 | 1.5077 | 1.4957 | 1.5277 | 0.2084 | 1.4075 | 1.4071 | 1.4349 | 0.2223 |
| F   |        |        |        |        |        |        |        |        |        |        |        |        |
| G   |        |        |        |        |        |        |        |        |        |        |        |        |
| H   |        |        |        |        |        |        |        |        |        |        |        |        |

| Cytotoxic | Replication |        |        |         | Replication |       |        |         | STD  |
|-----------|-------------|--------|--------|---------|-------------|-------|--------|---------|------|
|           | 1           | 2      | 3      | Average | 1           | 2     | 3      | Average |      |
| Ctrl      | 1.4244      | 1.2407 | 1.3786 | 1.3479  | 105.68      | 92.05 | 102.28 | 100.00  | 7.09 |
| JB1       | 1.1037      | 1.1197 | 1.0830 | 1.1021  | 81.88       | 83.07 | 80.35  | 81.77   | 1.37 |
| JB2       | 1.2311      | 1.2050 | 1.2351 | 1.2237  | 91.33       | 89.40 | 91.63  | 90.79   | 1.21 |
| JB3       | 1.2707      | 1.2527 | 1.2582 | 1.2605  | 94.27       | 92.94 | 93.35  | 93.52   | 0.68 |
| KD1       | 1.155       | 1.1343 | 1.1636 | 1.1510  | 85.69       | 84.15 | 86.33  | 85.39   | 1.12 |
| KD2       | 1.2697      | 1.2647 | 1.2580 | 1.2641  | 94.20       | 93.83 | 93.33  | 93.79   | 0.44 |
| KD3       | 1.2993      | 1.2873 | 1.3193 | 1.3020  | 96.39       | 95.50 | 97.88  | 96.59   | 1.20 |
| JK1       | 1.0524      | 1.0232 | 1.0244 | 1.0333  | 78.08       | 75.91 | 76.00  | 76.66   | 1.23 |
| JK2       | 1.1359      | 1.0768 | 1.0885 | 1.1004  | 84.27       | 79.89 | 80.76  | 81.64   | 2.32 |
| JK3       | 1.1852      | 1.1848 | 1.2126 | 1.1942  | 87.93       | 87.90 | 89.96  | 88.60   | 1.18 |



**Picture 2. The 96 well plate of 3T3 L1 cells**

Below is shown the cytotoxic test results of the test material, the statistical analysis of their mean and a further test Duncan.



**Table 2. Average Statistical analysis of measurement and Duncan post hoc test absorbance EEKD cytotoxic test, EEJB and combinations of the 3T3-L1 cells**

| Samples    | Absorbance   |              |              |
|------------|--------------|--------------|--------------|
|            | 100 µg/ml    | 50 µg/ml     | 10 µg/ml     |
| Extract JB | 81.77±1.36 a | 90.79±1.21 b | 93.52±0.68 c |
| Extract KD | 85.39±1.12 a | 93.79±0.44 b | 96.59±2.32 c |
| Extract JK | 76.66±1.23 a | 81.64±2.32 b | 88.60±1.18 c |

Data are shown as mean ± standard deviation.

Different letters in one column (concentration) showed significant at  $p < 0.05$

### Results of Treatment Activity Test against G6PDH

**Table 3. Mapping Plate of G6PDH.**

| Sample | 1     | 2     | 3       | 4       | 5       | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|-------|-------|---------|---------|---------|---|---|---|---|----|----|----|
| A      | Cal 1 | Cal 1 | Cal Pos | Cal Pos | Cal Pos |   |   |   |   |    |    |    |
| B      | Cal 2 | Cal 2 | Blank   | Blank   | Blank   |   |   |   |   |    |    |    |
| C      | Cal 3 | Cal 3 | Ctrl +  | Ctrl +  | Ctrl +  |   |   |   |   |    |    |    |
| D      | Cal 4 | Cal 4 | JB 1    | JB 1    | JB 1    |   |   |   |   |    |    |    |
| E      | Cal 5 | Cal 5 | JB 2    | JB 2    | JB 2    |   |   |   |   |    |    |    |
| F      | Cal 6 | Cal 6 | KD 1    | KD 1    | KD 1    |   |   |   |   |    |    |    |
| G      |       |       | KD 2    | KD 2    | KD 2    |   |   |   |   |    |    |    |
| H      |       |       |         |         |         |   |   |   |   |    |    |    |

