A Novel Eudesmane Glycosides Sesquiterpene from *Calendula officinalis* L.

Mohammad Waleed Mohammad Sadaka^{1, a*}

¹Department of Chemistry, Faculty of Applied Science, University of Kalamoon, Deir Atiyah, Syria.

^a waleed.sadaka@gmail.com

Keywords: *Calendula officinalis* L., eudesmane, sesquiterpene, lupane, kaempferol.

Abstract. The chromatographic separation of aqueous methanolic extract of *Calendula officinalis* led to the isolation of a new eudesmane sesquiterpene glycoside (1), together with two known compounds, a lupane ester (2) and flavonoid glucoside (3). Their structures were characterized to be 1 β ,3 β ,6 α ,8 α ,tetrahydroxyeudesm-4-ene 11-O- β -D-glucopyranoside (1), 3 β -E-caffeoyl lupeol (2), kaempferol-3-O-glucoside (3) by spectroscopic means.

1. Introduction

The genus *Calendula* belongs to the *Asteraceae* family and encloses more than 20 species distribution around the world. This plant is used in folk medicine for the treatment of digestive disorders, inflammation, emmenagogue, and as tonic, stimulant and antibacterial remedies. In the Flora of Syria, this genus is represented by 8 species [1]. Previous studies demonstrated therapeutic potentialities of *Calendula officinalis* L. such as anti-inflammatory activity [2-5], immunomodulatory [6-7], Hepatoprotective [8], wound-healing [9], antioxidant [10-12], antibacterial and antiparasitic activity [13-16]. The essential oil from *C. officinalis* has been also investigated [17-22].

2. Experimental

2.1. Materials and methods

2.1.1 Instrumentation

The ¹H and ¹³C NMR spectra were recorded on Bruker Ultra Shield 400 MHz spectrometer (faculty of Science, Al-Baath University, Homs, Syria) and reported in ppm. (δ) relative to TMS as internal standard and DMSO-*d*₆ as solvent. The IR spectra were measured on JASCO (FT-IR)-410 spectrophotometer (Medico Medical Company, Homs, Syria). MS spectra were recorded on Shimadzu GCMS-QP 2010 (Syrian atomic energy commission, Damascus, Syria). Silica gel 60 F₂₅₄ plates (Merck) were used for analytical and preparative TLC. Silica gel S (Merck; Mesh 230-400) was used for column chromatography (CC), and all the chemicals were purchased from Merck.

2.1.2 Plant material

Calendula officinalis L. were collected from Jeser alshoughur, Edlib, Syria in March 2008, and identified by Pro.Dr. Anwer Al-Khateeb, Department of Botany, College of Science, Damascus University, Damascus, Syria.

2.1.2 Extraction and Isolation

The air-dried aerial parts (2 kg) of plant material were extracted three times with aqueous methanol (80:20 MeOH:H₂O) at room temperature for 16 days. The combined extracts were evaporated under reduced pressure, to yield 17 g crude extract. The crude extract was fractionated by CC on silica gel (500 g) using gradient solutions of hexane, chloroform, methanol and water, respectively. Elution with CHCl₃-MeOH (8:2) yielded 40 fractions, Fr 28-32 afford 91 mg of unpurified (2), further purification by preparative TLC (90:10 CHCl₃-MeOH) afforded (24.5 mg) of

compound (2). Elution with CHCl_3 -MeOH (5:5) yielded 20 fractions, Fr 14-18 afford 87 mg of unpurified (1), farther purification by preparative TLC (50:50 CHCl_3 -MeOH) afforded (26 mg) of compound (2). Elution with MeOH yielded 36 fractions. Fr 5-8 afford 43 mg of unpurified (3), farther purification by preparative TLC (MeOH) afforded (17 mg) of compound (3).

