Hepatoprotective Constituents of the Seeds of *Combretum quadrangulare*

I Ketut ADNYANA,^a Yasuhiro TEZUKA,^a Arjun Hari BANSKOTA,^a Kim Qui TRAN,^b and Shigetoshi KADOTA^{*,a}

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University,^a 2630 Sugitani, Toyama 930-0194, Japan and National University Ho Chi Minh City,^b Ho Chi Minh City, Vietnam. Received May 29, 2000; accepted July 22, 2000

Hepatoprotective effect of MeOH, MeOH–H₂O (1 : 1) and H₂O extracts of *Combretum quadrangulare* seeds were examined on D-galactosamine (D-GalN)/tumor necrosis factor- α (TNF- α)-induced cell death in primary cultured mouse hepatocytes. The MeOH extract showed the strongest inhibitory effect on D-GalN/TNF- α -induced cell death (IC₅₀, 56.4 μ g/ml). Moreover, the MeOH extract also significantly lowered the serum glutamic pyruvic transaminase (sGPT) level on D-GalN/lipopolysaccharide (LPS)-induced liver injury in mice. Bioguided separation of the MeOH extract led to the isolation of 38 compounds of various classes including triterpene glucosides, lignans and catechin derivatives. Among the isolated triterpene glucosides, lupane-type (1–3; IC₅₀, 63.1, 59.8 and 76.2 μ M, respectively) and ursane-type (11, mixture of 12 and 14; IC₅₀, 30.2 and 34.6 μ M, respectively) compounds exhibited strong hepatoprotective activity. 1-O-Galloyl-6-O-(4-hydroxy-3,5-dimethoxy)benzoyl- β -D-glucose (26; IC₅₀, 7.2 μ M), methyl gallate (28; IC₅₀, 19.9 μ M), and (–)-epicatechin (31; IC₅₀, 71.2 μ M) also had a potent hepatoprotective effect on D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes.

Key words *Combretum quadrangulare*; Vietnamese medicinal plant; hepatoprotective effect; triterpene glucoside; catechin; 1-O-galloyl-6-O-(4-hydroxy-3,5-dimethoxy) benzoyl- β -D-glucose

Combretum (C.) species (Combretaceae) are widely used in folk medicine for the treatment of hepatitis, malaria, respiratory infections and cancer in different parts of Asia and Africa.¹⁾ *C. quadrangulare* is a tree indigenous to eastern Asia and is commonly known as “Tram bau” in Vietnam. The seeds, leaves and stem bark of the plant have been used in Vietnamese folk medicine as an antipyretic, antidiarrheal, antihepatitis and anthelmintic agent.²⁾ The seeds are administered orally together with ripe bananas as an anthelmintic for ascariasis and oxyuriasis,²⁾ but chemical investigations have been undertaken mainly on the leaves and flowers of *C. quadrangulare*.^{3,4)} In our continuing study on hepatoprotective natural products,^{4,5)} we found that a MeOH extract of the seeds of *C. quadrangulare* exhibits potent hepatoprotective activity on D-galactosamine (D-GalN)/tumor necrosis factor- α (TNF- α)-induced cell death in primary cultured mouse hepatocytes. Separation of the MeOH extract with column chromatography afforded thirty-eight compounds including eleven new (1–5, 11, 13, 15–17, 24) and eleven known (6–9, 12, 14, 18–22) triterpene glucosides, two known triterpenes (10, 23), β -sitosterol glucoside (25), a new (26) and two known (27, 28) gallic acid derivatives, four catechin derivatives (29–32), vitexin (33) and five lignans (34–38).⁶⁾ The hepatoprotective activity of the isolated compounds was further tested on D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes. In this paper, we report the hepatoprotective activity of extracts, fractions and constituents of the seeds of *C. quadrangulare*.

MATERIALS AND METHODS

Plant Material Seeds of *C. quadrangulare* Kurz were collected in Ho Chi Minh City, Vietnam in January 1998. A voucher sample (TMPW 19000) is preserved in the Museum for Materia Medica, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan.