Note. Cal : Standar. 1 : 0; 2 : 0.25; 3 : 0.5; 4 : 0.75; 5 : 1; dan 6 : 1.25. JB 1 : Jati belanda Extract 50 mg/ml. JB 2 : Jati belanda Extract 10 mg/ml. KD 1 : Extract Kedelai Detam 50 mg/ml. KD 2 : Detam 1 Soybean Extract 0 mg/ml. Ctrl + : control positif (perlakuan tanpa Extract).

**Table 4. Data Absorbance of G6PDH A<sub>1</sub> (T<sub>1</sub>)**

| Abs | 1      | 2      | 3      | 4      | 5      | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|--------|--------|--------|--------|--------|---|---|---|---|----|----|----|
| A   | 0.0632 | 0.0645 | 0.6350 | 0.6282 | 0.6322 |   |   |   |   |    |    |    |
| B   | 0.1322 | 0.1266 | 0.0651 | 0.0686 | 0.0688 |   |   |   |   |    |    |    |
| C   | 0.2072 | 0.1747 | 0.0949 | 0.0953 | 0.0957 |   |   |   |   |    |    |    |
| D   | 0.2463 | 0.2891 | 0.0832 | 0.0879 | 0.0857 |   |   |   |   |    |    |    |
| E   | 0.3735 | 0.3317 | 0.0993 | 0.0909 | 0.0965 |   |   |   |   |    |    |    |
| F   | 0.3789 | 0.3444 | 0.0927 | 0.0903 | 0.0924 |   |   |   |   |    |    |    |
| G   |        |        | 0.1035 | 0.107  | 0.1052 |   |   |   |   |    |    |    |
| H   |        |        |        |        |        |   |   |   |   |    |    |    |

**Table 5. Data Absorbance of G6PDH A<sub>2</sub> (T<sub>2</sub>)**

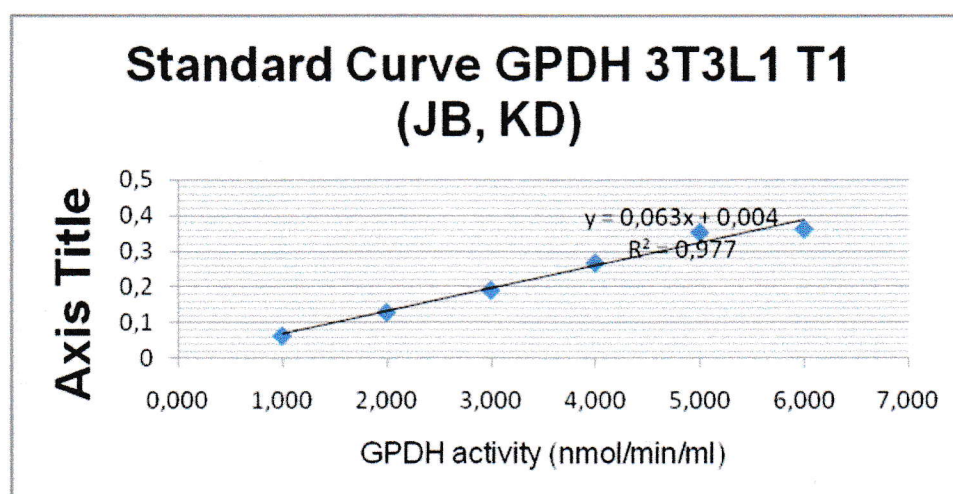
| Abs | 1      | 2      | 3      | 4      | 5      | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|-----|--------|--------|--------|--------|--------|---|---|---|---|----|----|----|
| A   | 0.0628 | 0.0635 | 1.0135 | 0.9684 | 1.0373 |   |   |   |   |    |    |    |
| B   | 0.1449 | 0.1413 | 0.0635 | 0.0697 | 0.0623 |   |   |   |   |    |    |    |
| C   | 0.2275 | 0.2044 | 0.2426 | 0.2611 | 0.2587 |   |   |   |   |    |    |    |
| D   | 0.2767 | 0.3290 | 0.1718 | 0.1722 | 0.1620 |   |   |   |   |    |    |    |
| E   | 0.4319 | 0.3942 | 0.2189 | 0.2195 | 0.2236 |   |   |   |   |    |    |    |



