Hypoglycemic Effect and Antioxidant Potential of Kaempferol-3,7-*O*-(α)-dirhamnoside from *Bauhinia forficata* Leaves

Eliandra de Sousa,[†] Leila Zanatta,[†] Ilana Seifriz,[†,‡] Tânia Beatriz Creczynski-Pasa,[‡] Moacir Geraldo Pizzolatti,[†] Bruno Szpoganicz,[†] and Fátima Regina Mena Barreto Silva*[,§]

Departamento de Química, Centro de Ciências Físicas e Matemáticas, Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, and Departamento de Bioquímica, Centro de Ciências Biológicas, Campus Universitário, Bairro Trindade. Cx. Postal 5069, CEP 88040-970, Florianópolis, SC, Brazil

Received November 25, 2003

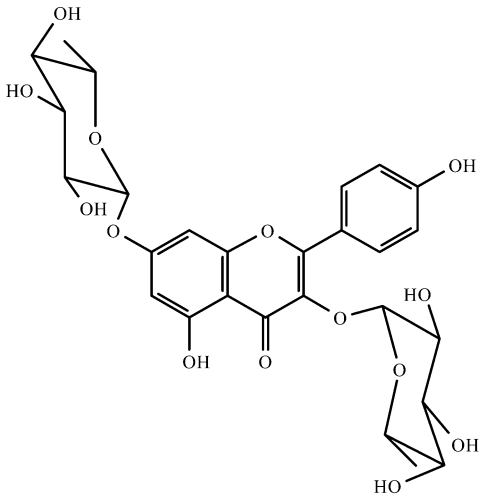
In vivo and in vitro treatments were carried out to investigate the effects of kaempferol-3,7-*O*-(α)-dirhamnoside (kaempferitrin), a major flavonoid compound of the *n*-butanol fraction from *Bauhinia forficata* leaves, on serum glucose levels, as well as its antioxidant potential. Oral administration of kaempferitrin led to a significant hypoglycemic effect in normal and in alloxan-induced diabetic rats. In normal rats, blood glucose lowering was observed only with the higher dose of kaempferitrin (200 mg/kg) at 1 h after treatment. However, the hypoglycemic effect of kaempferitrin in diabetic rats was evident at all doses tested (50, 100, and 200 mg/kg), and this profile was maintained throughout the period studied for both higher doses. Additionally, in glucose-fed hyperglycemic normal rats, the kaempferitrin failed to decrease blood glucose levels. In vitro antioxidant properties or action against reactive oxygen species of this compound was also evaluated. The compound showed high reactivity with 1,1-diphenyl-2-picryl hydrazyl (DPPH), IC_{50} of 84.0 ± 7.8 μM, inhibited myeloperoxidase activity with $K_{0.5}$ = 86 ± 9.9 μM, and decreased lipid peroxidation, induced by ascorbyl radical either in microsomes or in asolectin and phosphatidylcholine liposomes, with IC_{50}'s of 320 ± 14.1, 223 ± 8.3, and 112 ± 8.8 μM, respectively.

Diabetic disease is increasing rapidly and consumes vast amounts of resources in all countries. Both types of diabetes, mellitus and insulin resistant, are complex traits in which multiple gene effects and metabolic and environmental factors combine to contribute to the overall pathogenesis of these conditions. Type 1 diabetes results from β-cell destruction that is generally considered to be a multifactorial autoimmune process.[1] Plants used in folk medicine to treat diabetes mellitus represent a viable alternative for the control of this disease. Thus, the screening of plant compounds for an antidiabetic effect is mandatory.