Chemicals D-GalN and collagenase were purchased from Wako Pure Chemicals Industry (Osaka, Japan), and

lipopolysaccharide (LPS, *Escherichia coli* 055:B5) was from Difco Laboratories (Detroit, MI, U.S.A.). Mouse recombinant TNF- α , silibinin, William's E medium, bovine serum albumin (BSA), insulin, dexamethasone, penicillin G, streptomycin and 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.) and ethyleneglycol-O,O-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA) was from Fluka Chemie (Switzerland). Heat-inactivated calf serum and Hanks' balanced salt solution (HBSS) were obtained from Gibco BRL Products (Gaithersburg, MD, U.S.A.), and Falcon Primaria surface-modified polystyrene culture plates with 96 wells were from Becton Dickinson (Lincoln Park, NJ, U.S.A.).

Animals Male ddY mice, 6-weeks-old (30–34 g), were used for the D-GalN/LPS-induced liver injury model. All the animals were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan) and were maintained on a 12 h light/dark cycle in a temperature and humidity controlled room. The animals were fed with a laboratory pellet chow (CE-2; CLEA Japan Inc., Tokyo, Japan) and water *ad libitum* before the experiment.

D-GalN/LPS-Induced Liver Injury in Mice Liver injury was introduced by D-GalN/LPS in mice according to the method of Tiegs *et al.*⁷⁾ In each group 6 mice were used. After 12 h fasting, mice were given an i.p. injection of D-GalN (700 mg/kg) and LPS (20 μ g/kg). The MeOH extract of *C. quadrangulare* seeds was given *p.o.* of 100 or 500 mg/kg/d for one week before D-GalN/LPS challenge. Serum glutamic pyruvic transaminase (sGPT) levels were examined at 8 h post-injection of D-GalN/LPS to evaluate the extent of liver damage. The blood of three dead mice of the control group and one mouse from the 500 mg/kg MeOH extract-treated group (after 7 h of D-GalN/LPS challenge) was taken from the heart and tested to the experimental model.

TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes Mouse liver parenchymal cells were isolated according to the procedure described previously by

* To whom correspondence should be addressed. e-mail: kadota@ms.toyama-mpu.ac.jp

Chart 1. Structures of Compounds Isolated from the H₂O-fraction of MeOH Extract of *C. quadrangulare* Seeds

Seglen.⁸⁾ In brief, the liver was perfused with Ca²⁺-free HBSS containing 0.5% BSA and 5 μ M EGTA, then recirculated with collagenase solution composed of Ca²⁺-free HBSS, 0.075% collagenase, 4 μ M CaCl₂ and 0.005% trypsin inhibitor. Isolated hepatocytes were cultured in William's E medium supplemented with 10% calf serum, 100 IU/ml peni-

cillin G, 100 μ g/ml streptomycin, 100 μ M dexamethasone and 50 ng/ml insulin and incubated in a 96-well plastic plate (1.0×10^5 cells/well). After 2-h pre-incubation, the medium was replaced with fresh medium containing D-GalN (1.0 mM) and test specimens at various concentrations. Thirty minutes later, TNF- α (100 ng/ml) was added to each well, and the he-

Table 1. Hepatoprotective Effect of Different Extracts and Fractions of *C. quadrangulare* Seeds against D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes

Extract/fraction	Concentration ($\mu\text{g/ml}$)	Cell viability (% of normal)	Inhibition (% of control)	IC ₅₀ ($\mu\text{g/ml}$)
Normal		100.0 $\pm$ 11.7		
Control		41.5 $\pm$ 5.6		
MeOH extract	100	76.4 $\pm$ 8.3**	84.2	56.4
	50	59.7 $\pm$ 7.9**	44.0	
	25	52.4 $\pm$ 6.7*	26.4	
MeOH-H ₂ O extract	100	46.9 $\pm$ 3.1		
	50	44.6 $\pm$ 1.4		
	25	46.9 $\pm$ 5.0		
H ₂ O extract	100	48.1 $\pm$ 3.8		
	50	41.9 $\pm$ 6.5		
	25	44.4 $\pm$ 2.1		
H ₂ O-soluble fraction	100	84.2 $\pm$ 7.3**	103.0	39.3
	50	63.3 $\pm$ 9.2**	52.8	
	25	59.0 $\pm$ 7.5**	42.2	
MeOH-soluble fraction	100	77.7 $\pm$ 10.6**	87.4	42.1
	50	66.6 $\pm$ 5.7**	60.6	
	25	51.9 $\pm$ 3.2**	25.1	

Results are expressed as means $\pm$ S.D. ($n=4$; for normal and control, $n=8$). * $p<0.05$, ** $p<0.01$, significantly different from control.

