JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Antihyperglycemic Effect of Syringaldehyde in Streptozotocin-Induced Diabetic Rats

Chia-Hsin Huang,[†,‡] Mei-Fen Chen,[‡] Hsien-Hui Chung,[§] and Juei-Tang Cheng*[,§,⊥]

[†]Agricultural Research Institute, Council of Agriculture Executive, Taichung, Taiwan 40512

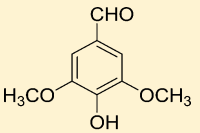
[‡]Department of Nursing and Department of Biological Science and Technology, Chung Hwa University of Medical Technology, Tainan, Taiwan 71741

[§]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan 70101

[⊥]Institute of Medical Science, College of Health Science, Chang Jung Christian University, Tainan, Taiwan 71101

Supporting Information

ABSTRACT: The antihyperglycemic effect of syringaldehyde (**1**), purified from the stems of *Hibiscus taiwanensis*, was investigated in streptozotocin-induced diabetic rats (STZ-diabetic rats) showing type-1 like diabetes mellitus. Bolus intravenous injection of **1** showed antihyperglycemic activity in a dose-dependent manner in STZ-diabetic rats. An effective dose of 7.2 mg/kg of **1** attenuated significantly the increase of plasma glucose induced by an intravenous glucose challenge test in normal rats. A glucose uptake test showed that **1** exhibits an increase of glucose uptake activity in a concentration-related manner. Moreover, an effect by **1** was shown for insulin sensitivity in STZ-diabetic rats. The compound was found to increase insulin sensitivity in STZ-diabetic rats. These results suggest that syringaldehyde (**1**) can increase glucose utilization and insulin sensitivity to lower plasma glucose in diabetic rats.



1

Diabetes mellitus (DM) and/or insulin resistance (IR) are ranked in the top 10 causes of mortality around the world. Both disorders often lead to disability through vascular complications, renal failure, blindness, and limb amputation, in addition to neurological complications and premature death.[1,2] However, novel treatments with fewer side effects than those presently available are needed for the management of this disorder.

Hibiscus taiwanensis S. Y. Hu (Malvaceae) is native to Taiwan. It is a moderately tall shrub and widely distributed throughout the island.[3] The stems and roots of *H. taiwanensis* have been used as anti-inflammatory, antifungal, antipyretic, and anthelmintic agents in traditional Chinese medicine.[4] From a crude methanol extract of the stems of *H. taiwanensis,* many compounds have been reported, including phenylpropanoid esters, myriceric acid, and other known compounds.[5] These active principles have been screened for cytotoxic activity against various carcinoma cell lines.[6] However, the effect on blood sugar of *H. taiwanensis* has not been investigated. Therefore, the chromatographic fractionation of the chemical constituents for their plasma glucose lowering action was carried out. Syringaldehyde (**1**) was purified from *H. taiwanensis* stems as an active principle, and its effects on glucose metabolism were further investigated.

In order to rule out any adverse pharmacokinetic effects, syringaldehyde (**1**) was studied by intravenous injection in animals. It was found that bolus injection of **1** can lower plasma glucose concentrations effectively in streptozotocin-diabetic (STZ-diabetic) rats. As shown in Figure 1, a dose-dependent increase of antihyperglycemic activity was observed in STZ-diabetic rats upon intravenous injection of **1** at the dose range 1.8−7.2 mg/kg. There was no additional effect of **1** with an increase in dosage beyond 7.2 mg/kg. The minimal and maximal plasma glucose-lowering activities of **1** in STZ-diabetic rats were 11.08 ± 1.0% at 1.8 mg/kg and 31.29 ± 2.07% at 7.2 mg/kg, respectively. In a previous study using the same conditions, it was demonstrated that the maximal glucose-