3. Results and Discussion

Compound (1) (Fig 1.) was isolated as a white amorphous powder. The IR spectrum of (1) showed absorptions for hydroxyl groups (3360 cm^{-1}), double bond (1460 cm^{-1}) and C-O (1120 cm^{-1}) functionalities. Compound (1) gave a molecular ion peak in its EI-MS spectrum at m/z 448, in agreement with the molecular formula $\text{C}_{21}\text{H}_{36}\text{O}_{10}$. The other prominent peaks were found to occur at m/z 430 $[\text{M}-\text{H}_2\text{O}]^+$, 412 $[\text{M}-2\text{H}_2\text{O}]^+$, 269 $[\text{M}-\text{glucose}]^+$, 227, 209. These data together with information from the ^{13}C NMR spectrum (Table 1.) that displays 21 signals. The multiplicities of carbon signals were determined by DEPT experiment. The ^{13}C -NMR spectra of (1) showed signals for four methyl carbons (δ 16.80, 18.20, 27.77 and 31.84), ascribed to C-14, C-15, C-12 and C-13 respectively, three methylene carbons, a ten methine carbons, five of it ascribed to eudesmane skeleton and five to sugar moiety. A four methine carbons in eudesmane skeleton are shifted to weak field due to connected to highly electronegative atom like oxygen [δ 73.28(C-1), 70.06 (C-3), 69.60 (C-6), 70.60 (C-8)]. ^{13}C -NMR spectra of (1) showed signals for four quaternary carbons, two of double bond [δ 126.80 (C-4) and 141.88 (C-5)], and one which are oxygenated [δ 82.30 (C-11)].

Fig 1. Eudesmane sesquiterpene glycoside

Taking into account the results from our comprehensive 1D and 2D NMR studies and previous knowledge derived from metabolites isolated from the genus *Calendula* it is evident that (1) possesses an eudesmane skeleton. The ^1H -NMR spectrum (Table 1.) of compound (1) showed four singlet at δ 1.10, 1.22, 1.32, and 2.08 belong to four methyl group, an anomeric proton signal at [δ 4.98 (d , $J = 7.6\text{ Hz}$, H-1')], indicate to β -D-glucopyranosyl moiety. The glycoside linkages of (1) were constructed on the basis of HMBC spectrum. In the HMBC experiment, correlations were observed between the 1'-H and the C-11 carbon, between the H-14 and the C-5, C-10 carbons, between the H-15 and the C-4 carbon, H-13, H-12 and the C-7, C-11 carbon. On the basis of the above evidence, and comparative ^1H -NMR data, and J coupling constant of (1) with related compounds [23-26], compound (1) was elucidated to be 1,3,6,8,tetrahydroxyeudesm-4-ene 11-O- β -D-glucopyranosid.

Compound (2) (Fig 2.) isolated as a white amorphous powder. The IR spectrum of (2) showed absorption band for hydroxyl groups 3425 cm^{-1} , carbonyl group 1675 cm^{-1} ($\nu\text{ C=O } \alpha,\beta$ -unsaturated ester), and double bond 1620 cm^{-1} functionalities. Compound (2) gave a molecular ion peak in its EI-mass spectrum at m/z 588, in agreement with the molecular formula $\text{C}_{39}\text{H}_{56}\text{O}_4$. The other prominent peaks were found to occur at m/z 426 $[\text{M}-\text{caffeoyl}]^+$, 385 $[\text{M}-\text{side chain}]^+$, 218, 207, 191, 163, 135, 109, 97, 93. The ^1H NMR spectrum of (2) displayed signals for typical pentacyclic triterpenoid methyl groups at δ 0.75, 0.87, 0.88, 0.93, 0.96, 1.05, and 1.69 ppm.) (δ C 18.10, 16.33, 28.02, 16.72, 14.41, 16.02 and 19.22 respectively according with HETCOR experiment). The

presence of a vinyl methyl singlet at δ 1.69 and two vinyl proton signals at δ 4.68 (*d*, $J = 2.2$ Hz) and 4.56 (*m*) with ^{13}C absorptions at δ 151.22 and 109.23 are characteristic of the isopropenyl group of triterpenes of the lupane type.