Fig. 1. Effect on D-GalN/LPS-Induced Liver Injury in Mice

The MeOH extract of seeds of *C. quadrangulare* (Cq500=500 mg/kg and Cq100=100 mg/kg) was *p.o.* administered to ddY mice (male, 6-weeks-old) once a day for one week. N: untreated mice; C: mice administered vehicle. Blood was collected for measurement at 8 h after D-GalN/LPS injection. Data are expressed as means $\pm$ S.E., $n=6$. * $p<0.05$, significantly different from control group.

patocyte viability was assessed 18 h thereafter by the MTT colorimetric reaction. MTT solution (0.05%, 100 μl) was added to each well after aspiration off the culture medium, and the mixture was incubated for 2 h. The formed formazan was dissolved in 100 μl DMSO and the amount was measured spectrophotometrically at 550 nm using a Perkin-Elmer HTS-7000 plate reader.

Statistical Analysis All values were expressed as means $\pm$ S.E. or S.D. Student's *t*-test for unpaired observations between control and tested samples was carried out to identify statistical differences; *p* values less than 0.05 were considered significantly different.

RESULTS AND DISCUSSION

In the majority of cases human hepatitis is triggered by an immunological response to viral infection, endotoxin or autoantigen,⁹⁾ and thus the liver injury model representing an immunological liver failure gives a more promising experimental basis for the evaluation of hepatoprotective agents. In the D-GalN/LPS-induced liver failure model in mice, TNF- α was secreted from LPS-stimulated macrophages and strongly induced hepatocyte apoptosis. The apoptosis triggers an in-

Table 2. Hepatoprotective Effect of Column Chromatography-Divided Fractions of H₂O-Soluble Fraction against D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes

Fraction	Concentration ($\mu\text{g/ml}$)	Cell viability (% of normal)	Inhibition (% of control)
Normal		100.0 $\pm$ 13.5	
Control		40.0 $\pm$ 3.2	
Fraction 1	100	53.6 $\pm$ 9.8*	34.0
	50	47.6 $\pm$ 7.3	19.0
Fraction 2	100	51.5 $\pm$ 1.5**	28.8
	50	50.1 $\pm$ 4.6**	25.3
Fraction 3	100	56.2 $\pm$ 3.9**	40.5
	50	48.0 $\pm$ 9.3*	20.0
Fraction 4	100	52.0 $\pm$ 3.2**	30.0
	50	55.2 $\pm$ 2.5**	38.0
Fraction 5	100	80.1 $\pm$ 5.5**	100.3
	50	71.1 $\pm$ 8.9**	77.8
Fraction 6	100	55.3 $\pm$ 7.0**	38.3
	50	40.7 $\pm$ 1.5	1.8
Fraction 7	100	60.6 $\pm$ 5.8**	51.5
	50	52.8 $\pm$ 12.7*	32.0

Results are expressed as means $\pm$ S.D. ($n=4$; for normal and control, $n=8$). * $p<0.05$, ** $p<0.01$, significantly different from control.

flammatory reaction and massive hepatocyte necrosis.¹⁰⁾ Thus, D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes is used as an *in vitro* model of immunological liver failure.

