SECONDARY METABOLITES ISOLATED FROM THE FUNGUS *MONASCUS KAOLIANG*-FERMENTED RICEMING-JEN CHENG¹, MING-DER WU¹, PING-SHIN YANG¹, JIH-JUNG CHEN²,
IH-SHENG CHEN³, YEN-LIN CHEN¹ AND GWO-FANG YUAN^{1*}¹Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), Hsinchu 300, Taiwan.²Graduate Institute of Pharmaceutical Technology & Department of Pharmacy, Tajen University, Pingtung 907, Taiwan.³Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University (KMU), Kaohsiung 807, Taiwan.^{*}First three authors share equal contributions to this paper

(Received: June 24, 2009 - Accepted: November 10, 2009)

ABSTRACT

A chemical study of the EtOAc-soluble ethanolic extract of *Monascus kaoliang* BCRC 31506 (Eurotiaceae) fermented rice led to the isolation of a new furan-3-one derivative along with 9 known compounds, monascin (2), ankaflavin (3), *N*-trans-feruloyltyramine (4), vanillic acid (5), 4-acetonyl-3,5-dimethoxy-*p*-quinol (6), (+)- β -eudesmol (7), *trans*-phytol (8), aesculetin dimethyl ether (9) and (–)-matairesinol (10). Structure 1 is proposed for monascuskaoliaone on the basis of spectroscopic evidence (NMR, UV, IR, and HRMS). None of the compounds exhibited significant scavenging activity toward DPPH radical in bioautography or spectroscopic assays.

Keywords: *Monascus kaoliang*; fungus; fermented Rice (red mold rice); furan derivative; monascuskaoliaone; DPPH.

INTRODUCTION

The filamentous fungi of the *Monascus* species have been used for a long time as a meat colorant, health food, and a Chinese folk medicine.¹ They comprise four represent species: *M. pilosus*, *M. purpureus*, *M. ruber*, and *M. anka*. These species belonged to the class Ascomycetes and the family Monascaceae.¹ Red mold rice (also called red yeast rice), which is also known as “koji”, “red koji”, “anka”, “Ang-kak”, and “ben-koji”, is obtained by the fermentation of rice (*Oryza sativa*) with fungi of the genus *Monascus*, mainly *M. pilosus*, *M. purpureus*, and *M. anka*, to produce a red-colored product.

The species of *Monascus* produced secondary metabolites such as pigments, Monacolin K, γ -aminobutyric acid (GABA), dimeric acid, and citrinin. Several secondary metabolites from *Monascus* sp. have recently been found to have some beneficial pharmacological effects in decreasing blood pressure, lowering plasma cholesterol levels and antibacterial activity.

Several secondary metabolites useful as food additives and pharmaceuticals have been reported by *Monascus* spp.¹⁻³ Polyketides, furanoidisophthalides, amino acids, azaphilones, pyranoidole alkaloids, benzenoids, furans, and fatty acids²⁻¹³ are widely distributed in the fungus *Monascus* species.

Some major pigments of red mold rice and some secondary metabolites have been identified, but knowledge of their biological and toxicological effects is fragmentary. Some minor compounds like furan derivatives or other type compounds except common pigments produced by *Monascus* sp. have received less concern on. Therefore, characterization of the components of red mold rice and their functionality still remain unclear and are worthy to be examined in further. In the course of our search for potential antioxidant metabolites from natural sources, we were intended to realize the secondary metabolites of red mold rice, and *M. kaoliang* BCRC 31506 has been found to be one of the active species. The antioxidant activity exhibited in the EtOAc-soluble fraction of the 70% EtOH extract of red fermented rice obtained with *M. kaoliang* BCRC 31506 were detected by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method¹⁴⁻¹⁵.

Bioassay-guided fractionation of the EtOAc-soluble fraction of a 70% EtOH extract of the red mold rice produced by *M. kaoliang* BCRC 31699 has resulted in the isolation of ten constituents, including a new furan derivative, monascuskaoliaone (1), together with nine known compounds. In this paper, we describe the isolation and structural elucidation of the new compound (Fig. 1), and the free radical scavenging activity of the isolates (1–10) by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method.

Figure 1. Structures of metabolites 1–10 isolated from *M. kaoliang* BCRC 31506.