|   |        |        |        |        |        |
|---|--------|--------|--------|--------|--------|
| F | 0.4525 | 0.4392 | 0.2249 | 0.2204 | 0.2208 |
| G |        |        | 0.2462 | 0.2456 | 0.2456 |

**Table 6. Standard Curve of G6PDH A<sub>1</sub> (T<sub>1</sub>)**

| Standar (nmol/μl) | 1      | 2      | average |
|-------------------|--------|--------|---------|
| 0.000             | 0.0632 | 0.0645 | 0.0639  |
| 0.250             | 0.1322 | 0.1266 | 0.1294  |
| 0.500             | 0.2072 | 0.1747 | 0.1910  |
| 0.750             | 0.2463 | 0.2891 | 0.2677  |
| 1.000             | 0.3735 | 0.3317 | 0.3526  |
| 1.250             | 0.3789 | 0.3444 | 0.3617  |

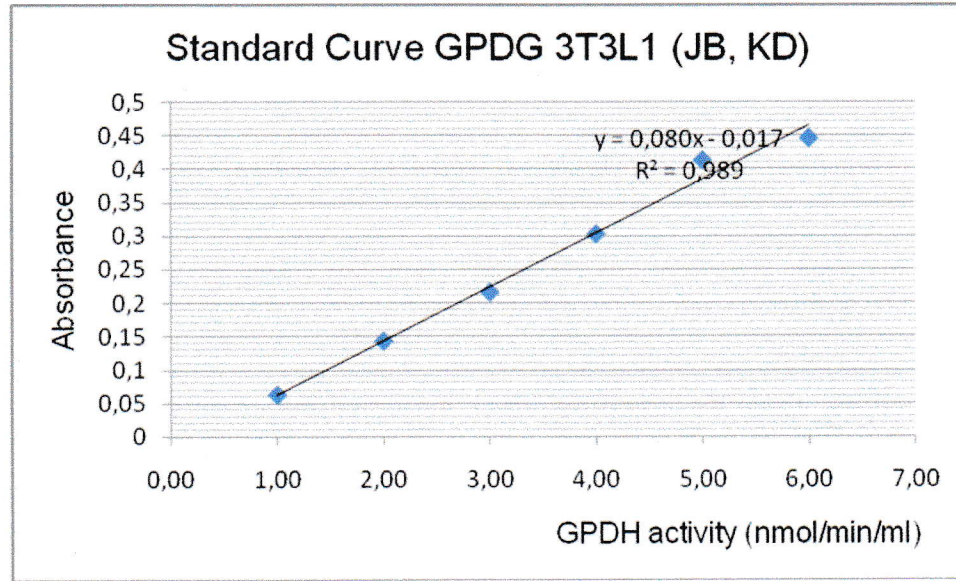


**Picture 2. Standard Curve of G6PDH A<sub>1</sub> (T<sub>1</sub>)**

**Table 7. Standard Curve of G6PDH A<sub>2</sub> (T<sub>2</sub>)**

| Standar<br>(nmol/μl) | 1      | 2      | Average |
|----------------------|--------|--------|---------|
| 0                    | 0.0628 | 0.0635 | 0.0632  |
| 0.25                 | 0.1449 | 0.1413 | 0.1431  |
| 0.5                  | 0.2275 | 0.2044 | 0.2160  |
| 0.75                 | 0.2767 | 0.3290 | 0.3029  |
| 1                    | 0.4319 | 0.3942 | 0.4131  |
| 1.25                 | 0.4525 | 0.4392 | 0.4459  |





**Picture 3. Standard Curve of G6PDH A<sub>2</sub> (T<sub>2</sub>).**

### Results of measurement of G6PDH

From the result in table 4 and 5, based on each standard curve in table 7 and 8, below were shown the absorbance data, G6PDH levels and Inhibition activities of G6PDH.