Using the D-GalN/TNF- α *in vitro* model, the hepatoprotective activity of the MeOH, MeOH-H₂O and H₂O extracts of seeds of *C. quadrangulare* were examined. The MeOH extract and its H₂O- and MeOH-soluble fractions showed hepatoprotective activity concentration-dependently with an IC₅₀ value of 56.4, 39.3, and 42.1 $\mu\text{g/ml}$, respectively (Table 1). Further, the hepatoprotective activity of the MeOH extract was tested in the D-GalN/LPS-induced liver injury model, and the *p.o.* administration of the MeOH extract at doses of 100 and 500 mg/kg for one week before D-GalN/LPS challenge significantly inhibited sGPT elevation, an indicator of liver injury, by 81.4 and 89.2%, respectively (Fig. 1). Seven fractions (frs. 1—7) obtained from the H₂O-

Table 3. Hepatoprotective Effect of Compounds Isolated from the H₂O-Soluble Fraction of MeOH Extract of *C. quadrangulare* Seeds against D-GalN/TNF- α -Induced Cell Death in Primary Cultured Mouse Hepatocytes

Compds.	Conc. (μ M)	Cell viability (% of normal)	% Inh ^{a)}	IC ₅₀ (μ M)	Compds.	Conc. (μ M)	Cell viability (% of normal)	% Inh ^{a)}	IC ₅₀ (μ M)
Normal 1		100.0 $\pm$ 5.6			Normal 4		100.0 $\pm$ 7.5		
Control 1		53.2 $\pm$ 4.3			Control 4		51.0 $\pm$ 3.8		
Silibinin	100	105.7 $\pm$ 1.9**	98.7	33.6	7	200	45.1 $\pm$ 3.1		
	50	86.7 $\pm$ 1.4**	63.0			100	42.4 $\pm$ 4.3		
	25	74.3 $\pm$ 4.7**	39.7			50	47.4 $\pm$ 2.7		
1	100	88.1 $\pm$ 12.4**	65.6	63.1	8	200	60.4 $\pm$ 2.6**	18.4	
	50	72.4 $\pm$ 8.8**	36.0			100	56.7 $\pm$ 5.7	11.3	
	25	69.8 $\pm$ 5.8**	31.2			50	54.4 $\pm$ 1.4	6.8	
2	100	93.2 $\pm$ 11.5**	75.1	59.8	10	200	56.5 $\pm$ 2.4*	10.7	
	50	70.5 $\pm$ 5.2**	32.5			100	54.2 $\pm$ 3.0	6.2	
	25	65.2 $\pm$ 3.0**	22.1			50	54.2 $\pm$ 4.1	6.2	
4	200	36.1 $\pm$ 1.0			14	200	58.7 $\pm$ 4.5*	15.0	
	100	34.9 $\pm$ 1.6				100	63.8 $\pm$ 7.5*	25.0	
	50	37.0 $\pm$ 1.5				50	55.2 $\pm$ 3.8	8.3	
11	100	95.6 $\pm$ 1.9**	79.7	30.2	19	200	67.7 $\pm$ 10.3**	32.7	
	50	90.1 $\pm$ 5.8**	69.4			100	67.1 $\pm$ 2.6**	31.6	
	25	85.7 $\pm$ 0.9**	61.1			50	56.2 $\pm$ 3.2	10.2	
	12.5	58.6 $\pm$ 0.5	10.2		25	200	44.4 $\pm$ 0.8		
17	200	72.9 $\pm$ 3.7**	37.0			100	43.4 $\pm$ 2.6		
	100	63.4 $\pm$ 2.5*	19.2			50	43.0 $\pm$ 2.5		
	50	58.1 $\pm$ 3.6	9.2		Normal 5		100.0 $\pm$ 5.4		
Normal 2		100.0 $\pm$ 12.3			Control 5		50.9 $\pm$ 4.2		