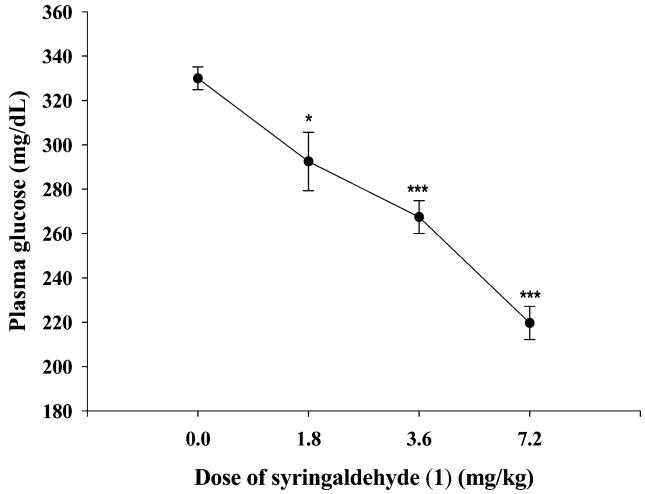


Figure 1. Antihyperglycemic action of syringaldehyde (**1**) in STZ-diabetic rats. Values (means ± SE) were obtained from eight animals through intravenous injection (iv) of syringaldehyde at the indicated dose. *$p < 0.05$ and ***$p < 0.001$ as compared with nontreated values (0 mg/kg).

Received: May 28, 2012
Published: August 10, 2012

dx.doi.org/10.1021/np3003723 | *J. Nat. Prod.* 2012, 75, 1465−1468

lowering effect of metformin, a clinically used antidiabetic agent, was 32 ± 5% at an oral dose of 100 mg/kg in STZ-diabetic rats.[7] By comparison with metformin, this indicated that 7.2 mg/kg of **1** is an effective dose for subsequent experiments. In preliminary screening, it was determined that the plasma glucose lowering activity at the same dose of **1** in normal Wistar rats (number size = 8) is 25.93 ± 5.63%, which seems slightly less than in STZ-diabetic rats. Thus, syringaldehyde (**1**) can be considered as a potential effective plasma glucose lowering agent.

An intravenous glucose challenge test[8] to characterize the ability of rats to clear glucose from the circulation was then carried out. Five minutes after glucose injection, the plasma glucose concentration was elevated in both vehicle- and **1**-treated rats (Figure 2). The increase of plasma glucose

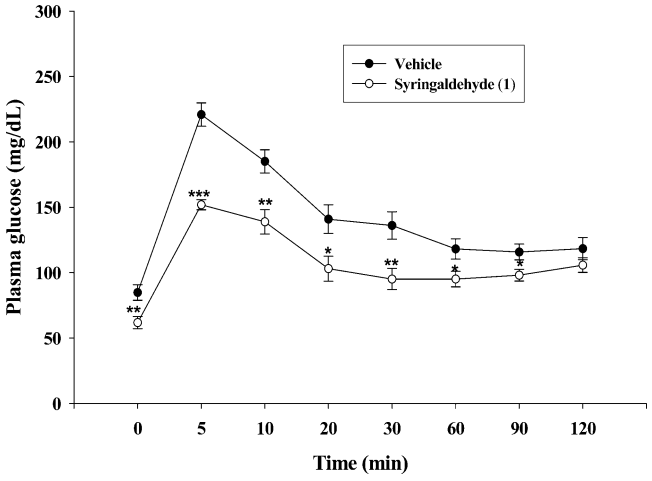


Figure 2. Effect of syringaldehyde (**1**) on plasma glucose levels in normal rats receiving an intravenous glucose challenge test. A solution of **1** at 7.2 mg/kg or the same volume of vehicle was injected intravenously into rats. After 30 min, plasma glucose values in blood samples were indicated as 0 min. Then, a glucose dose of 60.0 mg/kg was injected intravenously into each rat. Changes of plasma glucose at the indicated time were compared between the syringaldehyde-treated group (open circles) and the vehicle-treated group (solid circles). Values (means ± SE) of each group were obtained from eight animals. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ as compared with vehicle-treated group at the same time.

produced by the glucose injection was significantly lower in the rats pretreated with **1** after 5 min of glucose injection compared to the vehicle-treated rats. The plasma glucose in rats pretreated with **1** remained significantly lower after 30 min of glucose injection compared to the vehicle-treated group (Figure 2). No statistical difference was obtained for the plasma glucose concentration in rats that received a glucose injection 120 min later between the group treated with **1** and the vehicle-treated controls. This finding shows that **1** can enhance glucose utilization in vivo.

Skeletal muscle is the major site of glucose disposal.[9] Glucose transport, which depends on insulin-stimulated translocation of glucose carriers to the cell membrane, is the rate-limiting step in the carbohydrate metabolism of skeletal muscle.[10] Insulin-stimulated glucose uptake into skeletal muscle is the major site for the regulation of plasma glucose concentrations.[11] In the present study, cultured L6 myoblasts were used to identify the effect of **1** on glucose uptake as described previously.[12] It was found that **1** can increase glucose uptake in a concentration-dependent manner (Figure 3). Thus, an increase in glucose uptake may be considered as a potential mechanism for the observed glucose-lowering action of **1**.

