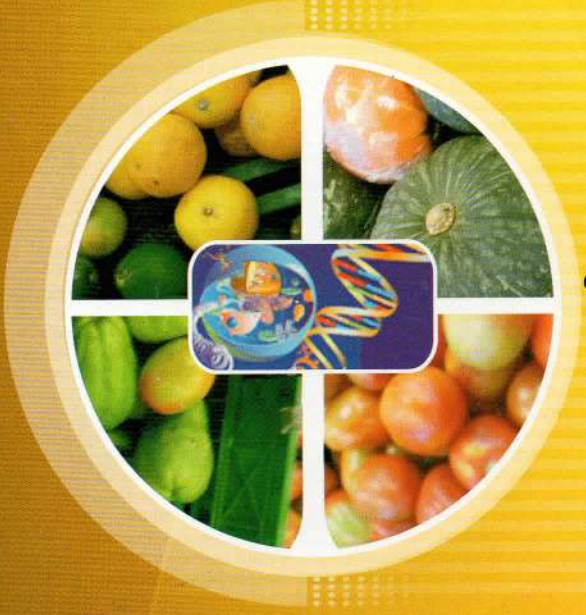
UNIVERSITAS
ATMA JAYA YOGYAKARTA
Fakultas Teknobiologi

PROCEEDING



1st International Seminar on

"Natural Resources Biotechnology: From Local to Global"

September 8th – 9th 2015
Faculty of Biotechnology
Universitas Atma Jaya Yogyakarta

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Welcome Speech
Chair of the Seminar Committee

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

It is a great pleasure to welcome all of you to the International Seminar "Natural Resources: From Local to Global". The Faculty of Biotechnology of Universitas Atma Jaya Yogyakarta runs this seminar to commemorate the 50th Anniversary of the Universitas Atma Jaya Anniversary and the 25th Anniversary of the Faculty of Biotechnology. Your presence is your present for the anniversary of our university and faculty as well.

The Anniversary is not the only reason to run this seminar. A greater reason is behind the seminar. Indonesia is rich in biodiversity. It is a challenge for us, as scientist, to maintain the biodiversity and to develop the potential of the biodiversity for the common good. Through this seminar, the scientific research on Indonesian biodiversity can be shared and probably the finding of the new research can inspire us for further exploration. Therefore, the seminars goal is to facilitate the spread of the research on local potential of biodiversity to the global level. Hopefully, it can attract more researchers to explore the wealth of local biodiversity.

The committee invites speakers who are expertise in the research concerning biodiversity. Our invited speakers are Assoc. Prof. Dr. Michael Murkovic from Graz University of Technology Austria (food scientist), Assoc.Prof. Worawidh Wajjwalku from Kasetsart University Bangkok Thailand (Veterinary disease biotechnology), Dr. Kathryn McMahon from Edith Cowan University Australia (Seagrass biotechnology), Prof. Marco Nemesio E. Montano, PhD from University of the Philippines (Seaweed biotechnology), Prof. Jun Kawabata from Hokkaido University Japan (food biochemist), Endang Semiarti, PhD from Universitas Gadjah Mada, Indonesia (Plant biotechnology), Ign. Pramana Yudha, PhD from Universitas Atma Jaya Yogyakarta (Conservation genetics), Dr Machmud Thohari from Technical Team for Environmental Biosafety, Ministry of Enviroment & Forestry Indonesia (Environmental Biosafety), Dr Harvey Glick from Asia Regulatory Policy & Scientific Affairs Monsanto Company (Regulatory Policy & Scientific Affairs Monsanto). It is a good opportunity to learn from the speakers to enhance and to update our knowledge. I hope this seminar is of benefit to all of us.

In conclusion, I wish you a successful seminar and a pleasant stay in Yogyakarta.

With kind regard
Coordinator of conference program

Dr. rer. nat. Yuliana Reni Swasti, S.TP., MP.

WELCOME SPEECH
DEAN
FACULTY OF BIOTECHNOLOGY
UNIVERSITAS ATMA JAYA YOGYAKARTA

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

On behalf of the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta and the Committee of the International Seminar, I would like to first of all to extend our heart-felt thanks for your presence at this Seminar. This seminar is so significant in a sense that it focuses on natural resources with local content but by utilizing biotechnology they will become global and worldwide products and services as well.