EXPERIMENTAL

General experimental procedure

All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco

P-1020 digital polarimeter, UV spectra were obtained on a Jasco UV-240 spectrophotometer in MeOH, and IR spectra (Neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. 1D (^1H , ^{13}C , DEPT) and 2D (COSY, NOESY, HSQC, HMBC) NMR spectra using CDCl_3 as solvent were recorded on a Varian Unity Plus 400 and Varian Mercury-400 (400 MHz for ^1H NMR, 100 MHz for ^{13}C NMR) spectrometer. Chemical shifts were internally referenced to the solvent signals in CDCl_3 (^1H , δ 7.26; ^{13}C , δ 77.0) with TMS as the internal standard. Low-resolution ESI-MS spectra were obtained on an API 3000 (Applied Biosystems) and high-resolution ESI-MS spectra on a Bruker Daltonics APEX II 30e spectrometer. Low-resolution EI-MS spectra were recorded on a Quattro GC/MS spectrometer having a direct inlet system. Silica gel (70-230, 230-400 mesh) (Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used for TLC and prep. TLC. For radical scavenging TLC autographic assay, DPPH (Sigma) was used as spray reagent.

Microorganism

Monascus kaoliang BCRC 31506 was used throughout this study, and specimens deposited at the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (FIRDI).

Cultivation and Preparation of red mold rice

Monascus kaoliang BCRC 31506 was maintained on potato dextrose agar (PDA; Difco). The strain was cultured on PDA slants at 25°C for 6 days and then the spores were harvested by sterile water. The spores (5×10^5) were seeded into 300 ml shake flasks containing 50 ml RGY medium (3% rice starch, 7% glycerol, 1.1% polypeptone, 3.2% soybean powder, 0.2% MgSO_4 , 0.2% NaNO_3), and cultivated with shaking (150 rpm) at 25°C for 3 days. After the mycelium enrichment step, an inoculum mixing 100 ml mycelium broth and 100 ml RGY medium was inoculated into plastic boxes (25 cm $\times$ 30 cm) containing 1 kg sterile rice and cultivated at 25°C for producing red mold rice (RMR; also called *beni-koji* in Japan). At day 7, 150 ml RGY medium was added for maintaining the growth of cells. After 28 days of cultivation, the RMR was harvested and lyophilized for the extraction of metabolites.

Extraction and Isolation

Red mold rice of the *M. kaoliang* BCRC 31506 (3.5 kg) were extracted three times with 70% EtOH at room temperature. The ethanol syrup extract was partitioned between EtOAc and H_2O (1:1) to afford EtOAc (fraction A, 5.2 g) and H_2O (fraction B, 18.2 g) soluble fractions. The EtOAc-soluble fraction (5.2 g) was chromatographed over silica gel (50 g, 70–230 mesh), eluting with *n*-hexane and enriched with EtOAc to produce 10 fractions (A1–A10). Fraction A-1 (500 mg) was subjected to silica gel (SiO_2 , 230–400 mesh; *n*-hexane–EtOAc, 10:1) to obtain (+)- β -eudesmol (7) (1.4 mg), and *trans*-phytol (8) (1.4 mg). Fr. A-2 (1.2 g) was chromatographed on silica gel (230–400 mesh), employing *n*-hexane containing increasing amounts of EtOAc as elute to produce 25 fractions (A-2-1–A-2-25). Fr. A-2-7 (125 mg) was purified by preparative TLC (*n*-hexane–EtOAc, 3:1) to afford monascin (2) (11.9 mg) and ankaflavin (3) (12.8 mg). Fraction A-3 (1.1 g) was chromatographed over silica gel, eluting with CH_2Cl_2 –MeOH (15:1) to obtain 20 fractions (A-3-1–A-3-20). Fraction A-3-15 (17.3 mg) was repeatedly purified by preparative TLC (*n*-hexane–acetone, 3:1) to afford monascuskaoiaone (1) (2.3 mg). Fraction A-3-17 (36.2 mg) was purified by preparative TLC (*n*-hexane–EtOAc, 3:1) to give 4-acetonyl-3,5-dimethoxy-*p*-quinol (6) (4.9 mg). Fraction A-5 (1.5 g) was resubjected to silica gel (CH_2Cl_2 –MeOH, 20:1) to afford 13 fractions (A-5-1–A-5-13). Fraction A-5-3 (60.7 mg) was repeatedly purified by preparative TLC (CH_2Cl_2 –MeOH, 13:1) to afford *N*-*trans*-feruloyltyramine (4) (3.9 mg), and vanillic acid (5) (2.4 mg). Fraction A-6 (700 mg) was resubjected to silica gel (CH_2Cl_2 –MeOH, 15:1) to afford 8 fractions (A-6-1–A-6-8). Fraction A-6-2 (120 mg), eluting with CH_2Cl_2 –EtOAc 1:1, was further separated using column chromatography to give aesculetin dimethyl ether (9) (1.7 mg) and (–)-matairesinol (10) (1.2 mg).