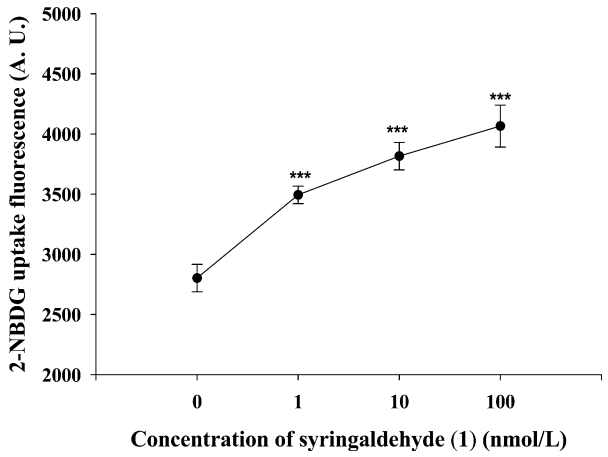


Figure 3. Effect of syringaldehyde (**1**) on glucose uptake in rat L6 myocytes. Cells were suspended in 0.2 mM 2-NBDG to incubate with syringaldehyde (**1**) at the indicated concentration or the same volume of vehicle (as 0 nmol/L) for 60 min. Values (means ± SEM) of absorbance units (AU) were obtained from each group of eight experiments; $***p < 0.001$ as compared with vehicle-treated group (0 nmol/L).

In addition, the possible mechanism for **1** to increase glucose uptake may be related to the regulation of GLUT4 in skeletal muscle. However, more experimentation is needed to find out the possible mechanism(s) for the increase of glucose uptake by **1**.

Insulin sensitivity is one of the major factors in the formation of insulin resistance.[13] Drugs useful in the treatment of diabetic disorders are believed to increase insulin sensitivity.[14] Thus, the effect of **1** on insulin sensitivity in STZ-diabetic rats was investigated. According to a previously used protocol,[15] a solution of **1** was injected intravenously into diabetic rats at 7.2 mg/kg three times per day for three days. Then, the responses were compared to exogenous insulin with the vehicle-treated group. As shown in Figure 4, responses to exogenous insulin were raised by treatment with **1** in STZ-diabetic rats. An increase of insulin sensitivity by **1** can thus be considered possible. Syringaldehyde increased the insulin sensitivity and seems more effective on the responses to insulin produced at higher concentrations. The above results suggest that **1** can increase the utilization of glucose in peripheral tissue via an insulin-independent mechanism. The glucose-lowering action of **1** in STZ-induced diabetic rats has not been published previously. Further studies are needed to elucidate the molecular mechanisms by which **1** regulates plasma glucose concentrations in the absence of insulin.

In conclusion, the data obtained suggest that intravenous injection of **1** can lower plasma glucose in STZ-diabetic rats through an increase of glucose utilization. Thus, **1** might be suitable as an adjuvant for the treatment of diabetic patients in the future.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting point was recorded on a Buchi B-545 melting point apparatus and is uncorrected. The optical rotation was measured on a JASCO DIP-1020 digital