**Table 8. Absorbance data, G6PDH levels and Inhibition activities of G6PDH.**

| Sampel  | Absorbance T1 |        |        | Abs T2 |        |        | AbsT2-T1      |               |               | Ave<br>rage | GPDH activity (n<br>mol/min/ml) |      |      | Ave<br>rage | GPDH inhibition activi<br>ty (%) |       |       | Ave<br>rage |
|---------|---------------|--------|--------|--------|--------|--------|---------------|---------------|---------------|-------------|---------------------------------|------|------|-------------|----------------------------------|-------|-------|-------------|
|         | 1             | 2      | 3      | 1      | 2      | 3      | (T2-T1)<br>-1 | (T2-T1)<br>-2 | (T2-T1)<br>-3 |             | 1                               | 2    | 3    |             | 1                                | 2     | 3     |             |
| Cal Pos | 0.5675        | 0.5607 | 0.5647 | 0.9483 | 0.9032 | 0.9721 | 0.3808        | 0.3425        | 0.4074        | 0.3769      | 0.63                            | 0.57 | 0.68 | 0.63        |                                  |       |       |             |
| Ctrl +  | 0.0274        | 0.0278 | 0.0282 | 0.1774 | 0.1959 | 0.1935 | 0.1500        | 0.1681        | 0.1653        | 0.1611      | 0.25                            | 0.28 | 0.28 | 0.27        | 7.41                             | -3.77 | -2.04 | 0.53        |
| JB 1    | 0.0157        | 0.0204 | 0.0182 | 0.1066 | 0.1070 | 0.0968 | 0.0909        | 0.0866        | 0.0786        | 0.0854      | 0.15                            | 0.14 | 0.13 | 0.14        | 43.89                            | 46.54 | 51.48 | 47.30       |
| JB 2    | 0.0318        | 0.0234 | 0.029  | 0.1537 | 0.1543 | 0.1584 | 0.1219        | 0.1309        | 0.1294        | 0.1274      | 0.20                            | 0.22 | 0.22 | 0.21        | 24.75                            | 19.20 | 20.12 | 21.36       |
| KD 1    | 0.0252        | 0.0228 | 0.0249 | 0.1597 | 0.1552 | 0.1556 | 0.1345        | 0.1324        | 0.1307        | 0.1325      | 0.22                            | 0.22 | 0.22 | 0.22        | 16.98                            | 18.27 | 19.32 | 18.19       |
| KD 2    | 0.0360        | 0.0395 | 0.0377 | 0.1810 | 0.1804 | 0.1804 | 0.1450        | 0.1409        | 0.1427        | 0.1429      | 0.24                            | 0.23 | 0.24 | 0.24        | 10.49                            | 13.02 | 11.91 | 11.81       |