Control 2		40.1 $\pm$ 6.3			23	200	74.4 $\pm$ 2.6**	46.2	
5	100	45.6 $\pm$ 2.7	14.0			100	69.7 $\pm$ 2.6**	37.0	
	50	44.7 $\pm$ 2.9	11.8			50	67.6 $\pm$ 0.8**	31.8	
	25	44.5 $\pm$ 3.7	11.3		27	200	54.5 $\pm$ 3.6	6.6	
13	100	51.0 $\pm$ 9.7*	27.5			100	54.3 $\pm$ 4.0	6.6	
	50	46.7 $\pm$ 3.5	16.8			50	55.1 $\pm$ 3.0	8.3	
	25	47.2 $\pm$ 4.0	17.7		31	200	101.2 $\pm$ 11.2**	98.9	71.2
15	100	49.6 $\pm$ 2.8*	24.0			100	80.5 $\pm$ 4.0**	58.1	
	50	45.0 $\pm$ 5.1	12.5			50	70.2 $\pm$ 5.2**	38.0	
	25	42.1 $\pm$ 4.3	5.3		30	200	67.9 $\pm$ 3.0**	33.5	
16	100	29.7 $\pm$ 0.8				100	66.3 $\pm$ 3.3**	30.3	
	50	26.2 $\pm$ 1.4				50	60.9 $\pm$ 1.6*	19.7	
	25	29.0 $\pm$ 0.6			34	200	67.0 $\pm$ 1.2**	31.6	
24	100	30.4 $\pm$ 1.3				100	70.5 $\pm$ 3.6**	38.5	
	50	31.8 $\pm$ 3.5				50	60.4 $\pm$ 2.7*	18.7	
	25	34.1 $\pm$ 4.6			35	200	58.8 $\pm$ 2.6	15.6	
Normal 3		100.0 $\pm$ 9.1				100	56.4 $\pm$ 1.5	10.8	
Control 3		49.5 $\pm$ 4.6				50	54.1 $\pm$ 2.7	6.2	
18	100	50.8 $\pm$ 7.7	2.6		36	200	44.6 $\pm$ 4.2		
	50	45.7 $\pm$ 5.4				100	47.5 $\pm$ 6.8		
	25	48.3 $\pm$ 4.3				50	43.0 $\pm$ 2.5		
20	100	60.8 $\pm$ 1.5**	22.8		Normal 6		100.0 $\pm$ 9.4		
	50	63.1 $\pm$ 5.3**	27.5		Control 6		43.6 $\pm$ 3.7		
	25	53.3 $\pm$ 6.7	7.7		3	100	70.0 $\pm$ 2.2**	60.6	76.2
	100	28.1 $\pm$ 2.9				50	57.9 $\pm$ 2.8**	32.8	
22	50	33.2 $\pm$ 2.7				25	50.8 $\pm$ 2.9**	16.5	
	25	30.2 $\pm$ 0.9			6	100	55.2 $\pm$ 5.8**	26.6	
26	50	97.2 $\pm$ 3.0**	96.5	7.2		50	46.9 $\pm$ 2.9	7.6	
	25	98.7 $\pm$ 4.0**	99.4			25	49.7 $\pm$ 3.7	14.0	
	12.5	79.5 $\pm$ 2.7**	60.5		9	100	77.6 $\pm$ 2.7**	77.9	55.7
	1	43.9 $\pm$ 2.2	-11.3			50	59.3 $\pm$ 2.0**	36.1	
28	100	98.6 $\pm$ 3.9**	99.3	19.9		25	53.3 $\pm$ 2.2**	23.5	
	50	95.6 $\pm$ 4.8**	93.3		12+14 ^{b)}	100	99.2 $\pm$ 9.0**	127.5	34.6
	25	82.1 $\pm$ 4.0**	65.9			50	70.5 $\pm$ 6.0**	61.7	
	12.5	61.9 $\pm$ 1.1**	25.1			25	58.9 $\pm$ 4.3**	35.2	
29	100	60.4 $\pm$ 7.5**	22.2		21	100	41.3 $\pm$ 5.5	-5.3	
	50	47.7 $\pm$ 3.0				50	49.9 $\pm$ 4.0	14.4	
	25	49.8 $\pm$ 4.4				25	47.9 $\pm$ 4.6	9.9	
32	100	56.1 $\pm$ 2.6*	13.3		37+38 ^{c)}	100	60.2 $\pm$ 3.3**	38.1	
	50	60.0 $\pm$ 5.7**	21.2			50	57.8 $\pm$ 2.2**	32.5	
	25	54.3 $\pm$ 2.5	9.7			25	51.0 $\pm$ 1.6*	23.7	
33	100	63.7 $\pm$ 2.3**	28.6						
	50	58.0 $\pm$ 5.8*	17.2						
	25	50.8 $\pm$ 2.2	2.7						