dx.doi.org/10.1021/np3003723 | J. Nat. Prod. 2012, 75, 1465−1468

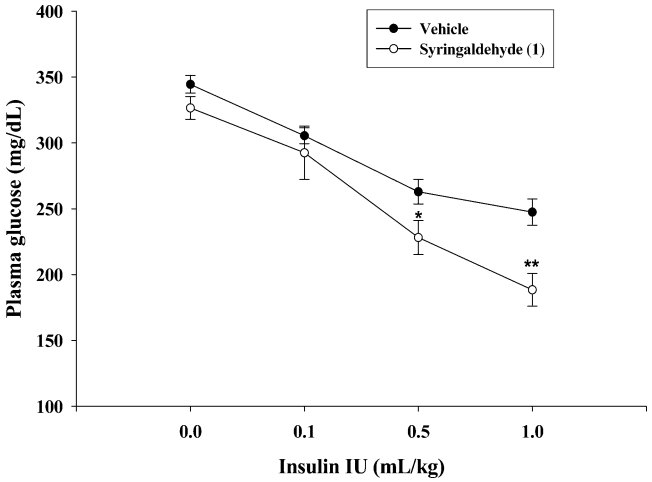


Figure 4. Effect of syringaldehyde (**1**) in an insulin challenge test in STZ-diabetic rats. Syringaldehyde (**1**) at 7.2 mg/kg was injected intravenously into STZ-diabetic rats three times daily for three days. Then, the animals were injected intravenously with exogenous insulin at the indicated dose. Changes of plasma glucose are expressed as open circles. Changes of plasma glucose in another group of STZ-diabetic rats receiving a similar treatment with vehicle at the same volume are shown as solid circles. Values (means ± SE) were obtained from each group of eight animals. *p < 0.05 and **p < 0.01 as compared with values from vehicle-treated group (solid circles) at the same dose of insulin.

polarimeter. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-500 (500 MHz) FT-NMR spectrometer in DMSO-d_6 solution, using the solvent as internal standard. The EIMS was determined on a Finnigan TSQ-700 mass spectrometer. Column chromatography was carried out with Diaion HP 20 (100−200 mesh, Mitsubishi Chemical Industries), MCI-gel CHP 20P (75−150 μm, Mitsubishi Chemical Industries), and Cosmosil C_{18}-OPN (75 μm, Nacalai Tesque, Inc.). TLC was conducted on silica gel plates (60 F-254, Merck), and 10% sulfuric acid solution was used as a visualizing agent on heating.

Plant Material. The stems of *Hibiscus taiwanensis* were provided by Hercet Co. Ltd. (Kaohsiung, Taiwan). The plant material was identified by Professor M. I. Wu, Kaohsiung Committee of Chinese Medicine (Kaohsiung, Taiwan). A voucher specimen (BT-H-00151) was deposited in the herbarium of the Agricultural Research Institute (Taichung, Taiwan).

Extraction and Isolation. Dried *H. taiwanensis* (500 g) stems were extracted with 60% aqueous acetone (ratio of solvent volume/dry weight about 2 mL/g) three times, each for 2 days, at room temperature. After evaporating the solvents under vacuum at 45 °C, a residue was obtained. This residue was dissolved in H_2O (1.5 L) and then extracted successively with CH_2C_{l2} (1 L × 3) and *n*-BuOH (1 L × 3). The *n*-BuOH extract (22% dry weight) was subjected to column chromatography over Diaion HP20 (15 × 120 cm) and eluted with a step gradient system (H_2O−MeOH, 0−100%) to give fractions A−E. Fraction B (2.37% dry weight) was divided into subfractions B1−B6 by passage over a Cosmosil C_{18}-OPN column (10 × 100 cm), eluted with MeOH−H_2O (from 10% to 70%). Syringaldehyde (**1**, 1.27 g) was obtained as colorless needles (methanol) from subfraction B2 (0.28% dry weight) by MCI-gel CHP 20P column (8 × 100 cm) chromatography, using MeOH−-H_2O (from 0% to 40%) as the solvent system. Syringaldehyde (**1**) exhibited mp 109−111 °C (lit. 109−110 °C)[16] and gave spectroscopic data (^{1}H NMR, EIMS) comparable to literature values.[17,18]

Animal Model. Male Wistar rats between the ages of 8 and 10 weeks (200−250 g body weight) were obtained from the Animal Center of National Cheng Kung University Medical College. Diabetic rats were prepared by giving an intravenous injection of streptozotocin (STZ) (Sigma Chemical Co., St. Louis, MO, USA) (60 mg/kg) to the fasting rats.[19] Rats with plasma glucose concentrations of 20 mmol/L or greater in addition to polyuria and other diabetic features were considered as having type-1-like diabetes mellitus. All studies were carried out two weeks after the injection of STZ. Animal procedures were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University and were performed according to the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

Effect of Syringaldehyde (1) on Plasma Glucose. A solution of syringaldehyde (**1**, purity >98%) was prepared by dissolving it with normal saline to a concentration of 5 mg/mL. The fasting STZ-diabetic rats received an intravenous injection of **1** at the desired doses, and blood samples (0.1 mL) were collected under sodium pentobarbital anesthesia (30.0 mg/kg, ip) from the tail vein for measurement of plasma glucose. In preliminary experiments, **1** at 7.2 mg/kg was found to produce a maximal plasma glucose lowering action in STZ-diabetic rats 60 min after intravenous injection. Thus, the effect of **1** on plasma glucose was determined using blood samples collected after 60 min. Control rats received a similar injection of vehicle at the same volume. The antihyperglycemic activity was calculated as a decreased percentage of the initial value according to the formula $[(G_iG_t)/G_i] \times 100\%$, where G_i is the initial glucose level and G_t is the plasma glucose concentration after treatment.[19]