Biotechnology has been developed very rapidly and it is believed to be "a new wave in the economic world". Biotechnology has contributed in all aspects of humans' life, such as food production, health, industry, environment, etc. The role of biotechnology for the betterment of human beings, however, is still need to be improved. Indonesia, with its huge biodiversity, has a potency to develop and applied biotechnology nationwide.

The role of biotechnology has increased rapidly. Many are believed that biotechnology has become an integral part of modern industries with high economic values. On the other hand, it needs to be closely managed in order to avoid its negative impacts. The are some example of negative impacts with relate to biotechnology application, such as intellectual property rights, genetically modified organisms (GMOs), environmental degradations, biodiversity issues, indigenous people knowledge, biosafety, etc.

The Seminar covers topics such as: Functional Foods, Food Biotechnology, Biopharmacy, Health/Medical Biotechnology, Environmental Biotechnology, Legal Aspect of Biotechnology, Bioinformatics, and Social-Economic Aspects of Biotechnology. This Seminar will be presented nine (9) invited speakers with different topics and expertise. There will be some papers and posters to be presented also in this Seminar from some participants from the Philippines and Indonesia.

Henceforth, in commemorating its 50th anniversary Universitas Atma Jaya Yogyakarta (UAJY) and 25th anniversary of Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta (UAJY) on September 2015, it is worthy and appropriate to explore the newest innovations in the field of research and development of biotechnology to be applied in many aspects for the betterment of human beings. The Seminar takes this opportunity to discuss and hopefully find ways to solve problems faced by human beings in the world.

I would like to take this opportunity to express my sincere thanks and gratitude to the Committee and in particular to the honorable speakers. Before closing this remarks, allow me to ask the Rector of Universitas Atma Jaya Yogyakarta to open this Seminar officially.

Finally, this is an opportune time for me to wish you all in the two (2) fruitful days of interesting and beneficial programs and hope you have a pleasant stay in Yogyakarta.

Thank you very much and may God bless us all. Amen.

Yogyakarta, 8 September 2015

Dean

Drs. B. Boy Rahardjo Sidharta, M.Sc

Brief Contents

Mixture of Sambiloto (*Andrographis panniculata* Nees.) and Salam (*Syzygium polyanthum* (Wight.) Walp.) Extract to Improve GLUT4 and PPAR-γ Expression in Hyperglicemic Wistar Rats

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Abstract

The increased prevalence of diabetes mellitus stimulates the ongoing study of molecular mechanisms of sambiloto (*Andrographis panniculata* Nees.) and salam (*Syzygium polyanthum* (Wight) Walp.) in relieving the disorder. The molecular study was done by treating hyperglicemia Wistar rats with a combination of sambiloto (AP) and salam (SP) extract. After 14 days of treatment, the rats were sacrificed and the liver organs were collected. The organs were stored in a solution of 10% buffered formalin to be blocked in paraffin afterwards. Subsequent molecular test was performed to identify GLUT4 and PPARγ gene expression by immunohistochemistry method. This study showed that administration of a combination of sambiloto and salam extracts (6:1) in rats was able to increase the expression level of GLUT4 which resembled the expression level of GLUT4 after insulin administration. It was also found that the expression level of PPARγ was increased after rats were treated by combination of AP and SP extracts (6:1). It showed that the increased expression of PPARγ resembles the expression of PPARγ after metformin stimulation. Interestingly, the administration of AP:SP extract (6:1) combination showed a level of GLUT4 expression that was not in line with PPARγ expression.

1. INTRODUCTION

The prevalence of diabetes mellitus (DM) that remains increased stimulates the ongoing study of molecular mechanisms of sambiloto and salam in relieving the disorder. In hyperglycemic condition, ROS (reactive oxygen species) from mitochondria electron transport was occurred. If this condition persisted, cellular organelles may be damaged and enzymes activities may also be disturbed. Thus, increased lipid peroxydation and insulin resistance may ultimately be stimulated. Increased level of ROS can be diminished by induction of PPAR-γ coactivator, PGC-1α (PPAR Gamma Coactivator-1a), MnSOD (manganese superoxide dismutase), and activation of AMPK (adenosine monophosphate activated protein kinase). The mechanism of action is carried out by metformin and pioglitazone. The thiazolidinedione (TZD) is another oral antidiabetic agent that acts as Peroxisome Proliferator Activated Receptorγ (PPARγ) agonis to stimulate insulin sensitivity in cells (Braissant *et al.*, 1996). Increase level of PPARγ may activate GLUT4, the gene

