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A New Antidiabetic Compound 8,9-dimethoxy Ellagic Acid from Sasaladaan (*Peperomia pellucida* L. Kunth)

Yasmiwar Susilawati[1*], Ricky Nugraha[3], Jegatheswaran Krishnan[1], Ahmad Muhtadi[2], Supriyatna Sutardjo[1] and Unang Supratman[3,]

[1] Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinagor 45363, Sumedang, Indonesia
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinagor 45363 Sumedang, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang Km. 21 Jatinagor 45363 Sumedang, Indonesia

ABSTRACT

Peperomia pellucida (L.) Kunth.) is a annual herb belongs to Piperaceae family and also know as sasaladaan or suruhan in Indonesia. These herbs has been known empirically used as blood sugar reducer. Previous research has proved that ethanol extract and ethyl acetat fraction have a good potential as antidiabetics. This study was conducted to obtain antidiabetic compounds of *P. pellucida* using chromatographic techniques guided by in vivo activity test. A new antidiabetic compound, 8,9-dimethoxy ellagic acid (**1**), was isolated from ethyl acetat fraction of *P. pellucida*. The chemical structure of **1** was determined by spectroscopic methods and comparison with those related compound previously reported. The 8,9-dimethoxy ellagic acid at dose of 100 mg/kg body weight produced significant antidiabetic activity in alloxan-induced hyperglycemic Swiss Webster mice.

Keywords: Antidiabetic, 8,9-dimethoxy-ellagic acid, *Peperomia pellucida*, Piperaceae.

Corresponding author

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INTRODUCTION

Diabetes mellitus is the largest endocrine disease worldwide characterized by elevated blood glucose levels and disturbance in carbohydrate, fat and protein metabolism [1]. Diabetic patients experience various vascular complications such as, atherosclerosis, diabetic nephropathy, retinopathy and neuropathy [2]. The currently available therapy for diabetic includes insulin and various oral antidiabetic agents such as the sulfonylureas, biguanides, thiazolidinediones and α-glucosidase inhibitors. Each of the oral antidiabetic agents is however, associated with a number of serious adverse effects [3,4]. Plant-based drugs have been known to be safe and cheaper. Many natural product and herbal medicines have been studied in the search for an effective management of diabetes mellitus and most of them have therapeutic claims [5,6,7]. As part of our continuing search for antidiabetic agents from Indonesian medicinal plant, the ethanolic extract of *Peperomia pellucida* showed a significant antidiabetic activity in alloxan-induced hyperglycemic Wistar rats.

P. pellucida known as "sasaladaan" in Indonesia is a annual herb that typically grows in wet rock crevices, is found from nothest to the south of Indonesia [8]. The plant is used in Indonesian folk medicine for the treatment of fever, headache, contused wound and skin diseases [9]. Previous phytochemical studies on the genus *Peperomia* have revealed the presence of a variety of compounds with interesting biological activities, including flavonoids [10,11], benzopyran derivatives [12,13], secolignans, lignans [14,15,16], A dimeric ArC2 compound, arylpropanoids, phenolic compounds [17,18,19] and essential oils [20]. Although secondary metabolites of other *Peperomia* species have been investigated previously, the antidiabetic compounds from the leaves of *P. pellucida* which growth in Indonesia is yet to be reported. The isolation and structure elucidation of the new antidiabetic compound from the leaves of *P. pellucida* are described herein.

MATERIALS AND METHODS

Plant material: The leaves of *Peperomia pellucida* were collected from Kawangluwuk village, South Congeang, Sumedang, Indonesia in September 2012 and identified by biologist Mr. Joko Kusmoro (Padjadjaran University). A voucher specimen (No. 01/HB-IX/2012) has been deposited at the Herbarium of the Department of Biology, Padjadjaran University, Sumedang, Indonesia.

Experimental animals: This experiment used animal models (mice) *Mus musculus* for antidiabetic activity test that has been approved by the ethics (ethical clearance) of the Health Research Ethics Committee, Faculty of Medicine, University of Padjadjaran, Bandung, West Java, Indonesia. Healthy albino mice of Swiss Webster strain weighing 25-30 g (weight of mice somewhat lower than standard, but the mice were otherwise healthy, 6-8 weeks old, took normal average daily diet, displayed normal daily activity and behavior). Mice of male sex were used, they were obtained from the Central Animal House, Department of Biology, Padjadjaran University. The animals were housed in standar polyprophylene cages and maintained under controlled room temperature (25 ± 5^{o}C) and humidity (55 ± 5%) with 12:12 hour light and dark cycle. The mice were fed commercially available mice normal pellet diet and water *ad libitum* [21].