Bauhinia forficata is one of the *Bauhinia* species most used as an antidiabetic herbal remedy in Brazil. This species is an arboreal plant of Asiatic origin that adapts well to the Brazilian climate.[2] Our earlier studies confirmed the hypoglycemic effect of the *n*-butanol fraction of *B. forficata* Link (Leguminosae) leaves in alloxan-induced diabetic rats.[3] However, in this fraction we have identified four different flavonoids with kaempferitrin (**1**) predominanting.[4]

Flavonoids are polyphenolic phytochemicals found in almost all types of plants from stems to roots to fruit. In *B. forficata* Link (Leguminosae), phytochemical and pharmacognostic investigations have demonstrated that kaempferitrin is present only in the leaves and absent from other parts of this plant.[5] Most studies have examined the effects of natural flavonoids in physiological and pathological conditions of glucose metabolism[3,6,7] as well as in lipid peroxidation.[8,9] Although not considered nutrients and thus essential for life, flavonoids have gained increasing recognition for their health-related qualities.

The relationship between reactive oxygen species (ROS) and many human diseases is well established. ROS have been implicated in over 200 disorders, including diabetes. It is still uncertain as to whether oxidative stress contributes to the origin of diabetes in humans; however, there is some evidence that in the diabetic or hyperglycemic state the antioxidant defenses are in a vulnerable condition.[10]



(1)

The present study was carried out to elucidate the acute effect of kaempferitrin on blood glucose lowering in normal, normal hyperglycemic, and alloxan-induced diabetic rats. Also, the in vitro antioxidant potential of kaempferitrin was analyzed by scavenging of free radicals, inhibition of a prooxidant enzyme, myeloperoxidase (MPO), and prevention of lipid peroxidation.

Results and Discussion

Table 1 shows the effect over time of 50, 100, and 200 mg/kg of kaempferitrin on blood glucose levels in normal fasted rats. Only the higher dose of kaempferitrin showed

* Corresponding author. E-mail: mena@mbox1.ufsc.br. Tel/Fax: +55-48.331.96.72/+55-48.225.20.79.
[†] Departamento de Química.
[‡] Departamento de Ciências Farmacêuticas.
[§] Departamento de Bioquímica.

10.1021/np030513u CCC: $27.50
Published on Web 04/21/2004

Table 1. Acute Effect of Kaempferitrin in Normal Rats[a]

	blood glucose level (mmol/L)				
time (h)	group I control	group II vehicle EtOH/H_2O	group III kaempferitrin 50 mg/kg	100 mg/kg	200 mg/kg
0	7.2 ± 0.13	6.1 ± 0.19	6.2 ± 0.23	6.0 ± 0.2	5.9 ± 0.67
1	6.5 ± 0.09	6.0 ± 0.15	5.5 ± 0.21	5.4 ± 0.35	4.5 ± 0.34[b]
2	6.4 ± 0.35	5.9 ± 0.23	5.9 ± 0.19	5.5 ± 0.34	5.0 ± 0.09
3	7.2 ± 0.22	6.4 ± 0.19	6.2 ± 0.13	6.2 ± 0.23	5.1 ± 0.27

[a] Values are expressed as mean ± SEM; $n = 6$ in duplicates for each group. [b] Statistically significant difference from the corresponding zero time value, $P \leq 0.05$.

Table 2. Acute Effect of Kaempferitrin in Diabetic Rats[a]

	blood glucose level (mmol/l)				
time (h)	group I diabetic	group II vehicle EtOH/H_2O	group III kaempferitrin 50 mg/kg	100 mg/kg	200 mg/kg
0	28.0 ± 0.15	28.4 ± 0.42	27.1 ± 0.45	30.2 ± 0.53	28.4 ± 0.41
1	27.0 ± 0.61	28.6 ± 0.77	23.5 ± 0.44[b]	24.3 ± 0.19[b]	22.4 ± 0.44[b]
2	29.8 ± 0.31	28.4 ± 0.37	25.2 ± 0.43[b]	24.0 ± 0.67[b]	22.2 ± 0.83[b]
3	29.0 ± 0.2	27.6 ± 1.41	25.3 ± 0.55	24.0 ± 0.72[b]	22.4 ± 0.36[b]

[a] Values are expressed as mean ± SEM; $n = 6$ in duplicates for each group. [b] Statistically significant difference from the corresponding zero time value, $P \leq 0.05$.

a hypoglycemic effect at 1 h after treatment as compared to the respective zero time.