that plays role in regulating transport of glucose to the adipose tissue and skeletal muscle, as a response of increase insulin level in the blood (Watson *et al.*, 2004). It was mentioned that both sambiloto and salam are effective in reducing blood glucose level (Widjajakusuma *et al.*, 2010). Therefore, this molecular study was conducted to explore the efficacy of a mixture of sambiloto and salam extract in increasing PPARγ and GLUT4 in hyperglycemic Wistar rats.

2. METHODS

2.1. Preparation of Extracts

Fresh Andrographis paniculata Nees. herbs (AP) and Syzygium polyanthum Wight. (Walp.) (SP) leaves that obtained from Pandaan, East Java were cleaned, cut, dryed, and water extracted (Kaneria *et al.*, 2012). The water extracts was spray dried in order to obtain the dried extracts.

2.2. General Procedures

Healthy Wistar rats that were aged 2-3 months, weighted 200 – 250 g, blood glucose level of 65-100 mg/dl. The rats were randomly grouped into 8 groups of 6, namely: negative control group (K I); normal control group (K II); positive control group I (K III) – insulin; positive control group II (K IV) - metformin 9 mg/kg BW; treatment group I (K V) - AP extract; treatment group II (K VI) - SP extract; treatment group III - mixture of AP:SP extracts (6:1); treatment group IV (K VIII) – AP:SP mixture extract (2:1); treatment group V (K IX) – AP:SP mixture extract (1:2); treatment group VI (K X) – AP:SP mixture extract (1:6). Dose of extract was 200 mg/kg BW. Prior to the treatment, rats were administered by monohidrate alloxan 150 mg/kg BW through intraperitoneal route in order to reach the hyperglycemic condition (blood glucose level of 300-400 mg/dl). When hyperglycemic condition was achieved, mixture of *AP and SP* extracts was given for 14 days. After 14 days of treatment, the hyperglycemic rats were sacrificed and the liver was collected. The organs were stored in a solution of 10% buffered formalin to be blocked in paraffin afterwards.

2.3. Immunohistochemistry of GLUT 4 and PPARγ

The rats livers were paraffine blocked and sliced into 3-4 µm and incubated overnight 450 °C, then deparaffined with xylene. Subsequently, washed with PBS (phosphate buffer saline) and incubated in 3 % hydrogen peroxide (H_2O_2 in methanol). The antigen retrieval was conducted by incubating the slides in the citrate buffer pH 6. The 0,1% protease was added in 37 °C, digested with 100 µg/ml proteinase K in buffer (0,01 mol/l trish HCl pH 7,8 0,005 mol/l EDTA dan 0,5 % SDS) and incubated in mouse serum. Subsequently, the normal mouse serum was cleaned by primer antibody (1 : 50) of IgG1 anti GLUT 4 and PPARγ monoclonal antibody, in separate. Afterwards, incubation with secondary antibody IgG goat anti rat was conducted, streptavidin-peroxidase was dropped, and incubated in DAB for 5-15 minutes. Counterstain with hematoxylin eosin and rehidration were conducted with addition of absolute ethanol, 95% ethanol, 80% ethanol and xylol. The medium mounting was conducted lastly by adding gliserol gelatin prior to closing by deck glass.

3. RESULTS AND DISCUSSION

3.1. GLUT 4 Expression

This study showed that administration of AP and SP mixture extracts (6:1) in rats was able to increase the expression level of GLUT4 (figure 1 in brown) which resembled the expression level of GLUT4 after insulin administration (table 1). This study indicated that the mixture extracts were able to stimulate glucose transport to the cells by the high level of GLUT4 expression similar as insulin stimulation. This result is in line to the study of Widjayakusuma *et al.*, 2010 who reported the reduction of blood glucose level in rats after administrtion with mixture extracts of Ap and Sp.