Monascuskaoiaone (1). Yellowish oil; $[\alpha]_{\text{D}}^{25} = \pm 0$ (c 0.09, CHCl_3); UV (MeOH): 224 (3.95) nm; IR (Neat): 3400, 1695, cm^{-1} ; ESI-MS m/z 305 $[\text{M}+\text{Na}]^+$; HR-ESI-MS m/z 305.2092 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{17}\text{H}_{30}\text{O}_3\text{Na}$, 305.2093); ^1H - and ^{13}C -NMR spectra are shown in Table 1.

Table 1. NMR data (CDCl_3) of compound 1. δ in ppm, J in Hz.

No.	1 ^a	
	$\delta(\text{C})^b$	$\delta(\text{H})$
1	–	–
2	91.4 (s)	–
3	207.1 (s)	–
4	103.5 (d)	5.48 (1H, s)
5	189.1 (s)	–
6	34.1 (t)	2.79 (2H, t, $J = 6.2$ Hz)
7	59.3 (t)	3.96 (1H, t, $J = 6.2$ Hz)
8	36.6 (t)	1.72 (2H, m)
9	23.0 (t)	1.23 (2H, br. s)
10	29.3~29.6 (t)	1.23 (2H, br. s)
11	29.3~29.6 (t)	1.23 (2H, br. s)
12	29.3~29.6 (t)	1.23 (2H, br. s)
13	29.3~29.6 (t)	1.23 (2H, br. s)
14	29.3~29.6 (t)	1.23 (2H, br. s)
15	31.9 (t)	1.23 (2H, br. s)
16	22.7 (t)	1.23 (2H, br. s)
17	14.1 (q)	0.88 (3H, t, $J = 6.8$ Hz)
Me-2	22.0 (q)	1.35 (3H, s)

^a All spectra were recorded at 400 MHz (^1H) and 100 MHz (^{13}C); assignment were aided by 2D-NMR, COSY, HSQC and HMBC experiments; ^b ^{13}C -NMR multiplicities were determined by DEPT experiment.

Reduction of DPPH Radical. After developing and drying, TLC plates were sprayed with a 0.2% DPPH (Aldrich-Sigma) solution in MeOH. The plates were examined 30 min after spraying. Extracts and compounds showing a yellow-on-purple spot were regarded as antioxidant qualities¹⁴⁻¹⁵. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample.

Determination of the Scavenging Effect on DPPH Radical. The radical scavenging activity of the test compounds was examined with the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, as described previously¹⁵. α -Tocopherol (= vitamin E) (Sigma) was used as control. 50 μl of a solution containing the isolated compounds (final concentration was 50 μM) to be tested was added to 5 ml of a 1.0×10^{-4} M MeOH solution of DPPH. The reaction mixture was shaken vigorously, and its absorbance at 517 nm was determined after 40 min incubation in a dark room. Decreasing DPPH solution absorbance indicates an increase in DPPH radical-scavenging activity. The DPPH solution, without sample solutions, was used as a control. All tests were run in triplicate and averaged. This activity is given as % DPPH radical-scavenging and is calculated in the equation: % DPPH radical-scavenging = (Control absorbance—sample absorbance/Control absorbance) $\times$ 100. The data were expressed as a mean of three experiments.

RESULTS AND DISCUSSION

The EtOAc extracts, prepared from the 70% EtOH extract of the red mold rice produced by *M. kaoliang* BCRC 31506 were fractionated by a combination of open column chromatography (silica gel), MPLC, and prep. TLC to obtain the furan derivative (1), azaphilones (2 and 3), amide (4), benzenoid (5),

quinone (**6**), diterpenoids (**7** and **8**), coumarin (**9**), and lignan (**10**). Compounds **2**–**10** were identified by comparing their physical and spectroscopic data with those reported in the literature and by direct comparison with authentic samples on TLC. The new structure of **1** was identified based on the following evidences.