Intravenous Glucose Challenge Test. An intravenous glucose challenge test (IVGCT) was performed according to a method previously described.[8] Briefly, the basal plasma glucose concentration was obtained from samples from the tail vein of Wistar rats under anesthesia with sodium pentobarbital (30.0 mg/kg, ip) before the IVGCT. A solution of **1** at 7.2 mg/kg or the same volume of saline was injected into the tail vein of rats. Then, 30 min later, blood samples (0.1 mL) from the tail vein were drawn and indicated as 0 min. Next, a glucose dose of 60.0 mg/kg was injected through the femoral vein of the rats. Rats receiving a similar injection of saline at the same volume were used as control. Blood samples (0.1 mL) from the tail vein were drawn at 5, 10, 20, 30, 60, 90, and 120 min following the glucose injection for the measurement of the plasma glucose concentrations. Rats were maintained under anesthesia by pentobarbital throughout the procedure.

Determination of Plasma Glucose. Blood samples (0.1 mL) were collected using a chilled syringe containing 10 IU heparin from the tail vein of rats under anesthesia with sodium pentobarbital (30.0 mg/kg, ip). Blood samples were then centrifuged at 13 000 rpm for 3 min, and an aliquot (15 μL) of plasma was added to 1.5 mL of Glucose Kit Reagent (Biosystems S.A., Barcelona, Spain) and incubated at 37 °C in a water bath (Yamato-BT-25, Tokyo, Japan) for 10 min. The concentration of plasma glucose was then estimated via an analyzer (Quik-Lab, Ames, Miles Inc., Elkhart, IN, USA), and samples were run in duplicate.

Uptake of 2-NBDG into Rat L6 Myocytes. The glucose uptake test was carried out using 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) as a fluorescence indicator according to previous reports,[20,21] with some modifications used.

The L6 cells were cultured in a 10 cm dish for 48 h. The number of L6 cells in each assay was 1 × 10 ^{6} cells/mL. The medium was removed, and the cells were washed gently with phosphate buffer solution (PBS). Cells were detached from the dish by using a trypsin treatment, suspended in 0.2 mM 2-NBDG and **1** at the indicated concentration in PBS, and then incubated in a 37 °C water bath for 60 min in the dark. The cells were centrifuged (4 °C, 5000 × g, 10 min) to discard the supernatant. The pellet was washed three times with cold PBS and subjected to ice cooling. The pellet was suspended in 1 mL of PBS. The fluorescence intensity in cell suspension was evaluated using a fluorescence spectrofluorometer (Hitachi F-2000, Tokyo, Japan), with excitation and emission wavelengths of 488 and 520 nm, respectively. The intensity of fluorescence reflected the uptake of 2-NBDG in the cells.[22]

Measurement of Insulin Sensitivity in Rats. STZ-diabetic rats were used to investigate the response to exogenous insulin. These rats received an injection of long-acting human insulin at 1 IU/kg once daily to normalize the insulin sensitivity. Then, three days later, the

dx.doi.org/10.1021/np3003723 | *J. Nat. Prod.* 2012, 75, 1465−1468

STZ-diabetic rats were divided into two groups. One group received the intravenous injection of **1** at 7.2 mg/kg, three times daily, and another group received similar treatment with the same volume of saline. After three days of treatment, all rats were challenged with exogenous insulin. According to a previous method,[15] an intravenous insulin challenge test was performed by giving 0.1 to 1.0 IU/kg of short-acting human insulin to these STZ-diabetic rats. Blood samples (0.2 mL) from the femoral vein were drawn at 30 min following the intravenous insulin challenge test for the measurement of plasma glucose concentrations.