Table 1. GLUT4 Expression (n=6) GLUT4 Expression in the liver of hyperglycemic Wistar Rats

	Groups	Means±SD
K I	Negative control	179±11
K II	Normal control	51±18
K III	Insulin	231±15
K IV	Metformin	54±20
K V	Ap (*Andrographis paniculata*)	141±23
K VI	Sp (*Syzygium polyanthum*)	151±23
K VII	Ap:Sp (6:1)	234±11
K VIII	Ap:Sp (2:1)	62±18
K IX	Ap:Sp (1:2)	70±15
K X	Ap:Sp (1:6)	43±18

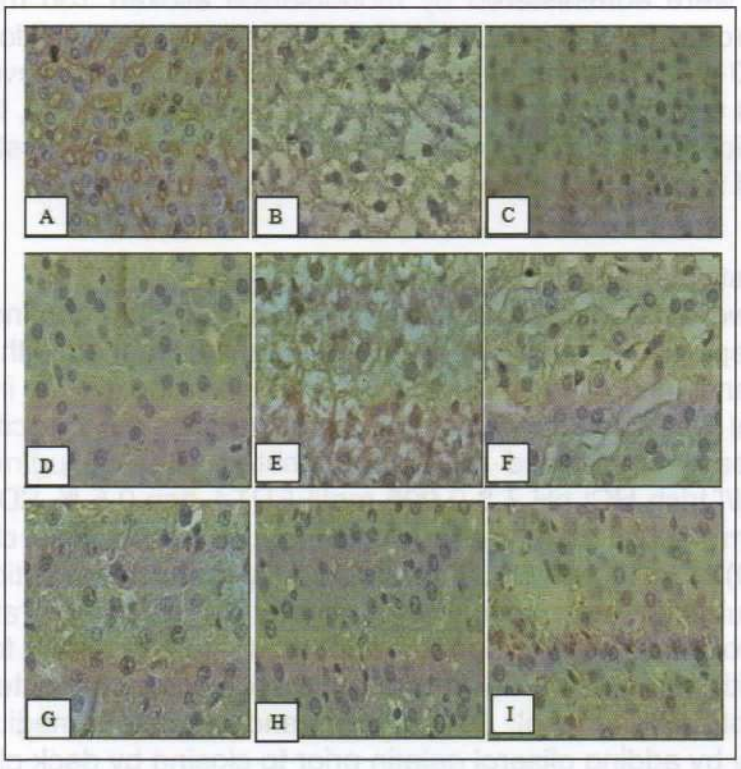

Figure 1. GLUT4 Expression in the liver of hyperglycemic Wistar Rats
(A) Negative control group **(B)** Normal group **(C)** Insulin group **(D)** Metformin group **(E)** Apgroup **(F)** Sp group **(G)** Ap:Sp=6:1 **(H)** Ap:Sp=2:1 **(I)** Ap:Sp=2:1

GLUT4 is a glucose transporter that was exspressed by adipose tissue, muscle, and liver cells (Fukumoto *et al.*, 1989). GLUT4 is activated and facilitates glucose transport to the adipose tissue and muscle when insulin hormone is released. It was proved that insulin was the main regulator of GLUT4 mRNA expression in adipose tissue (Garvey *et al.*, 1989). This study found that GLUT4 was expressed in relatively same level as insulin, thus it was predicted that active compound of AP is able to induce glucose transport by stimulating the activation of GLUT4. The expression of GLUT4 was also increase in high-fat-fructose-fed rats (Nugroho *et al.*, 2011). Moreover, AP was also increase glucose cellular uptake in STZ-diabetics-induced rats by increasing the GLUT4 mRNA and protein level (Zhang *et al.*, 2009). Andrographolide, the active compound of AP also reported its ability to increase glucose uptake into the isolated ileus muscle of STZ-diabetic-induceds rat (Yu *et al.*,, 2008).

3.2. PPARγ expression

This study also showed the increased expression level of PPARγ after rats were treated by mixture of sambiloto and salam extracts (6:1). It showed that the increased expression of PPARγ resembles the expression of PPARγ after metformin stimulation (table 2).

Table 2. PPARγ Expression

Groups		Means±SD
K I	Negative control	164±11
K II	Normal control	169±9
K III	Insulin	183±8
K IV	Metformin	147±24
K V	Ap (*Andrographis paniculata*)	178±13
K VI	Sp (*Syzygium polyanthum*)	150±19
K VII	Ap:Sp (6:1)	141±12

Table 2 showed relative high expression of PPARγ that maybe indication of high reactivity of monoclonal antibody used in the immmunohistochemistry process. Moreover, this result maybe also an indication that PPARγ expression need to be studied in other organs or tissues such as adipose tissue, pancreas, or muscle tissue that are related to glucose transport. In figure 2, the PPARγ expression can be seen brown in colour.

