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Uncovering potential of Indonesian medicinal plants on glucose uptake enhancement and lipid suppression in 3T3-L1 adipocytes

Lucy Lahrita, Eisuke Kato *, Jun Kawabata

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo 060-8589, Japan

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ABSTRACT

Ethnopharmacological relevance: 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 screen have been traditionally used in Indonesian medicine for the treatment of diabetes and its complications.
Aim of the study: To investigate the ability of the selected plants to both increase insulin sensitivity through the enhancement of glucose uptake after insulin induction in adipocytes and suppress lipid production in the same target cells.
Materials and methods: Dried Indonesian medicinal plants were extracted with 50% (v/v) aq. methanol. The extracts were dissolved in 50% DMSO when tested in 3T3-L1 adipocytes. The screening platform consists of insulin-induced glucose uptake, lipid accumulation, and cell viability. Initially, an enzymatic fluorescence assay was designed to demonstrate the enhancement of 2-deoxyglucose (2-DG) uptake after insulin induction. Different concentrations of the extracts that enhanced glucose uptake were subjected to lipid accumulation assay using Oil Red O staining. Potential extracts based on lipid suppression were subsequently assessed by CCK-8 cell viability assay to distinguish lipid reduction activity from cytotoxicity.
Results: Out of 59 plants, 13 plants demonstrated their ability to increase glucose uptake in 3T3-L1 adipocytes after insulin induction, and 4 of these plants' extracts suppressed lipid production of the cells. The CCK-8 assay results of those 4 plant extracts suggest that the lipid inhibition activity of *Eurycoma longifolia* Jack (root) and *Piper nigrum* L. (fruits) extracts is not attributed to their cytotoxicity in the adipose cells. Both of the plant extracts increased glucose uptake by more than 200% at 50 μg/mL and suppressed lipid accumulation in a concentration-dependent manner.
Conclusions: Screening of selected Indonesian medicinal plants has uncovered the potentials of *E. longifolia* Jack (root) and *P. nigrum* L. (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 the ability of both plants to suppress lipid production would provide additional benefits in the treatment of diabetes.

1. Introduction

In 2014, the International Diabetes Federation (IDF) reported that 387 million of the world's population was living with diabetes, and this number is anticipated to rise beyond 592 million within the next two decades (International Diabetes Federation, 2014). As a complex metabolic disorder, type-2 diabetes mellitus (T2DM) is associated with impaired insulin release and insulin resistance, which result in high levels of glucose in the blood. Meanwhile, obesity involves an abnormal accumulation of adipose tissue, characterized by an increasing adipocyte number (hyperplasia) and size (hypertrophy). The association between obesity and T2DM, which has been well-recognized for decades, is mainly due to the insulin resistance of target cells, which affects glucose utilization and energy production.

Insulin sensitizers are commonly prescribed to treat insulin resistance, the primary clinical issue in T2DM. Thiazolidinediones (TZDs) reduce insulin resistance by activating peroxisome proliferator

Abbreviations: 2DG, 2-deoxyglucose; BSA, bovine serum albumin; DEX, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; G6PDH, glucose 6-phosphate dehydrogenase; IBMX, 3-isobutyl-1-methylxanthine; IDF, International Diabetes Federation; KRPH, Krebs–Ringer–phosphate–Hepes; PBS, phosphate buffered saline; TEA, triethanolamine

* Corresponding author. Tel./fax: +81 11 706 2496.
E-mail address: eikato@chem.agr.hokudai.ac.jp (E. Kato).

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activated receptor gamma (PPARγ). Clinical studies undertaken by Mayerson et al. (2002) and Virtanen et al. (2003) reported that TZDs reduce plasma insulin and increase glucose uptake. However, these improvements result in weight gain and undesirable lipid profiles. Therefore, there is on-going debate as to whether it is appropriate to use TZDs for the treatment of obesity associated with T2DM. In agreement with the reported results of TZDs, non-TZD PPARγ agonists from natural compounds have the capacity to activate PPARγ (Yang et al., 2007; Choi et al., 2009; Han et al., 2006). However, one consequence is the promotion of adipogenesis. For this reason, there is a growing need to identify novel bioactive compounds from local medicinal plants that have the capacity to increase insulin sensitivity and reduce lipid production at the same time.

This study investigates and identifies plants with dual active functions that could serve as anti-diabetes and anti-obesity agents. Those plants listed in Table 1 were selected for use in this study

Table 1
List of selected Indonesian medicinal plants for the treatment of diabetes and its complications.