Statistical Analysis. The plasma glucose lowering activity was determined in fasted rats that received intravenous injection of **1** under anesthesia. Data are expressed as the means (SEM) for the number (n) of animals in the group as indicated in the figures. Repeated measures of analysis of variance (ANOVA) were used to analyze the changes in plasma glucose and other parameters. Dunnett range posthoc comparisons were used to determine the source of significant differences, where appropriate ($p < 0.05$ was considered statistically significant).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H NMR and ESIMS spectra of **1** and results are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +886-6-278-5123. Fax: +886-6-278-5178. E-mail: jtcheng@mail.cjcu.edu.tw.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We gratefully acknowledge the financial support from Council of Agriculture Executive in Taiwan for a grant numbered CAE101-1,2,2-S-a7. We also thank Miss M. J. Wang for her skillful technical assistance.

■ REFERENCES

(1) Mori, T.; Ogawa, S.; Cowely, A. W., Jr.; Ito, S. *Clin. Exp. Pharmacol. Physiol.* **2012**, *39*, 125−131.
(2) Grote, C. W.; Morris, J. K.; Ryals, J. M.; Geiger, P. C.; Wright, D. E. *Exp. Diabetes Res.* **2011**, *2011*, 212571.
(3) Liao, J. C. *Flora of Taiwan*; Editorial Committee of the Flora of Taiwan: Taipei, 1993; Vol. 3, p 743.
(4) Gan, W. S. *Manual of Medicinal Plants in Taiwan*; National Research Institute of Chinese Medicine: Taipei, 1965; Vol. 3, p 516.
(5) Wu, P. L.; Wu, T. S.; He, C. X.; Su, C. H.; Lee, K. H. *Chem. Pharm. Bull.* **2005**, *53*, 56−59.
(6) Wu, P. L.; Chuang, T. H.; He, C. X.; Wu, T. S. *Bioorg. Med. Chem.* **2004**, *12*, 2193−2197.
(7) Cheng, J. T.; Huang, C. C.; Liu, I. M.; Tzeng, T. F.; Chang, C. J. *Diabetes* **2006**, *55*, 819−825.
(8) Liu, I. M.; Chi, T. C.; Hsu, F. L.; Chen, C. F.; Cheng, J. T. *Planta Med.* **1999**, *65*, 712−714.
(9) Baron, A. D.; Brechtel, G.; Wallace, P.; Edelman, S. V. *Am. J. Physiol.* **1988**, *255*, E769−E774.
(10) Ziel, F. H.; Venkatesan, N.; Davidson, M. B. *Diabetes* **1988**, *37*, 885−890.
(11) Hollenbeck, C.; Reaven, G. M. *J. Clin. Endocrinol. Metab.* **1987**, *64*, 1169−1173.
(12) Zou, C.; Wang, Y.; Shen, Z. *J. Biochem. Biophys. Methods* **2005**, *64*, 207−215.
(13) Conte, C.; Fabbrini, E.; Kars, M.; Mittendorfer, B.; Patterson, B. W.; Klein, S. *Diabetes Care* **2012**, *35*, 1316−1321.
(14) Yokozawa, T.; Kim, H. J.; Yamabe, N.; Okamoto, T.; Cho, E. J. *J. Pharm. Pharmacol.* **2007**, *59*, 1271−1278.
(15) Chiu, Y. J.; Chung, H. H.; Yeh, C. H.; Cheng, J. T.; Lo, S. H. *Phytother. Res.* **2011**, *25*, 1306−1312.
(16) Pearl, I. A. *J. Am. Chem. Soc.* **1948**, *70*, 1746−1748.
(17) Tripathi, A. K.; Sama, J. K.; Taneja, S. C. *Indian J. Chem.* **2010**, *49B*, 379−381.
(18) Lee, C. K.; Chang, M. H. *J. Chin. Chem. Soc.* **2000**, *47*, 555−560.
(19) Huang, W. J.; Niu, H. S.; Lin, M. H.; Cheng, J. T.; Hsu, F. L. *J. Nat. Prod.* **2010**, *73*, 1170−1172.
(20) Alonso-Castro, A. J.; Zapata-Bustos, R.; Dominguez, F.; Garcia-Carranca, A.; Salazar-Olivo, L. A. *Phytomedicine* **2011**, *18*, 926−933.
(21) Alonso-Castro, A. J.; Salazar-Olivo, L. A. *J. Ethnopharmacol.* **2008**, *118*, 252−256.
(22) Bhaskar, J. J.; Salimath, P. V.; Nandini, C. D. *J. Sci. Food Agric.* **2011**, *91*, 1482−1487.