Runus

GPDH=  $\left[ \frac{B}{(T2-T1) \times V} \right] \times \text{sampel dilution}$

B : T2-T1

T1 : 0 menit

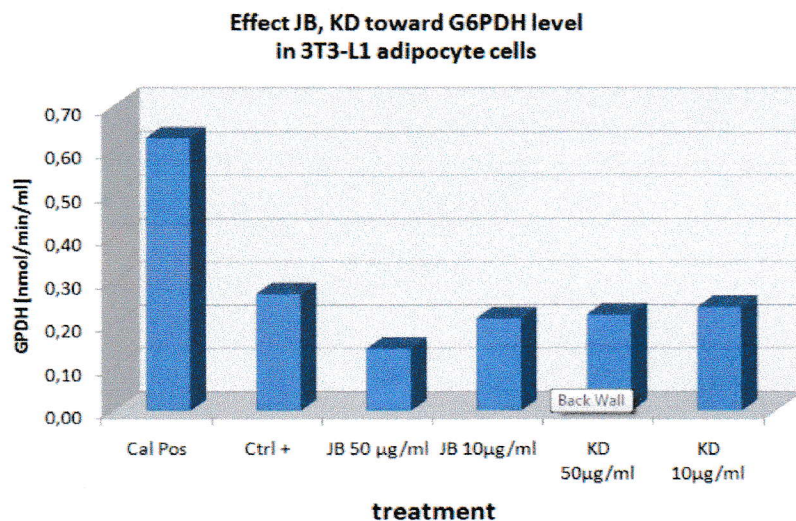
T2 : 30 menit

V : volume sampel (ml) (20  $\mu$ l = 0.02 ml) sampel dilution = 1x

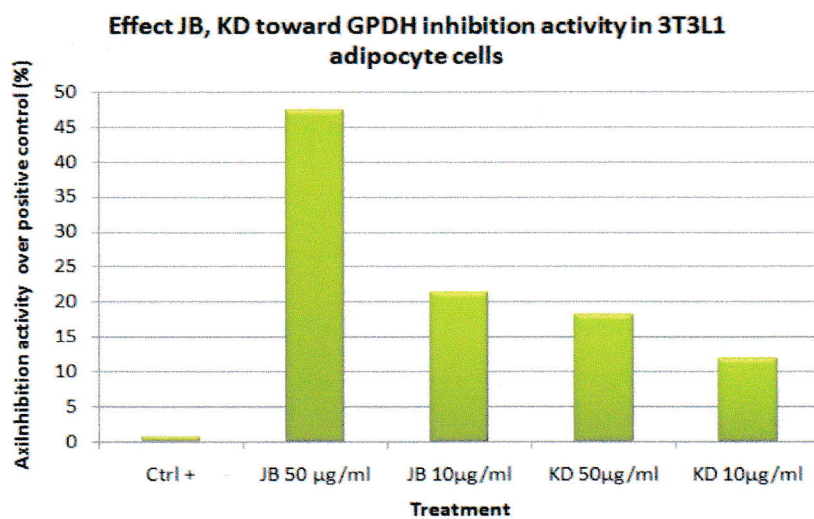
**Table 9. Analysis and Statistics Average of 3 measurements Duncan post hoc test activities against G6PDH levels and Inhibition activity (%) of EEDS, EEJB and combinations in 3T3-L1 cells.**

| Samples                  | GPDH<br>GPDH (nmol/min/ml) | GPDH (%)           |
|--------------------------|----------------------------|--------------------|
| Extract JB 50 $\mu$ g/ml | 0.14 $\pm$ 0.01 a          | 47.30 $\pm$ 3.85 e |
| Extract JB 10 $\mu$ g/ml | 0.21 $\pm$ 0.01 b          | 21.36 $\pm$ 2.97 d |
| Extract KD50 $\mu$ g/ml  | 0.22 $\pm$ 0.00 bc         | 18.19 $\pm$ 1.17 c |
| Extract KD 10 $\mu$ g/ml | 0.24 $\pm$ 0.01 c          | 11.81 $\pm$ 1.27 b |
| Positive control         | 0.27 $\pm$ 0.02 d          | 0.00 $\pm$ 0.00 a  |





**Figure 4. Graph of G6PDH levels as Treatment Effects of 3T3-L1 cells**



**Figure 5. Graph of G6PDH inhibition activities in 3T3-L1 cells**

## Conclusion

The Extract Ethanol of Detam 1 Soybean and Extract Ethanol of Jati Belanda concentration of 50 µg/ml and 10 µg/ml is proved to be safe toward 3T3-L1 cells. Extract Ethanol of Jati Belanda concentration of 50 µg/ml has the best effect in GPDH inhibition activity.



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