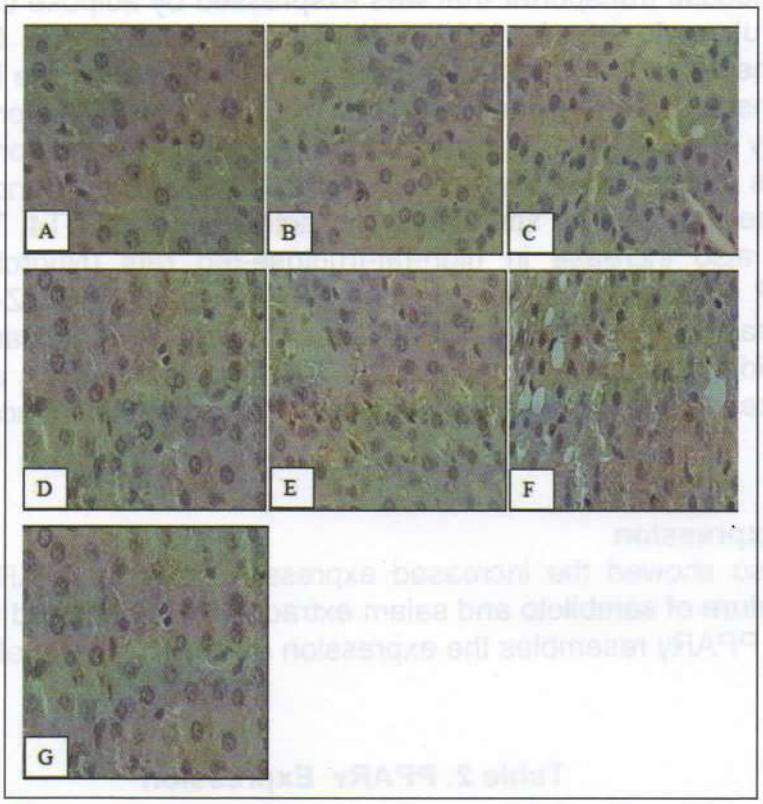

Figure 2. PPAR-γ Expression in the liver of hyperglycemic Wistar Rats
(A) Negative control group **(B)** Normal group **(C)** Insulin group **(D)** Metformin group **(E)** Ap group **(F)** Sp group **(G)** Ap:Sp=6:1

PPARγ expression expressed in adipose and muscle tissue. PPARγ promotes tcel differentiation adipose cells (Braissant *et al.*, 1996). GLUT4 activation during adipogenesis is closely related to the role of PPARγ (Wu Z *et al.*, 1998). The role of PPARγ to GLUT4 expression was shown in a study of wild ginseng in reducing body weight (Ollah *et al.*, 2008). In the study, wild ginseng administration of dose 100 and 200 mg/kg for 4 weeks, revealed the lowering blood glucose level and increasing of PPARγ and GLUT4 expression, as well as insulin receptor in muscle and liver. Active compounds of SP was previously studied and revealed that campest-4-en-3-one, exhibited a significant protein tyrosine phosphatase 1B inhibitory (PTP1B) activity (Syaifudin *et al.*, 2012). PTP1B is an enzyme that found in insulin-targetting tissue (liver, muscle and adipose) that pays a role as a negative regulator in insulin signal transduction (Byon *et al.*, 1998).

Interestingly, the administration of AP and SP mixture extract (6:1) showed a level of GLUT4 expression that was not in line with PPAR-γ expression. The expressed PPAR-γ gene that were stimulated by the mixture extract maybe expressed in the different form as was previously studied, thus no relation with the level of GLUT4 was observed. These results explained that there maybe another gene expression than PPARγ was expressed and induced activation of GLUT4 following the administration of Ap and Sp mixture extract.

4. CONCLUSIONS

Mixture extracts of *Andrographis paniculata* Nees and *Syzygium polyanthum* Wight. (Walp.) able to increase GLUT4 expression in alloxanne-diabetic induced.

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