Plant species	Batch number	Local name	Family	Part used	References	liGUE[a] activity
Alpinia galanga (L.) Willd.	AG1314.073	Lengkuas	Zingiberaceae	Rhizome	Hariana (2006)	51.8 ± 7.6
Alstonia scholaris (L.) R. Br.	AS1314.004	Pule	Apocynaceae	Bark	Elfahmi et al. (2014)	66.4 ± 1.3
Andrographis paniculata (Burm. f.) Nees	AP1314.042	Sambiloto	Acanthaceae	Whole plant	Elfahmi et al. (2014)	129.2 ± 4.4
Areca catechu L.	AC1314.006	Pinang	Arecaceae	Fruits	Ghate et al. (2014)	143.2 ± 9.2
Averrhoa bilimbi L.	AB1314.070	Belimbing wuluh	Oxalidaceae	Leaves	Pushparaj et al. (2000)	78.9 ± 15.8
Azadirachta indica A.Juss.	AI1314.051	Mimba	Meliaceae	Leaves	Atangwho et al. (2012)	113.3 ± 8.0
Baeckea frutescens L.	BF1314.023	Jungrahab	Myrtaceae	Bark	Dalimartha (2000)	68.6 ± 15.8
Blumea balsamifera (L.) DC.	BB1314.007	Sembung	Asteraceae	Leaves	Elfahmi et al. (2014)	72.2 ± 8.7
Brucea javanica (L.) Merr.	BJ1314.031	Buah Makasar	Simaroubaceae	Seeds	NoorShahida et al. (2009)	37.4 ± 8.2
Caesalpinia sappan L.	CS1314.17	Kayu secang	Fabaceae	Wood	Tong et al. (2013)	161.1 ± 19.9
Carica papaya L.	CP1314.049	Pepaya	Caricaceae	Leaves	Juárez-Rojop et al. (2012)	91.1 ± 9.3
Centella asiatica (L.) Urb.	CA1314.041	Pegagan	Apiaceae	Whole plant	Elfahmi et al. (2014)	65.9 ± 3.6
Cinnamomum burmanni (Nees & T.Nees) Blume	CB1314.065	Kayu manis	Lauraceae	Bark	Hariana (2006)	98.0 ± 15.8
Cinnamomum cassia (L.) J.Presl	CC1314.015	Kembang lawang	Lauraceae	Fruits	Hariana (2006)	70.8 ± 11.7
Clerodendrum serratum (L.) Moon	CS1314.013	Senggugu	Verbenaceae	Roots	Hariana (2006)	21.8 ± 2.7
Coriandrum sativum L.	CS1314.068	Ketumbar	Apiaceae	Seeds	Rajeshwari et al. (2011)	64.9 ± 26.7
Curcuma aeruginosa Roxb.	CA1314.039	Temu ireng	Zingiberaceae	Rhizomes	Hariana (2006)	63.6 ± 14.6
Curcuma heyneana Valeton & Zijp	CH1314.038	Temu giring	Zingiberaceae	Rhizome	Hariana (2006)	79.6 ± 15.1
Curcuma zanthorrhiza Roxb.	CX1314.040	Temulawak	Zingiberaceae	Rhizome	Elfahmi et al. (2014)	55.8 ± 0.1
Curcuma zedoaria (Christm.) Roscoe	CZ1213.001	Temu putih	Zingiberaceae	Rhizome	Hariana (2006)	95.0 ± 15.2
Cymbopogon citratus (DC.) Stapf	CC1314.053	Sere minyak	Poaceae	Leaves	Hariana (2006)	105.5 ± 27.9
Elephantopus scaber L.	E1113.001	Tapak liman	Asteraceae	Leaves	Hariana (2006)	101.0 ± 32.9
Eleutherine americana (Aubl.) Merr. ex K.Heyne	EA1314.055	Bawang sabrang	Iridaceae	Bulb	Febrinda et al. (2014)	130.9 ± 9.3
Eugenia aromatica (L.) Baill.	EA1314.050	Cengkeh	Myrtaceae	Leaves	Hariana (2006)	119.1 ± 8.5
Eugenia polyantha Barb. Rodr.	EP1314.056	Daun salam	Myrthaceae	Leaves	Dalimartha (2000)	102.9 ± 21.2
Eurycoma longifolia Jack	EL1314.057	Pasak bumi	Simaroubaceae	Roots	Husen et al. (2004)	223.1 ± 15.7
Foeniculum vulgare Mill.	FW1314.003	Adas-Jogja	Apiaceae	Seeds	Hariana (2006)	12.9 ± 5.3
Guazuma ulmifolia Lam.	GU1314.033	Jati Belanda	Sterculiaceae	Leaves	Elfahmi et al. (2014)	68.2 ± 14.1
Gynura procumbens (Lour.) Merr.	GP1314.058	Sambung nyawa	Asteraceae	Leaves	Wijayakusuma (2006)	64.4 ± 7.2
Helicteres isora L.	HI1314.063	Kayu ulet	Sterculiaceae	Fruits	Hariana (2006)	110.8 ± 6.3