The administration of alloxan (60 mg/kg) to the fasted rats markedly increased blood glucose levels as compared to both groups I in Tables 1 and 2. Also, in these groups, the vehicle did not show a significant difference in blood glucose levels. The oral administration with doses of 50, 100, and 200 mg/kg of kaempferitrin significantly reduced the hyperglycemia in diabetic rats with all doses studied when compared to the respective zero time. Both 100 and 200 mg/kg doses of the compound caused a significant lowering of blood glucose levels throughout the period studied, and the maximum hypoglycemic effect was at 2 h after kaempferitrin treatment (Table 2). It is, therefore, conceivable that the hypoglycemic principle(s) of kaempferitrin exert a prominent and direct effect in diabetic animals. In these groups, kaempferitrin cannot act indirectly by stimulating the release of insulin since alloxan treatment causes destruction of β cells. These results are in agreement with Russo et al.,[11] who observed no differences in insulin levels between normal and diabetic patients in response to either acutely or chronically administered *B. forficata* leaf extracts. However, the hypoglycemia produced by the higher dose of kaempferitrin in normal animals may be due to a slight potentiation of the insulin action.

Within 30 min of starting the glucose tolerance test, blood glucose concentration was significantly increased from its initial level. This hyperglycemia was maintained until 90 min and then started to decrease, returning to its initial value at 180 min (Table 3). The 100 mg/kg dose of kaempferitrin that produced a hypoglycemic effect in diabetic animals was tested against a glucose tolerance curve. The hyperglycemic time course (from 0 to 180 min) observed on the glucose tolerance curve was unchanged in the presence of kaempferitrin. Tolbutamide (100 mg/kg) administered to normal glucose-fed rats showed a typical hypoglycemic effect from 60 to 180 min, producing lower glucose levels than the control group.

It is generally accepted that the sulfonylureas, including tolbutamide, produce hypoglycemia in normal animals by stimulating the pancreatic β cells to release more insulin. These drugs, however, do not decrease blood glucose in alloxan-diabetic animals.[12] In glucose-fed hyperglycemic normal rats, tolbutamide (100 mg/kg) was able to return the glycemia to control levels (Table 3). Also, kaempferitrin had no influence over the glucose intestinal absorption. Considering together the results from Tables 1, 2, and 3 we propose that the kaempferitrin hypoglycemic mechanism involves an insulin-like effect on peripheral glucose consumption, inhibition of glucose reabsorption by the kidney, a delay in insulin catabolism (inhibition of insulinases), and/or potentiation of the effect of residual insulin.

Table 3. Acute Effect of Kaempferitrin on Oral Glucose Tolerance[a]

	blood glucose level (mmol/L)			
time (min)	group I control	group II glucose 4 g/kg	group III glucose + kaempferitrin (100 mg/kg)	group IV glucose + tolbutamide (100 mg/kg)
0	6.9 ± 0.23	7.1 ± 0.19	6.5 ± 0.75	6.7 ± 0.19
30	6.6 ± 0.42	8.8 ± 0.37[b]	9.5 ± 1.22[b]	7.8 ± 0.35[b]
60	6.5 ± 0.11	8.8 ± 0.29[b]	8.8 ± 0.44[b]	6.3 ± 0.13
90	6.6 ± 0.18	9.2 ± 0.3[b]	8.2 ± 0.3[b]	5.9 ± 0.32
180	7.0 ± 0.23	7.9 ± 0.13	6.7 ± 0.71	5.2 ± 0.29[b]

[a] Values are expressed as mean ± SEM; $n = 6$ in duplicates for each group. [b] Statistically significant difference from the corresponding normal group; $P \leq 0.05$.

Table 4. Inhibition of MPO Activity by Kaempferitrin and Action as DPPH Scavenger[a]

assay	$K_{0.5}$ (μM)	IC_{50} (μM)
DPPH reactivity		84.0 ± 7.8
MPO activity	86.0 ± 9.9	

[a] Values are expressed as mean ± SEM; $n = 3$ in duplicates for each group.