Compound **1** was obtained as yellowish optically inactive oil ($[\alpha]^{22D} = \pm 0$ (c 0.09, CHCl_3)). The molecular formula of compound **1** was determined as $\text{C}_{17}\text{H}_{30}\text{O}_3$ from its HR-ESI-MS data (m/z 305.2092 ($[\text{M}+\text{Na}]^+$; calc. 305.2093)) as well as from its ^{13}C -NMR and DEPT, requiring 3 degree of unsaturation. UV absorption at 224 nm and IR absorption bands are due to the presence of hydroxyl (3400 cm^{-1}), and a conjugated carbonyl (1695 cm^{-1}) groups, suggesting the presence of a furanoid nucleus^{16–18} as in the case of terrefuranone¹⁸. The presence of a conjugated carbonyl group was revealed by an IR absorption, along with a resonance signal in the ^{13}C -NMR spectrum at δ_c 207.1.

Analysis of the ^1H NMR spectrum of **1** (Table 1) revealed a typical A_2X_2 pattern signals at δ_H 2.79 (2H, t, $J = 6.2$ Hz, H-6) and 3.96 (2H, t, $J = 6.2$ Hz, H-7), one 3H singlet at δ_H 1.35 assigned to a methyl group on a quaternary carbon (C-2), one olefinic proton deshield by a conjugated carbonyl observed at δ_H 5.48 (1H, s, H-4), a decyl moiety appeared at δ_H 0.88 (3H, t, $J = 6.8$, Me-17), 1.23 (16H, br s, CH_2 -9–16), and 1.72 (2H, m, CH_2 -8), indicating that **1** was probably a furan derivative possessing a conjugated carbonyl group¹⁶. The ^{13}C -NMR and DEPT spectrum showed that **1** had a total of 17 carbons for two Me, eleven CH_2 (two for a $\text{CH}_2\text{CH}_2\text{OH}$ moiety), one CH, and three quaternary C-atoms including one $\text{C}=\text{O}$ (δ_c 207.1) and a $\text{C}=\text{C}$ bond (δ_c 189.1). The carbon of the **1** were assigned, from ^{13}C -NMR, DEPT and HSQC experiments, as two methyls at δ_c 14.1 (C-17) and 22.0 (Me-2), eleven methylenes at δ_c 22.7 (C-16), 23.0 (C-9), 29.3–29.6 (C-10–14), 31.9 (C-15), 34.1 (C-6), 36.6 (C-8), and 59.3 (C-7), one olefinic carbon at δ_c 103.5 (C-4), and three quaternary carbons at δ_c 91.4 (C-2), 189.1 (C-5), and 207.1 (C-3). The above data also pointed to a furan derivative skeleton.

The above observation accompanied by the ^1H , ^1H -COSY (Fig. 2), and ^1H , ^1H -LRCOSY spectrum of **1** established the presence of the partial substructures: fragments, **1a**, **1b**, and **1c**, for compound **1** (Fig. 5). The entire skeleton of **1** was constructed by the aid of HMBC spectrum (Fig. 3).

Figure 2. Important COSY ($\rightarrow$) contacts of **1**.

Figure 3. Key HMBC ($\rightarrow$) correlations of **1**.

The ^1H , ^{13}C -NMR long-range 2J and 3J HMBC correlations of the signal at δ_H 1.35 (Me-2), with the carbon signal at δ_c 91.4 (C-2) and 36.6 (C-8), helped to establish the connections of fragments **1a** and **1b** at C-2. In addition, the cross peak between δ_H 2.79 (H-6)/ δ_c 103.5 (C-4), as well as δ_H 5.48 (H-4)/ δ_c 34.1 (C-6), suggest that fragments **1b** and **1c** were linked together at C-5 (Fig. 3). The other key correlations of HMBC were illustrated in Fig. 3.