Chemical materials:

Chromatographic separations were carried out on silica gel 60 (Merck). PTLC glass plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). TLC plates were precoated with silica gel GF_{254} (Merck, 0.25 mm). Antidiabetic activity test was performed by *blood glucose level measurement using gluco-test strips (FreeStyle Optium H).*

Instruments:

The UV-Visible spectrum was obtained on UV-Shimadzu 1800 series spektrofotometer. Optical rotations were recorded on an ATAGO AP-300 polarimeter. The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Water UP-LC MS/MS instruments. ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard

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Methods:

Procedure 1:

***Extraction and isolation*:** Dried powder leaves (4.4 kg) of *P. pellucida* were extracted by soxhlet apparatus with *n*-hexane, EtOAc, *n*-BuOH and EtOH, successively. Evaporation of these extract under reduced pressure resulted in the crude extracts of *n*-hexane (117 g), EtOAc (125 g), *n*-BuOH (86 g) and EtOH (104 g), respectively. The ethyl acetate extract produced significant antidiabetic activity in alloxan-induced hyperglycemic Wistar rat at dose of 250 mg/kg body weight. A portion of the EtOAc (120 g) was subjected to vacuum liquid chromatography (VLC) using gradient elution of *n*-hexane-EtOAc-MeOH to afford 12 fractions (Ea1-Ea12). Fraction Ea3 (20 g) was subjected to vacuum liquid chromatography (VLC) using gradient elution of *n*-hexane-EtOAc-MeOH to afford 10 fractions (Ea3-1-Ea3-10). Fraction Ea3-1 (3.3 g) was subjected to column chromatography of silica gel, eluted with CH_2Cl_2-EtOAc (9:1) to afford 8 fractions (Ea3-1.1-Ea3-1.8). Fraction Ea3-1.3 (500 mg) was preparative TLC on silica gel GF_{254}, eluted with *n*-hexane:EtoAc (7:3) and CH_2Cl_2:EtOAc (9.5:0.5) to give **1** (11 mg).

Procedure 2:

Induction of diabetes: Leaving aside six mice for normal control group, diabetes was induced in 24 mice by a single intraperitoneal injection of alloxan monohydrate in the dose of 245 mg/kg body weight. The fasting blood glucose was determined after 24 hours [21,22]. Eighteen mice showing a blood glucose level of >170 mg/100 mL were taken for the study.

Experimental Design for Antidiabetic Study: A total of 24 animals were equally divided into four groups with six animals in each group [23]:

Group-A: Normal control (Normal saline; 10 mL/kg/d)
Group-B: Diabetic control (Normal saline; 10 mL/kg/d)
Group-C: Diabetic Test (**1**; 100 mg/kg/d)
Group D: Diabetic Standar (Glibenclamide; 0.7mg/kg/d)

The above drugs were administrated orally once daily for three days. The blood glucose level were measured everyday until the fifth day for each group.

Method of blood glucose estimation: Blood glucose estimation was carried out by Glucose Oxidase Method (GOD-PAP) using blood glucose test strips (FreeStyle Optium H). In order to assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the mice using scalpel blade 7 days after induction. A sample of the mice's venous blood was collected on a reagent strip 7 days after the diabetes induction procedure for blood glucose level determination using portable glucose analyzer. In this study, mice with glucose levels above 170 mg/dL were considered as having severe diabetes [21].

RESULTS AND DISCUSSION

The dried and powdered leaves of *P. pellucida* was extracted with *n*-hexane, ethyl acetate, *n*-butanol and ethanol succesively. All the extracts were evaluated for antidiabetic activity at dose of 250 mg/kg body weight in alloxan-induced hyperglycemic Wiss albino mice; the ethyl acetate showed strongest antidiabetic activity. By using the antidiabetic assay *in vivo* in alloxan-induced hyperglycemic Swiss Webster albino mice, the ethyl acetate extract was subjected to multiple chromatographic steps, using silica gel G_{60} and preparative TLC to afford a new benzopyran compound (**1**) (Figure 1).

Compound **1** was obtained as a pale yellowish powder, completely dissolved in methanol and showed flourencence under UV light at λ 384, 329, 278 and 248 nm, $[\alpha]^{D}_{20}$ + 24.7° (*c* 0.2, MeOH). The molecular formula, $C_{15}H_{12}O_4$, was determined from LC-MS/MS (*m/z* 257.3145 $[M+H]^+$, calcd for $C_{15}H_{12}O_4$ 256.3674) and NMR spectrosopic data (Table 1), requiring ten degrees of unsaturation. The UV spectrum showed absorption maximum at λ_{max} 384, 329, and 207 nm, indicated the presence of conjugated double bond. The IR spectrum showed the presence of carbonyl lactone (1718 cm^{-1}), conjugated double bond (1612 cm^{-1})and ether (1089 cm^{-}

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[1]) functionalities. The ^{1}H-NMR spectrum (Table 1) showed the presence of two aromatik proton singlet at [δ_H 7.15 (1H, s, H-10) and 7.82 (1H, s, H-7)] due to tetrasubstituted benzene ring, four other aromatic protons at [δ_H 7.57 (1H, d, *J*=7.2 Hz, H-4), 7.91 (1H, d, *J*=6.7 Hz, H-1), 7.70 (1H, dd, *J*=6.7, 7.8 Hz, H-3) and 9.26 (1H, dd, *J*=7.2, 7.8 Hz, H-2)] from disubstituted benxene ring, and two methoxyl signal at [δ_H 4.13 (3H, s) and 4.20 (3H, s)]. In the ^{13}C NMR spectrum (Table 1), 15 carbon signals appeared, which were assigned by DEPT and HMQC experiments as one carbonyl lactone (δ_C 169.2), six sp^2 methines (δ_C 129.8, 128.3, 128.2, 126.3, 110.4, and 105.4), four sp^2 quarternary carbons (δ_C 127.6, 136.2, 122.7 and 121.5), two quartenary oxyaryl carbons (δ_C 152.1 and 155.6) and two methoxyl carbons (δ_C 60.5 and 57.2). These functionatilities accounted for seven out of the ten indices of hydrogen deficiency.