Another relevant point is that the hypoglycemic effect of kaempferitrin in diabetic rats was markedly more potent (around four times) when compared to the *n*-butanol *B. forficata* fraction using a similar protocol (see Silva et al.[3] and Table 2), thus emphasizing the importance of kaempferitrin in that fraction.

The antioxidant potential was first analyzed by observing the reactivity of the compound with DPPH, a stable radical (violet colored). As the radical picks up an electron in the presence of a free radical scavenger, the absorption decreases, and the resulting decolorization is stoichiometrically related to the number of electrons gained. The compound showed a very high reactivity with this radical with an IC_{50} of 84 ± 7.8 μM (Table 4).

Second, the effect of kaempferitrin on MPO activity, a pro-oxidant enzyme, was analyzed. The compound inhibited the MPO activity with a $K_{0.5}$ of 86 ± 9.9 μM (Table 4). One hundred percent of enzyme activity was determined as 4.0 ± 0.5 units/min/mg protein. Each point of the curve was monitored kinetically. The maximal enzyme inhibition was obtained with 400 μM of the compound.

As a third approach to analyze the antioxidant potential of the compound, the protection indices of lipid peroxidation for kaempferitrin in membranes of endoplasmic reticulum (microsomes), asolectin, and phosphatidylcholine liposomes were obtained (Table 5). Peroxidation was induced by ascorbyl and hydroxyl radicals as described in the Experimental Section. The compound protected against lipid peroxidation, although with a different potency for each of the membranes tested with both free radicals. Comparing the IC_{50}'s of kaempferitrin against lipid peroxidation induced by ascorbyl and hydroxyl, we observed that the antioxidant potential of the compound was much higher against the latter. The IC_{50}'s for ascorbyl radical were 320

Table 5. Effect of Kaempferitrin. Action against Lipid Peroxidation[a]

	ascorbyl radical IC_{50} (μM)		hydroxyl radical IC_{50} (μM)	
membrane	kaempferitrin	quercetin	kaempferitrin	quercetin
liver microsomes	320.0 ± 14.1	125 ± 5.0	85.0 ± 3.5	7.5 ± 0.7
ASO liposomes	223.0 ± 8.3	80 ± 5.7	n.d.	n.d.
PC liposomes	112.0 ± 8.8	80 ± 6.0	n.d.	n.d.

[a] Values are expressed as mean ± SEM; $n = 3$ in duplicates for each group. n.d. = not determined.

± 14.1, 223 ± 8.3, and 112 ± 8.8 μM, respectively, and for hydroxyl radical in liver microsomes it was 85 ± 3.5 μM (Table 5). The data shown in Table 5 indicate that the protective potential of the antioxidant is dependent on the lipid membrane constitution, as clearly shown through the IC_{50} of kaempferitrin against lipid peroxidation induced by ascorbyl in three different lipid bilayers. In parallel, as a positive control of lipoperoxidation protection, we measured the effect of quercetin, a flavonoid with known antioxidant properties, as described by Saija et al.[13] and references therein, also shown in Table 5. As expected, quercetin showed a strong action against lipid peroxidation with an IC_{50} in liver microsomes of 125.0 ± 5.0 μM and essentially the same value, 80 ± 6.0 μM, in ASO and PC liposomes. This indicates that kaempferitrin is only 2−3 times less potent than quercetin, a strong and well-characterized antioxidant.

Kaempferitrin presented an antioxidant potential at very low concentrations compared to the doses used in the in vivo analysis of its hyperglycemic effect: (i) it inhibited the lipid peroxidation in different membrane models, indirectly demonstrating that distinct tissues, containing membranes of distinct lipid composition, can be protected, which may be of particular importance in some pathological conditions, including diabetes; (ii) it also inhibited a pro-oxidant enzyme, myeloperoxidase, which plays a central role in infection and inflammation, converting hydrogen peroxide and chloride to hypochloric acid (HOCl), and although HOCl has an important role in killing microorganisms, it also possesses high reactivity and the ability to damage biomolecules by oxidation, both directly and in its decomposed form, chlorine gas (Cl_2);[10] (iii) it reacted with DPPH, as expected for flavonoids.[14]

