学位論文審査の要旨

博士の専攻分野の名称　　博士（農学）　　氏　名　　Lucy Lahrita

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学位論文題名

Anti-obesity study of Indonesian medicinal plants: an *in vitro* study in adipocytes

（脂肪細胞を対象としたインドネシア薬用植物の抗肥満研究）

This thesis consists of 163 pages, 4 chapters, 89 figures, 24 tables, with 2 reference articles.

Obesity has become a global health problem due to its association with major life-threatening diseases including cardiovascular diseases, type 2 diabetes and numerous cancers. Over the past decades, only few anti-obesity drugs have been developed and approved. In fact, some drugs have been withdrawn from the market due to their serious side-effects. With the alarming rise of obese individuals globally, and in view of the dissatisfactions with synthetic drugs, there is a growing shift towards natural product-based medications.

In the thesis, potential of anti-obesogenic activity of Indonesian medicinal plant materials is investigated. Two plants, *Eurycoma longifolia* and *Brucea javanica* (both from family Simaroubaceae) are selected to investigate their lipolytic activity, a bioactivity to breakdown triglyceride into glycerol in adipocytes. Eight lipolytic compounds are isolated from the selected plants and are further studied for their cellular mechanisms of actions. In addition, screening of Indonesian medicinal plants is conducted to explore anti-lipogenesis plant material which is another anti-obesogenic activity.

1. Study on lipolytic activity of *E. longifolia*

Based on glycerol release assay, the extract of *E. longifolia* (EL) was shown to exert lipolytic activity in a concentration-dependent manner. Molecular mechanistic study using several specific inhibitors of lipolytic signaling pathways showed that protein kinase A (PKA) and extracellular signal-regulated kinase (ERK) are involved in the lipolytic activity of EL. Immunoblotting analysis is accompanied to confirm the activates PKA and ERK through enhanced phosphorylation.

Based on bioassay-guided fractionation, two lipolytic compounds, eurycomanone and 13β,21-epoxyeurycomanone, were isolated from EL. Eurycomanone enhanced lipolysis in adipocytes with an EC_{50} of 14.6 μM, while 13β,21-epoxyeurycomanone had a stronger

activity with an EC_{50} of 8.6 μM. Molecular mechanistic study showed that inhibition of PKA totally diminish their lipolytic activity. Furthermore, immunoblotting analysis confirmed the enhancement of phosphorylation in PKA through the stimulation by both compounds. These results indicate that the isolated compounds are the active principle of EL which induces lipolysis through PKA activation.

Additionally, eurycomanone and 13β,21-epoxyeurycomanone were shown to reduce lipid accumulation and exert lipolytic activity in WT-1 brown adipocytes that suggest a potential of these compounds to stimulate thermogenesis.

2. Study on lipolytic activity of *B. javanica*

The extract of *B. javanica* (BJ) was demonstrated to show strong lipolytic activity in white adipocytes. Involvement of PKA was confirmed through inhibitory challenge employing specific PKA inhibitor. Bioassay-guided fractionation of BJ lead to the isolation of six lipolytic compounds including brucein A, brusatol, brucenatinol, brucein B, 3'-hydroybrucein A, and bruceine C. These isolated compounds demonstrated stronger lipolytic activity compared to two isolated compounds from *E. longifolia*.

3. Anti-lipogenesis screening of Indonesian medicinal plants

The 76 selected Indonesian plant extracts were subjected to two bioassays, namely lipid accumulation and glycerol release assays. Four plants were shown to both reduce lipid accumulation and enhance lipolysis, *B. javanica*, *Melaleuca leucadendra*, *Zingiber purpureum*, and *Clerodendrum serratum*. Seven plants enhanced lipolysis but not reduced lipid accumulation, *Leucas lavandulifolia*, *Mentha arvensis*, *Pimpinella anisum*, *E. longifolia*, *Piper crocatum*, *Guazuma ulmifolia*, and *Sapindus rarak*. One plant reduced lipid accumulation but did not enhance lipolysis. The plant, *Momordica charantia*, is found as a potential candidate which reduces lipid synthesis instead of enhancing its breakdown.

The findings of this study provided significant contributions to the body of knowledge in anti-obesity related activity of Indonesian medicinal plants. Not only has this study provided scientific basis and rationale for the use of those potential plants for medicinal purposes, but it has also provided a possibility of multiple mode of effect to address this global health problem.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.