The above assignments were further confirmed by significant NOESY correlations (Fig. 4) between the H-atom Me-2 (δ_H 1.35) and CH_2 -8 (δ_H 1.72), between CH_2 -6 (δ_H 2.79) and CH_2 -7 (δ_H 3.96), and between H-4 (δ_H 5.48) and CH_2 -6 (δ_H 2.79) (Fig. 3) indicated that the hydroxyethyl ($-\text{CH}_2\text{CH}_2\text{OH}$) group was attached to C-5, and a decyl moiety to C-2. Because of the optical inactivity ($[\alpha]^{22D} = \pm 0$ (c 0.09, CHCl_3)), **1** was concluded to be racemic. On the basis of these data, the structure of compound **1** was, thus, deduced as 2-decyl-5-(2-hydroxyethyl)-2-methylfuran-3(2H)-one, and named monascuskaolione.

Figure 4. Major NOESY ($\leftrightarrow$) correlations of **1**.

Figure 5. The partial substructures: fragments, **1a**, **1b**, and **1c**, for compound **1**.

The other known isolates, monascin (**2**)¹⁹, ankaflavin (**3**)¹⁹, *N*-trans-feruloyltyramine (**4**)²⁰, vanillic acid (**5**)²¹, 4-acetyl-3,5-dimethoxy-*p*-quinol (**6**)²², (+)- β -eudesmol (**7**)²³, *trans*-phytol (**8**)²⁴, aesculetin dimethyl ether (**9**)²⁵ and (–)-matairesinol (**10**)²⁶ were readily identified by comparison of their spectral data (UV, IR, ^1H NMR, MS) with the data from the corresponding values in the literature. Among them, all known compounds except **2** and **3**, were isolated from *Monascus* spp for the first time.

The free radical scavenging effect of the isolated compounds (**1**–**10**) was determined using the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging system. By using DPPH as a TLC spray reagent^{14–15}, compounds **4**, **5**, and **10** (5, 10 μg) appeared as strong yellow spots against a purple background, while compound **6** displayed moderate yellow spots, and other compounds did not react with the radical. The free radical scavenging effects of the isolates, compounds **4**, **5**, and **10**, corresponding to the intensity of quenching of the DPPH radical, were further evaluated by spectroscopic assay. At a concentration of 50 μM , the test compounds, *N*-trans-feruloyltyramine (**4**), vanillic acid (**5**), and (–)-matairesinol (**10**) showed moderate to strong DPPH radical scavenging activity, with 58, 70, and 75 % inhibition. (–)-Matairesinol (**10**) exhibited the most active metabolite in this study.

Several biological activities of *Monascus* extracts and the isolated compounds, such as antioxidant, and cytotoxic, have been reported previously²⁷. On the basis of this information, free radical scavenging activities of compounds **1**–**10** were determined using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging system, by comparing α -tocopherol, which was commonly used in the food industry as antioxidant (85 % inhibition, at 50 μM). The radical scavenging effects of antioxidants on the DPPH radical are considered to be due to their H donating character. A phenolic hydroxyl (PhOH) or carboxylic acid (COOH) moieties have been recognized to function as proton donors.

Reviewing the past literature regarding *Monascus* species^{1–13} reveals that azaphilone, furanoisophthalides, amino acid, and polyketides are the major

metabolites. In our previous studies, we have reported over fifteen metabolites, together with their DPPH free radical scavenging activity from *M. pilosus*⁵. In the course of our search for potential antioxidant metabolites from natural sources, and *M. kaoliang* BCRC 31506 has been found to be one of the active species. In this successive study, we focused on the minor secondary metabolites appearing in the EtOAc-soluble fraction of a 70% EtOH extract of the red mold rice produced by *M. kaoliang* BCRC 31506. The new metabolite **1**, found in this study is novel, naturally occurring compounds. It is worthy to mention that this is the first report of a furan-3-one derivative isolated from this *Monascus* spp. Compound **1** did not show any scavenging activity against the DPPH radical because of its non-phenolic structure. Further study to realize the different biological activities of *Monascus* spp and related secondary metabolites are under in progress.

