

## Uncovering the Potential of Indonesian Medicinal Plants for the Dual Actions of Anti-diabetes and Anti-obesity

Japan



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**Duration:** April to August 2014

**Total cost:** Scholarship from the Japan's Ministry of Education, Culture, Sport, Science and Technology (MEXT)

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## Summary

As obesity is a key factor in the development of type 2 diabetes, lowering lipid accumulation in adipose tissues is as important as increasing insulin sensitivity in diabetic patients. The selected plant extracts used in this screening have been traditionally used in Indonesian medicine for the treatment of diabetes and its complications. This study aimed to investigate the ability of the selected plants to increase both insulin sensitivity through the enhancement of glucose uptake after insulin induction in adipocytes and to suppress lipid production in the same target cells. For this purpose, dried Indonesian medicinal plants were screened accordingly. The screening platform consisted of insulin-induced glucose uptake, lipid accumulation, and cell viability. Out of 59 plants tested, 13 plants demonstrated their ability to increase glucose uptake in 3T3-L1 adipocytes after insulin induction, and four of these extracts

suppressed lipid production of the cells. The CCK-8 assay results of those four plant extracts suggest that the lipid inhibition activity of pasak bumi *Eurycoma longifolia* Jack (root) and black pepper *Piper nigrum* L. (fruits) extracts are not attributable to their cytotoxicity in the adipose cells. Both plant extracts increased glucose uptake by more than 200% at 50 µg/mL and suppressed lipid accumulation in a concentration-dependent manner. This indicates that screening of selected Indonesian medicinal plants has uncovered the potentials of pasak bumi root and black pepper fruits with dual active functions: increasing insulin sensitivity through the enhancement of glucose uptake, and reducing lipid accumulation in adipose cells. These findings suggest that both plants could provide additional benefits in the treatment of diabetes.

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## Background and Justification

Lowering lipid accumulation in adipose tissues (fat cells) is as important as enhancing insulin sensitivity in obesity-related diabetic patients due to the association between obesity and insulin resistance. This relationship presents major health hazards, including morbid obesity and cardiovascular complications. There is therefore a growing need for a medicine with two-fold properties.

For this reason, the study of the treatment of metabolic disorders using traditional forms of medicine has been intensified recently, as synthetic medicines for treatment of diabetes often cause undesirable side effects and certain drug groups even exacerbate obesity conditions. In addition, pharmacologists are now aware that the concept of 'one disease, one target, one drug' does not always lead to successful cures. To address the challenge of chronic and degenerative diseases, the holistic approach of traditional medicines represents a novel solution.

Indonesian traditional medicine, known as jamu, is used to treat various diseases, not only in rural areas, but also in urban settings. Unlike traditional Chinese medicine, jamu prescriptions are not well documented.

This study identified and investigated plants with dual active functions that could serve as anti-diabetes and anti-obesity agents. These plants were selected for use in this study because they are commonly used by local herbal industries in Indonesia. Other plants that are often used locally for treating diabetes complications were also selected. Certain plants in this study were expected to have unique mechanisms that could increase glucose uptake, while simultaneously suppressing lipid production.

To the best of our knowledge, this work is the first to report the *in vitro* screening of Indonesian medicinal plants for the dual activity of insulin-induced glucose uptake enhancement and lipid-lowering activity in 3T3-L1 adipocyte model cells (Lahrita et al., 2015). The findings of the study were expected to corroborate the traditional application of Indonesian jamu medicines, in treating diabetes and its complications, thus contributing to a more complete understanding of their efficacy.

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## Description

Dried samples of Indonesian plants were collected from the Central Java region and identified by a herbalist.

The processes surrounding adipocyte differentiation have been well studied using 3T3-L1 cells, cell lines that are committed to the adipocyte lineage. The 3T3-L1 preadipocytes differentiate into mature adipocyte cells and accumulating triglyceride lipid droplets. Needless to say, 3T3-L1 adipocytes have been used as a well-established cell culture system that can be employed to study adipogenesis, fatty acid metabolism, and insulin-regulated trafficking.