The antioxidant potential of flavonoids is particularly important because they are easily available in the plant kingdom and are able to protect against stress phenomena such as lipid peroxidation, as shown for a variety of kaempferol derivatives having high antioxidant selectivity[9] and for quercetin, which was able to protect cells against H_2O_2-induced oxidative stress and calcium dysregulation.[15] Table 5 also shows that kaempferitrin has an antioxidant potential comparable to that of quercetin. The effectiveness of the protection against lipid peroxidation is probably because flavonoids, in general, are able to interact with lipid membranes, as shown by Saija et al.[13]

Since we were convinced regarding the importance of a drug treatment against multiple targets, in this case with hypoglycemic and antioxidant actions, and also considering that the model used in this work provokes an artificial oxidative stress in the animals,[10,16] we evaluated the antioxidant potential of kaempferitrin only in vitro.

We conclude that kaempferitrin, purified from the *n*-butanol fraction of *B. forficata* leaves, was effective in decreasing blood glucose levels in normal and diabetic rats after an acute treatment, and this effect was not related to reduced intestinal absorption. A further conclusion is that the fraction possesses a strong antioxidant potential, preventing the in vitro lipid peroxidation in different lipid bilayers, induced by hydroxyl and ascorbyl radicals, as well as acting as a free radical scavenger and inhibitor of pro-oxidant enzymes.

Since the *n*-butanol fraction contains at least three different glycosylated kaempferols, studies are underway, with isolated compounds, to elucidate the structure−function relationships.

Experimental Section

General Experimental Procedures. Tolbutamide, alloxan, quercetin, *o*-dianisidine, asolectin, l-α-phosphatidylcholine, cholate, deoxycholate, and ascorbate were purchased from Sigma Chemical Company (St. Louis, MO). Glucose, salts, H_2O_2, and solvents were purchased from Merck AG (Darmstadt, Germany); dialysis membrane was obtained from DIANORM GMBH, Munich, Germany.

Plant Material. The leaves of *B. forficata* Link were collected in the summer of 1999 at Orleans, Brazil, and identified by Prof. Daniel de Barcelos Falkenberg. A voucher specimen of the plant (FLOR-31271) has been deposited in the herbarium of the Botany Department at Universidade Federal de Santa Catarina, Florianópolis, Brazil.

Extraction and Isolation. The air-dried plant material was ground to a powder and extensively extracted with 8:1 EtOH−H_2O by maceration at room temperature. The aqueous alcoholic extract was concentrated to the desired level and stored at 4 °C for 24 h, before being filtered to withdraw a waxy residue and an aqueous suspension, which was successively extracted with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-BuOH fraction was subjected to column chromatography over silica gel 60, eluting with a gradient of EtOAc to MeOH, to give three fractions that showed a positive reaction to $FeCl_3$ on TLC. These fractions were then purified by elution through a further flash column chromatography step over silica gel to give kaempferitrin as a pale yellow amorphous powder.[4]

Experimental Animals. Male Wistar rat (160−190 g) were used. They were bred in our animal facility and housed in an air-conditioned room (approximately 24 °C) with controlled lighting (lights on from 06:00 to 20:00 h). All the animals were maintained with pelleted food (Nuvital, Nuvilab CR1, Curitiba, PR, Brazil) and tap water available ad libitum. Fasted animals were deprived of food for at least 16 h but allowed free access to water. All the animals were carefully monitored and maintained in accordance with ethical recommendations of the Brazilian Veterinary Medicine Council and the Brazilian College for Animal Experimentation.

Diabetes was induced by a single intravenous injection of 60 mg/kg of alloxan monohydrate (0.9% NaCl) in overnight fasted rats.[3] We assessed the diabetic state by measuring body weight and blood glucose levels at 3 days thereafter. Blood samples from the retro-orbital vein plexus were collected and centrifuged, and blood glucose serum levels were determined by using the glucose oxidase method.[17] A blood glucose range of 25−30.5 mmol/L was used for the experiment. All in vivo treatments were made orally by gavages.