ACKNOWLEDGMENTS

The first two authors (M.-J. Cheng and M.-D. Wu) contributed equally to this work and should both be regarded as first author of this publication. This investigation was supported by a grant from the Ministry of Economic Affairs of Republic of China (Grant No. 94-EC-17-A-17-R7-0563)

REFERENCES

1. J. Ma, Y. Li, Q. Ye, J. Li, Y. Hua, D. Ju, D. Zhang, R. Cooper, M. Chang, *J. Agric. Food Chem.* **48**, 5220, (2000).
2. T. Akihisa, S. Mafune, M. Ukiya, Y. Kimura, K. Yasukawa, T. Suzuki, H. Tokuda, N. Tanabe, T. Fukuoka, *J. Nat. Prod.* **67**, 479, (2004).
3. T. Akihisa, H. Tokuda, K. Yasukawa, M. Ukiya, A. Kiyota, N. Sakamoto, T. Suzuki, N. Tanabe, H. Nishino, *J. Agric. Food Chem.* **53**, 562, (2005).
4. Blanc P.J., Loret M.O. and Goma G., *Biotechnology Letters* **17**, 291, (1995).
5. M. J. Cheng, M. D. Wu, I. S. Chen, G. F. Yuan, *Chem. Pharm. Bull.* **56**, 394, (2008).
6. Z. Huang, Y. Xu, L. Li, Y. Li, *J. Agric. Food Chem.* **56**, 112, (2008).
7. S. Jongrungruangchok, P. Kittakoop, B. Yongsmith, R. Bavovada, S. Tanasupawat, N. Lartpornmatulee, Y. Thebtaranonth, *Phytochemistry* **65**, 2569, (2004).
8. P. Juzlová, L. Martínková, V. Kren, *J. Ind. Microbiol.* **16**, 163, (1996).
9. P. Juzlová, T. Rezanka, L. Martínková, V. Kren, *Phytochemistry* **43**, 151, (1996).
10. H. Nozaki, S. Date, H. Kondo, H. Kiyohara, D. Takaoka, T. Tada, M. Nakayama, *Agric. Biol. Chem.* **55**, 899, (1991).
11. K. Sato, Y. Goda, S. S. Sakamoto, H. Shibata, T. Maitani, T. Yamada, *Chem. Pharm. Bull.* **45**, 227, (1997).
12. D. Wild, G. Tóth, H. U. Humpf, *J. Agric. Food Chem.* **50**, 3999, (2002).
13. D. Wild, G. Tóth, H. U. Humpf, *J. Agric. Food Chem.* **51**, 5493, (2003).
14. M. Cuendet, K. Hostettman, O. Potterat, W. Dyatmiko, *Helv. Chim. Acta* **80**, 1144, (1997).
15. T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, T. Yasuhara, *Chem. Pharm. Bull.* **37**, 2016, (1989).
16. I. Zabetakis, J. W. Gramshaw, D. S. Robinson, *Food Chem.* **65**, 139, (1999).
17. T. Furumoto, T. Hamasaki, H. Nakajima, *Tetrahedron Lett.* **38**, 5523, (1997).
18. J. He, E. M. Kithsiri Wijeratne, B. P. Bashyal, J. Zhan, C. J. Seliga, M. X. Liu, E. E. Pierson, L. S. Pierson, H. D. VanEtten, A. A. Leslie Gunatilaka, *J. Nat. Prod.* **67**, 1985, (2004).
19. L. L. Li, J. P. Chen, L. Y. Kong, *Zhong Guo Tian Ran Yao Wu* **4**, 32, (2006).
20. C. Y. Chen, F. R. Chang, Y. C. Wu, *J. Chin. Chem. Soc.* **44**, 313, (1997).
21. Y. L. Lin, W. J. Tsai, I. S. Chen, Y. H., *J. Chin. Chem. Soc.* **45**, 213, (1998).
22. T. S. Wu, C. C. Yang, P. L. Wu, L. K. Liu, *Phytochemistry* **40**, 1247, (1995).
23. S. D. Joland, B. N. Timmermann, J. J. Hoffmann, R. B. Bates, F. A. Camou, T. J. Siahannm, *Phytochemistry* **27**, 2199, (1998).
24. I. L. Tsai, Y. F. Jeng, C. Y. Duh, I. S. Chen, *Chin. Pharm. J.* **53**, 291, (2001).
25. J. J. Chen, C. S. Yang, C. F. Peng, I. S. Chen I.S., C. L. Miaw, *J. Nat. Prod.* **71**, 1016, (2008).
26. B. Gözler, G. Arar, T. Gözler, M. Hesse, *Phytochemistry* **31**, 2473, (1992).
27. K. Yasukawa, M. Takahashi, X. Yamanouchi, M. Takido, *Oncology* **53**, 247, (1996).