Kaempferia angustifolia Roscoe	KA1314.037	Kunci pepet	Zingiberaceae	Rhizome	Elfahmi et al. (2014)	22.9 ± 0.2
Kaempferia galanga L.	KG1314.059	Kencur	Zingiberaceae	Rhizome	Elfahmi et al. (2014)	89.0 ± 13.6
Leucaena leucocephala (Lam.) de Wit	LL1314.075	Lamtoro	Fabaceae	Fruit	Wijayakusuma (2006)	59.7 ± 19.2
Leucas lavandulifolia Sm.	LL1314.014	Leng-lengan	Labiatae	Whole plant	Hariana (2006)	35.0 ± 6.4
Melaleuca leucadendra (L.) L.	ML1314.024	Merica bolong	Myrtaceae	Fruits	Elfahmi et al. (2014)	46.7 ± 6.7
Melastoma polyanthum Burm. f.	MP1314.019	Senggani	Melastomataceae	Roots	Hariana (2006)	56.5 ± 2.1
Mimosa pigra L.	MP1314.018	Putri malu	Fabaceae	Whole plant	Dalimartha (2000)	42.5 ± 6.8
Momordica charantia L.	MC1314.043	Pare	Cucurbitaceae	Fruits	Wijayakusuma (2006)	79.0 ± 14.6
Morinda citrifolia L.	MC1314.029	Mengkudu	Rubiaceae	Fruits	Wijayakusuma (2006)	53.8 ± 6.9
Moringa oleifera Lam.	MO1314.071	Kelor	Moringaceae	Leaves	Yassa and Tohamy (2014)	128.0 ± 1.7
Myristica fragrans Houtt.	MY1013.001	Pala	Myristicaceae	Fruits	Hariana (2006)	106.1 ± 2.5
Nigella sativa L.	NS1314.028	Jintan hitam	Ranunculaceae	Seeds	Dalimartha (2000)	26.3 ± 5.5
Ocimum gratissimum L.	OG1314.067	Selasih	Lamiaceae	Seeds	Elfahmi et al. (2014)	92.5 ± 24.0
Orthosiphon aristatus (Blume) Miq.	OA1314.061	Kumis kucing	Lamiaceae	Whole plant	Elfahmi et al. (2014)	51.5 ± 2.9
Phaleria macrocarpa (Scheff.) Boerl.	PM1314.034	Mahkota dewa	Thymelaeaceae	Fruits	Wijayakusuma (2006)	86.7 ± 8.7
Phyllanthus urinaria L.	PU1314.012	Meniran	Phyllanthaceae	Whole plant	Dalimartha (2000)	105.5 ± 21.9
Physalis angulata L.	PA1314.32	Ciplukan	Solanaceae	Whole plant	Wijayakusuma (2006)	99.9 ± 32.6
Pimpinella anisum L.	PA1314.045	Adas manis	Apiaceae	Seeds	Rajeshwari et al. (2011)	280.6 ± 0.6
Piper betle L.	PB1314.062	Sirih	Piperaceae	Leaves	Arambewela et al. (2005)	159.6 ± 17.9
Piper crocatum Ruiz & Pav.	PC1314.066	Sirih merah	Piperaceae	Leaves	Safithri and Fahma (2008)	188.5 ± 9.2
Piper nigrum L.	PN1314.027	Lada hitam	Piperaceae	Fruits	Onyesife et al. (2014a)	212.3 ± 1.2
Plantago major L.	PM1314.072	Daun sendok	Plantaginaceae	Leaves	Wijayakusuma (2006)	106.1 ± 3.5
Ruellia tuberosa L.	RT 1314.001	Ceplikan	Acanthaceae	Fruits	Dalimartha (2000)	57.5 ± 26.0
Swietenia mahagoni (L.) Jacq.	SM1314.020	Mahoni	Meliaceae	Seeds	Elfahmi et al. (2014)	45.5 ± 6.5
Syzygium aromaticum (L.) Merr. & L.M.Perry	SA1314.022	Cengkeh	Myrtaceae	Fruits	Elfahmi et al. (2014)	225.3 ± 0.7
Tamarindus indica L.	TI1314.047	Asam Jawa	Fabaceae	Fruits	Hariana (2006)	13.4 ± 7.5
Terminalia bellirica (Gaertn.) Roxb.	TB1314.009	Joho	Combretaceae	Fruits	Sabu and Kuttan (2002)	276.6 ± 6.4
Tinospora crispa (L.) Hook. f. & Thomson	TC1314.021	Brotowali	Menispermaceae	Bark	Elfahmi et al. (2014)	213.9 ± 5.1
Typhonium flagelliforme (Lodd.) Blume	TF1314.005	Keladi tikus	Araceae	Rhizome	Hariana (2006)	29.3 ± 9.9
Rosiglitazone 1 μM						178.3 ± 10.22

Concentration of tested plant extracts: 50 μg/mL. liGUE, insulin-induced glucose uptake enhancement.

[a] Percentage of values, relative to control. Data are expressed as the mean ± SD ($n=3$) and are representative of at least three independent experiments.