Bioassay. Normal animals fasted overnight were randomly divided into three groups of six rats: group I served as control; group II received the vehicle, 0.5 mL of 1% EtOH−H_2O; group III received kaempferitrin at the following doses: 50, 100, and 200 mg/kg. Animals for which development of hyperglycemia was confirmed (around 95%) 72 h after the alloxan injection were randomized into three groups of six rats: group I, diabetic rats; group II diabetic rats that received vehicle; and group III, diabetic rats that received the kaempferitrin at the following doses: 50, 100, and 200 mg/kg. Serum glucose levels were measured at zero time (before receiving the compound) and 1, 2, and 3 h following the treatment.

Fasted rats were divided into four groups of six animals: group I, control rats; group II, rats receiving glucose (4 g/kg); group III, rats receiving glucose (4 g/kg) plus kaempferitrin (100 mg/kg); and group IV, rats receiving glucose (4 g/kg) plus tolbutamide (100 mg/kg). Blood samples were collected just

prior to and at 30, 60, 90, and 180 min after the glucose loading, and blood glucose levels were measured.

DPPH Reactivity. This assay was carried out with a medium containing an EtOH solution of 200 μM DPPH. The free radical exhibits a potent absorption at 515 nm, and the antioxidants decrease the amount of the free radical, as described by Vivot et al.[14]

Assay for Myeloperoxidase Activity. Biological material, such as rat lungs, was homogenized in an ice-cold phosphate buffer as previously described by Rao et al.[18] as a source of the enzyme. The enzyme activity was determined by the slope of the absorption curve set at 450 nm in the presence of *o*-dianisidine−2HCl and H_2O_2 and calculated on the basis of a standard curve obtained for a commercial enzyme.

Liver Microsomal Preparations. Liver microsomes were obtained from rat liver by differential centrifugation with calcium aggregation, according to Schenkman and Cinti.[19] The fractions obtained were immediately placed in a freezer at −70 °C for the later determination of antioxidant activity. The protein concentration was determined according to Lowry et al.[20]

Liposomes Preparation. Bilayer liposomes were prepared by cholate dialysis as described previously by Creczynski-Pasa and Gräber.[21] The method consists of the solubilization of the phospholipids with cholate and deoxycholate followed by a dialysis procedure. PC = l-α-phosphatidylcholine from fresh egg yolk; ASO = asolectin = L-α-phosphatidylcholine + another lipid such as phosphatidylinositol or phosphatidylethanolamine, from soybean.

Lipid Peroxidation Induced by Ascorbyl Radical and by Hydroxyl Radical. Lipid peroxidation was induced by the addition of 25 μM $FeSO_4$ and 500 μM ascorbate, for ascorbate radical, according to Sanz et al.[8] and by the addition of 25 μM $FeCl_2$, 2.8 mM H_2O_2, and 100 μM sodium ascorbate in a medium containing 2 mg microsomal protein/mL 0.1 M KH_2PO_4 at pH 7.4, for hydroxyl radical. When the lipid peroxidation was assayed in liposomes, the lipid concentration was 12.5 mg/mL, and in microsomes protein concentration was fixed at 2 mg/mL. The amount of TBARS was measured according to Bird and Draper[22] and calculated using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Statistical Analysis. Data were expressed as mean ± SEM. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Bonferroni post-test using the software GraphPAD InStat, Version 1.12[a]. Differences were considered to be significant at $P \leq 0.05$.

Acknowledgment. This work was supported by grants from Funpesquisa, Universidade Federal de Santa Catarina-UFSC, Conselho Nacional de Desenvolvimento Científico e Tecnológico-Brasil (CNPq), and Biobrás Bioquímica do Brasil S/A. E.d.S. thanks CAPES for fellowships to develop her master's degree in the Chemistry graduation program at UFSC, and L.Z. thanks the PIBIC-CNPq/UFSC fellowship program. I.S. held a doctoral studentship through the Chemistry post-graduate program at UFSC. The authors express their appreciation to Dr. G. P. Cuttle for assistance with the English correction of the manuscript.