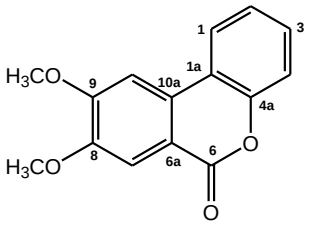

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Figure 1: Chemical structure of compound **1**.

Table 1: NMR data (500 MHz for ^{1}H and 125 MHz for ^{13}C, in asetone-d_6)

Position	^{13}C NMR, δ_C (mult.)	δH NMR, δ_H (integral, mult., *J* Hz)
1	129.8 (d)	7.91 (1H, d, 6.7)
1a	127.6 (s)	-
2	128.3 (d)	9.26 (1H, dd, 7.2, 7.8)
3	128.2 (d)	7.70 (1H, dd, 6.7, 7.8)
4	126.3 (d)	7.57 (1H, d, 7.2)
4a	136.2 (s)	-
6	169.2 (s)	-
6a	122.7 (s)	-
7	110.4 (d)	7.82 (1H, s)
8	152.1 (s)	-
9	155.6 (s)	-
10	105.4 (d)	7.15 (1H, s)
10a	121.5 (s)	-
11	60.4 (q)	4.13 (3H, s)
12	57.2 (q)	4.20 (3H, s)].

The remaining three hydrogen deficiency were consistent with benzopyran structure [17,18]. Comparison of the NMR data for **1** with those urolithin A [17-19] indicated that compound **1** was an analogue of this later compound. The main difference is additional methoxyl groups at C-8 and C-9, indicating that compound **1** is 8,9-dimethoxy of ellagic acid. The gross structure of **1** was deduced from ^{1}H-^{1}H COSY and HMBC spectra (Figure 2).

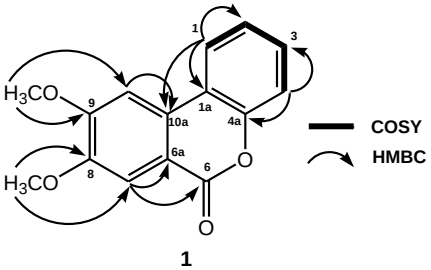

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Figure 2: Selected ^{1}H-^{1}H COSY and HMBC correlations for **1**.

In the HMBC spectrum, the correlations arising from methoxyl protons at dH 4.13 and 4.20 to C-9 (δ_C 155.6), C-10 (δ_C 105.4), C-8 (δ_C 152.1), and C-7 (δ_C 110.4), enable the assignment of the two methoxyl groups was located at C-8 and C-9, respectively. Proton aromatic signals at δ_H 7.91 and 9.26 were coupled to each other and were correlated to C-1a (δ_C 127.6) and C-10a (δ_C 121.5), whereas other proton aromatic signals at δ_H 7.57 and 7.70 were coupled to each other and were correlated to C-3 (δ_C 128.2) and C-4a (δ_C 136.2), suggested that benzopyran ring was located at C-4a, C-1a, C-10a and C-6a. The location of benzopyran ring was supported also from correlation from aromatic signals at δ_H 7.82 to C-6a (δ_C 122.7) and C-6 (δ_C 169.2) and δ_H 7.15 to C-10a (δ_C 121.5).

Compound **1** was evaluated for its antidiabetic activity in alloxan-induced hyperglycemic mice. Compound **1** exhibited 33.74% ($p<0.01$) blood glucose lowering in normoglycemic model at dose of 100 mg/kg.

8,9-dimethoxy of ellagic acid (1)

Yellowish amorphous powder.
$[\alpha]^{D}_{20}$ -24.7° (*c*, 0.2, MeOH)
UV (MeOH) λ_{max}: 384, 329, 278 and 248 nm.
IR (KBr) ν_{max}: 1718, 1612, 1089 cm^{-1}
^{1}H NMR (acetone-d_6, 500 MHz): Table 1
^{13}C NMR (acetone-d_6, 125 MHz): Table 1
LC-MS/MS: (positive ion mode), *m/z* 257.3145 $[M+H]^+$, calcd. for $C_{15}H_{12}O_4$, 256.3674).

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CONCLUSIONS

Based above data and biogenetic point of view the occurance benzopyran compound in this genus, consequently compound **1** was determined as a new benzoypran derivative and was named 6H-dibenzo[b,d]-pyrano-6-one-8,9-dimethoxy or 8,9-dimethoxy of ellagic acid. Compound **1** exhibited 33.74% ($p<0.01$) blood glucose lowering in glycemic model at dose of 100 mg/kg.

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