### ***Insulin-induced glucose uptake-enhancing assay***

On day 8 after differentiation, 50 µg/mL of each of the plant extracts was added to mature adipocytes. The reference compound used in this study was rosiglitazone, which acts as an insulin sensitizer, while the control cells were treated with 0.5% of a standard solvent. Insulin stimulation and glucose uptake were conducted on day 12, followed by an enzymatic fluorescence assay. On day 12, the cells were washed twice with KRPH buffer and incubated in a serum-free D-MEM medium. After being washed twice in a buffer solution and incubated with or without to perform insulin stimulation in the same buffer. The cells were then incubated with 2-DG solution. To remove excess 2-DG and halt its uptake, the cells were washed with cold KRPH buffer three times.

After washing again to stop the reaction, cells were lysed adding 50  $\mu\text{L}$  of Sodium hydroxide (NaOH) to each sample. The cell lysate was frozen and then heated to 85°C for 45 minutes. The lysate was neutralized by adding hydrochloric acid (HCl) and 350  $\mu\text{L}$  of a second buffer was subsequently added. The measurement of 2-DG uptake was done using an enzymatic fluorescence assay and normalization of protein using the Bradford protein assay.

### **Lipid accumulation assay**

For lipid accumulation studies, plant extracts were added to 3T3-L1 cells after differentiation induction.

To detect the accumulated lipid droplets, a standard Oil Red O staining assay was performed when the 3T3-L1 adipocytes reached maturity.

### **Cytotoxicity assay**

Cell viability was tested using the cell counting kit solution (CCK-8) from Dojindo Molecular Laboratories Inc. (Kumamoto, Japan).

All experiments were undertaken at least in triplicate under identical conditions.

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## **Results and Discussion**

### **Screening of plant extracts for insulin-induced glucose uptake-enhancing activity**

Fifty-nine plant extracts were screened for their ability to enhance glucose uptake upon the induction of insulin against fully differentiated 3T3-L1 cells. Out of 59 plants, 13 plant extracts that demonstrated insulin-induced glucose uptake-enhancing activity at the concentration of 50  $\mu\text{g}/\text{mL}$  were assessed, including green chiretta *Andrographis paniculata* (Burm.f.) Nees (whole plant), areca palm *Areca catechu* L. (fruits), sappanwood *Caesalpinia sappan* L. (wood), *Eleutherine bulbosa* syn. *americana* (Aubl.) Merr. ex K. Heyne (bulb part), pasak bumi *Eurycoma longifolia* Jack (root), moringa *Moringa oleifera* Lam. (leaves), betel *Piper betle* L. (leaves), red betel *P. crocatum* Ruiz & Pav. (leaves), black pepper *P. nigrum* L. (fruits), clove *Syzygium aromaticum* (L.) Merr. & L.M. Perry (fruits), beleric *Terminalia bellirica* (Gaertn.) Roxb. (fruits), *Tinospora crispa* (L.) Hook. f. & Thomson (bark), and aniseed *Pimpinella anisum* L. (seeds).

### **Lipid suppression from glucose uptake-enhancing plants**

Overproduction of lipids in adipose tissue is considered a sign of dyslipidaemia, which could lead to obesity and exacerbate type 2 diabetes mellitus conditions. For this reason, studies on the regulation of adipogenesis inform the medical community of the best

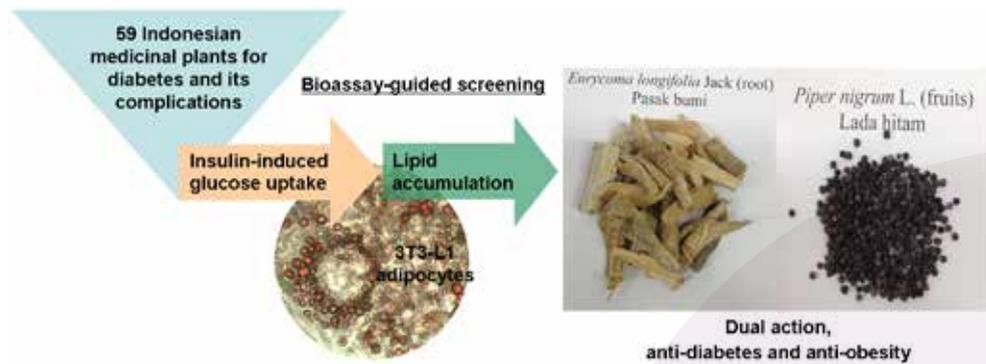
method for treating obesity-associated diabetes. The 13 plant extracts that exerted insulin-induced glucose uptake-enhancing activity were subjected to a lipid accumulation assay to examine their biological effects on the lipid production of adipocytes. Various concentrations of the extracts were added on day 4 after the induction of differentiation, to investigate their effects on intracellular lipid formation (lipo-genesis).