References and Notes

(1) Verge, C. F.; Gianani, R.; Yu, L.; Pietropaolo, M.; Smith, T.; Jackson, R. A.; Soelder, J. S.; Eisenbarth, G. S. *Diabetes* **1995**, *44*, 1176−1179.
(2) Miyake, E. T.; Akisue, G.; Akisue, M. K. *Rev. Bras. Farm.* **1986**, *1*, 56−68.
(3) Silva, F. R. M. B.; Szpoganicz, B.; Pizzolatti, M. G.; Willrich, M. A. V.; De Sousa, E. *J. Ethnopharmacol.* **2002**, *83*, 33−37.
(4) Pizzolatti, M. G.; Cunha, J. A.; Szpoganicz, B.; De Sousa, E.; Braz-Filho, R.; Schripsema, J. *Quím. Nova* **2003**, *26*, 466−469.
(5) Da Silva, K. L.; Biavatti, M. W.; Leite, S. N.; Yunes, R. A.; Monache, F. D.; Cechinel-Filho, V. *Z. Naturforsch.* **2000**, *55*, 478−480.
(6) Zarzuelo, A.; Jiménez, I.; Gámez, M. J.; Utrilla, P.; Fernandez, I.; Torres, M. I.; Osuma, I. *Life Sci.* **1996**, *58*, 2311−2316.
(7) Pepato, M. T.; Keller, E. H.; Baviera, A. M.; Kettelhut, I. C.; Vendramini, R. C.; Brunetti, I. L. *J. Ethnopharmacol.* **2002**, *81*, 191−197.
(8) Sanz, M. J.; Ferrandiz, M. L.; Cejudo, M.; Terencio, M. C.; Gil, B.; Bustos, G.; Ubeda, A.; Gunasegaran, R.; Alcaraz, M. J. *Xenobiotica* **1994**, *24*, 589−599.
(9) Cos, P.; Calomme, M.; Sindambiwe, J. B.; De Bruyne, T.; Cimanga, K.; Pieters, L.; Vlietinck, A. J.; Vande Berghe, D. *Planta Med.* **2001**, *67*, 515−519.
(10) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*, 3rd ed., Oxford University Press: New York, 2000.
(11) Russo, E. M. K.; Reichelt, A. J.; De-Sá, J. R.; Furnaletto, R. P.; Moisés, R. C. S.; Kasamatsu, T. S.; Chacra, A. R. *Braz. J. Med. Biol. Res.* **1990**, *23*, 11−20.
(12) Goth, M. D. *Medical Pharmacology*, 9th ed.; C.V. Mosby: Saint Louis, MO, 1985; pp 471−480.
(13) Saija, A.; Sclese, M.; Lanza, M.; Marzullo, D.; Bonina, F.; Castelli, F. *Free Radical Biol. Med.* **1995**, *9*, 481−486.
(14) Vivot, E.; Munoz, J. D.; Cruanes, M. C.; Cruanes, M. J.; Tapia, A.; Hirschmann, G. S.; Martinez, E.; Di Sapio, O.; Gattuso, M.; Zacchino, S. *J. Ethnopharmacol.* **2001**, *76*, 65−71.
(15) Wang, H.; Joseph, J. *Free Radical Biol. Med.* **1999**, *87*, 683−694.
(16) Rerup, C. C. *Pharmacol. Rev.* **1970**, *22* 485−519.
(17) Varley, H.; Gowenlock, A. H.; Bell, M. In *Practical Biochemistry*, 5th ed.; Heinemann I. W., Ed.; London, 1976; Vol. 1, pp 389−391.
(18) Rao, T. S.; Yu, S. S.; Djuric, S. W.; Isakson, P. C. *J. Lipid Mediators Cell Signal.* **1994**, *10*, 213−228.
(19) Schenkman, J. B.; Cinti, D. L. *Methods Enzymol.* **1978**, *52*, 83−89.
(20) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265−275.
(21) Creczynski-Pasa, T. B.; Gräber, P. *FEBS Lett.* **1994**, *350*, 195−198.
(22) Bird, R. P.; Draper, A. H. *Methods Enzymol.* **1984**, *105*, 295−305.

NP030513U