Results are expressed as means $\pm$ S.D. (n=4; for normal and control, n=8). **p<0.01, *p<0.05, significantly different from control. a) % of inhibition was calculated by the formula [(% cell viability of sample - % of cell viability control) / % of control] $\times$ 100. b), c) Mixture of the 3 : 2 and 1 : 1 ratio, respectively.

soluble fraction by Sephadex LH-20 column chromatography exhibited significant hepatoprotective activities with various intensities (Table 2). Fractions 2—7 were then separated to give thirty-eight compounds including twenty-four triterpenoids (1—24).

All isolated compounds were tested on the D-GalN/TNF- α *in vitro* model, and the majority of them exhibited hepatoprotective activity (Table 3). Considering the type of triterpenes, all the lupane-type triterpene glucosides (1—3) possessed potent hepatoprotective activity with an IC₅₀ value of 63.1, 59.8, and 76.2 μ M, respectively. Most of the oleanane-type compounds (17, 19, 20, 23) also possessed mild hepatoprotective activity, while only half of ursane-type triterpene glucosides (6, 8, 9, 11, 14, a mixture of 12 and 14) showed hepatoprotective activity in the same experimental model. Quadranoside V (11), an ursane-type triterpene glucoside with a conjugated double bond, exhibited potent hepatoprotective activity with an IC₅₀ value of 30.2 μ M, whereas the ursane-type triterpene glucosides (15, 16) bearing double bonds at C-12 and C-19(29) did not have any protective effect. This might suggest that the conjugated double bond plays an important role in the hepatoprotection, and this was supported by the fact that 13 and 14, having the double bonds at C-12 and C-19(20), possessed mild hepatoprotective activity. The hepatoprotective effect of 11 and the mixture of 12 and 14 was stronger than or similar to the clinically-used positive control, silibinin (IC₅₀, 33.6 μ M). Ursane-type triterpene glucosides (6, 8, 9) with only one double bond at C-12 also showed various extents of hepatoprotective activity. Among them, 9 possessed the strongest hepatoprotective activity with an IC₅₀ value of 55.7 μ M (Table 3).

Among the remaining compounds, 26, 28 and 31 showed potent hepatoprotective activity with an IC₅₀ value of 7.2, 19.9, and 71.2 μ M, respectively, and compounds 29, 30, 32 and 33 showed mild hepatoprotective activity, while compounds 25 and 27 did not show any. Catechin and its derivatives were reported to have antioxidative activity on *tert*-butyl hydroperoxide-induced lipid peroxidation of rat liver homogenates,¹¹⁾ and on iron-increased lipid peroxidation of hepatocytes cultures.¹²⁾ On the other hand, increasing evidence indicates that the total cellular balance between reactive oxygen species and antioxidants possibly affects the signaling mechanisms of various responses to TNF- α .¹³⁾ Thus, the hepatoprotective activity of these phenolic compounds could be due to their antioxidative activity. Among the isolated lignans, in the other hand, (–)-lyoniresinol (34) and a mixture of 37 and 38 showed significant hepatoprotective effect and nudiposide (38) was previously reported to have hepatoprotective activity on D-GalN-cytotoxicity in primary cultured rat hepatocytes.¹⁴⁾

In conclusion, lupane-type triterpenes possessed potent protective activity against D-GalN/TNF- α -induced cell death in primary cultured mouse hepatocytes, and the conjugated

double bond within ursane-type triterpene glucosides seemed to enhance the hepatoprotective activity. Among the phenolic compounds, catechin and gallic acid derivatives and some of the lignans also had significant hepatoprotective effect. The hepatoprotective activity of the H₂O-soluble fraction of the MeOH extract of seeds of *C. quadrangulare* would be due to these constituents.

Acknowledgment This work was supported in part by a Grant-in-Aid for International Scientific Research (No. 09041177) from the Ministry of Education, Science, Sports, and Culture, Tokyo, Japan.