Lipid accumulation assay demonstrated that treatment in 3T3-L1 adipocytes with four of the plant extracts substantially reduced the accumulation of intracellular lipids in a concentration-dependent manner. They were *A. paniculata* (Burm.f.), Nees (whole plant), *E. longifolia* Jack (root), *P. betle* L. (leaves), and *P. nigrum* L. (fruits).

### Cytotoxicity

It is important to examine cytotoxicity when observing potential lipid-suppressing activity in order to distinguish between selectivity of action and cell death. Out of the four tested plants in which lipid reduction had previously been demonstrated, there were two species that exerted their lipid-lowering activity without causing cytotoxicity, namely pasak bumi root and black pepper fruits.

Collectively, the glucose uptake, lipid accumulation, and cytotoxicity results demonstrate the potential of pasak bumi root and black pepper fruits as anti-diabetic agents for the regulation of glucose and lipid metabolism (Fig. 1).



**Figure 1:** Bioassay scheme of screening Indonesian traditional medicines for dual actions for the potential treatment of type 2 diabetes.

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## Partnerships

If there is a field where people can think globally and act locally, it is traditional medicine, and the path to knowledge and innovation has always been travelled best in company. More things can be achieved in this field when people work together than when they work alone, because in the end, research on exploring traditional medicine tends to be an interdisciplinary process that requires collaboration among colleagues within an institute or with other institutes beyond national borders. For this reason, Hokkaido University (Japan) has research interest in Indonesian traditional medicine. Through intellectual knowledge exchange, academic institutions can synergize research strengths in exploring traditional medicine for the benefit of public health.

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## Impact

This study has uncovered the potential of both pasak bumi root and black pepper fruits to increase insulin sensitivity and suppress lipid production without causing significant cell death in the 3T3-L1 adipose cells.

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## Replicability

With more than 20,000 plant species, Indonesia is rich in biodiversity for treatment of diabetes and other disorders. Following the Amazon rain forests, Indonesia has the second greatest biodiversity in the world, reflected in the high number of indigenous medicinal plants. To promote biodiversity for the benefits of public health, Indonesia needs to assist pharmacists and scientists to develop proper intellectual property rights (IPR), indigenous knowledge, benefit sharing, efficacy and safety for the further development of Indonesian medicinal plants.

So far, the country lacks this kind of legislation. Jamu has been handed down from one generation to the next based on traditional knowledge and community experience. When new plant-derived therapeutics based on indigenous knowledge are explored, it is important that the benefit be returned to native populations and the local governments from which the research material was obtained.

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## Lessons Learned

This work provides a scientific basis for the use of pasak bumi root and black pepper fruits in obesity-associated diabetes mellitus. Further studies of both plants should be conducted to investigate their cellular mechanisms as well as the isolation of their bioactive constituents.

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## Future Plans

It is expected that the bioactive components of the plants could become lead compounds in the field of new drug discovery as well as functional foods. To expand the project, the findings of this study could be used to promote the traditional application of jamu in treating obesity-related diabetes diseases. We plan to publicize this encouraging result with other research institutes in Asia through workshops and international seminars.

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## Publication

- Lahrita, L., Kato, E. & Kawabata, J. (2015). Uncovering potential of Indonesian medicinal plants on glucose uptake enhancement and lipid suppression in 3T3-L1 adipocytes. *J. Ethnopharmacol.* 168, 229-236.

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## Acknowledgement

The author thanks the Elsevier Publisher for the opportunity to disseminate this seminal work to a wider scientific community in the field of traditional medicine for the benefit of public health.