REFERENCES

- 1) Pettit G. R., Singh S. B., Boyd M. R., Hamel E., Pettit R. K., Schmidt J. M., Hogan F., *J. Med. Chem.*, **38**, 1666—1672 (1995).
- 2) Tran K. (ed.), "Medicinal Plants in Vietnam," WHO Regional Office for the Western Pacific Manila, and Institute of Materia Medica Hanoi, Science and Technology Publishing House, Hanoi, 1990, p. 119.
- 3) a) Castleden I. R., Hall S. R., Nimgirawath S., Thadaniti S., White A. H., *Aust. J. Chem.*, **38**, 1177—1185 (1985); b) Ganzera M., Ellmerer E. P., Stuppner H., *Phytochemistry*, **49**, 835—838 (1998).
- 4) a) Banskota A. H., Tezuka Y., Phung L. K., Tran K. Q., Saiki I., Miwa Y., Taga T., Kadota S., *Bioorg. Med. Chem. Lett.*, **8**, 3519—3524 (1998); b) Banskota A. H., Tezuka Y., Tran K. Q., Tanaka K., Saiki I., Kadota S., *J. Nat. Prod.*, **63**, 57—64 (2000); c) *Idem*, *Chem. Pharm. Bull.*, **48**, 496—504 (2000).
- 5) a) Xiong Q., Hase K., Tezuka Y., Tani T., Namba T., Kadota S., *Planta Med.*, **64**, 120—125 (1998); b) Xiong Q., Hase K., Tezuka Y., Namba T., Kadota S., *Life Sci.*, **65**, 421—430 (1999); c) Banskota A. H., Tezuka Y., Adnyana I. K., Xiong Q., Hase K., Tran K. Q., Tanaka K., Saiki I., Kadota S., *Biol. Pharm. Bull.*, **23**, 456—460 (2000); d) Banskota A. H., Tezuka Y., Adnyana I. K., Midorikawa K., Matsushige K., Message D., Huertas A. A. G., Kadota S., *J. Ethnopharmacol.*, **72**, 239—246 (2000).
- 6) a) Adnyana I. K., Tezuka Y., Banskota A. H., Xiong Q., Tran K. Q., Kadota S., *J. Nat. Prod.*, **63**, 496—500 (2000); b) Adnyana I. K., Tezuka Y., Awale S., Banskota A. H., Tran K. Q., Kadota S., *Chem. Pharm. Bull.*, **48**, 1114—1120 (2000); c) *Idem*, *Planta Med.*, in press.
- 7) Tiegs G., Wolter M., Wendel A., *Biochem. Pharmacol.*, **38**, 627—631 (1989).
- 8) Seglen P. O., *Methods Cell Biol.*, **13**, 29—83 (1976).
- 9) Dudley F. J., Fox R. A., *Lancet*, **I**, 723—726 (1968); Almeida J. D., Waterson A. P., *Lancet*, **II**, 983—986 (1969).
- 10) a) Leist M., Gantner F., Bohlinger I., Yiegs G., Germann P. G., Wendel A., *Am. J. Pathol.*, **146**, 1220—1234 (1995); b) Jaeschke H., Fisher M. A., Lawson J. A., Simmons C. A., Farhood A., Jones D. A., *J. Immunol.*, **160**, 3480—3486 (1998).
- 11) Yoshino K., Hara Y., Sano M., Tomita I., *Biol. Pharm. Bull.*, **17**, 146—149 (1994).
- 12) Morel I., Lescoat G., Cogrel P., Sergent O., Pasdeloup N., Brissot P., Cillard P., Cillard J., *Biochem. Pharmacol.*, **45**, 13—19 (1993).
- 13) a) Slater A. F., Nobel C. S., Orrenius S., *Biochim. Biophys. Acta*, **1271**, 59—62 (1995); b) Manna S. K., Zhang H. J., Yan T., Oberley L. W., Aggarwal B. B., *J. Biol. Chem.*, **273**, 13245—13254 (1998).
- 14) Matsuda H., Ishikado A., Nishida N., Kiyofumi N., Fujiwara H., Kobayashi Y., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 2939—2944 (1998).