Table 1. ^{13}C -NMR and ^1H -NMR Data of Compound (1), (400 MHz, $\text{DMSO-}d_6$)

No	C	DEPT	H
1	73.28	CH	3.62 , <i>dd</i> , $J = 4.8$, 12 Hz
2	31.40	CH_2	1.86 , <i>m</i> 2.24 , <i>brd</i> , $J = 12.8$ Hz
3	70.06	CH	4.34 , <i>dd</i> , $J = 6.0$, 10.4 Hz
4	126.80	C	---
5	141.88	C	---
6	69.60	CH	3.92 , <i>d</i> , $J = 6.8$ Hz
7	54.24	CH	1.96 , <i>d</i> , $J = 6.2$ Hz
8	70.60	CH	4.08 , <i>dd</i> , $J = 8.8$, 4 Hz
9	46.40	CH_2	1.70 , <i>dd</i> , $J = 6.6$, 10.2 Hz 2.18 , <i>dd</i> , $J = 6.4$, 10.2 Hz
10	38.62	C	---
11	82.30	C	---
12	27.77	CH_3	1.32 , <i>s</i>
13	31.84	CH_3	1.22 , <i>s</i>
14	16.80	CH_3	1.10 , <i>s</i>
15	18.20	CH_3	2.08 , <i>s</i>
1'	97.00	CH	4.98 , <i>d</i> , $J = 7.6$ Hz
2'	71.90	CH	3.72 , <i>t</i> , $J = 6$ Hz
3'	77.50	CH	3.98 , <i>t</i> , $J = 6.6$ Hz
4'	74.50	CH	3.49 , <i>t</i> , $J = 6.2$ Hz
5'	76.50	CH	3.73 , <i>t</i> , $J = 6.4$ Hz
6'	62.50	CH_2	3.71 , <i>m</i> 3.86 , <i>dd</i> , $J = 11.4$, 6 Hz
	OH		3.21
	OH		3.11
	OH		4.78

Fig 2. Lupane-3-*O*-caffeoyle

^1H -NMR spectrum also showed a multiplet at (δ 4.64 ppm.) due to a hydroxymethine group (δ C 81.21). The assignment of the signals of the methyl groups and the remaining ^1H and ^{13}C signals

was performed by analysis of HETCOR, HMBC, and COSY experiments, and the results were consistent with a lupine-type triterpene [27-29]. The EI-MS fragments at m/z 163 $[\text{C}_9\text{H}_7\text{O}_3]^+$, 135 $[\text{C}_8\text{H}_7\text{O}_2]^+$, and 109 $[\text{C}_6\text{H}_5\text{O}_2]^+$ indicated the presence of caffeoyl unity in the side chain of (2). The presence of a trans-caffeoyl moiety was confirmed by ^1H - and ^{13}C -NMR resonance spectral data (Table 2).

Table 2. ^{13}C -NMR and ^1H -NMR Data of Compound (2), (400 MHz, $\text{DMSO}-d_6$)

No	C	DEPT	H
1	38.24	CH_2	1.66, <i>m</i> , 1.70 <i>m</i>
2	23.91	CH_2	1.70 <i>m</i>
3	81.21	CH	4.64 <i>m</i>
4	38.14	C	---
5	55.40	CH	0.80 <i>m</i>
6	18.21	CH_2	1.43 <i>m</i> , 1.54 <i>m</i>
7	34.22	CH_2	1.43 <i>m</i>
8	40.93	C	---
9	50.42	CH	1.30 <i>m</i>
10	37.25	C	---
11	21.24	CH_2	1.24 <i>m</i> , 1.46 <i>m</i>
12	25.22	CH_2	1.18 <i>m</i>
13	38.12	CH	1.69 <i>m</i>
14	42.91	C	---
15	27.51	CH_2	1.92
16	35.63	CH_2	1.50 <i>m</i> , 1.52 <i>m</i>
17	43.00	C	---
18	48.23	CH	1.37 <i>m</i>
19	48.01	CH	2.38 <i>m</i> (<i>dt</i> , $J= 11.1, 5.7$ Hz)
20	151.22	C	----
21	29.81	CH_2	1.88 <i>m</i> , 1.94 <i>m</i>
22	40.00	CH_2	1.20 <i>m</i> , 1.40 <i>m</i>
23	28.02	CH_3	0.88 <i>s</i>
24	16.33	CH_3	0.87 <i>s</i>
25	16.72	CH_3	0.93 <i>s</i>
26	16.02	CH_3	1.05 <i>s</i>
27	14.41	CH_3	0.96 <i>s</i>
28	18.10	CH_3	0.75 <i>s</i>
29	109.23	CH_2	4.68 <i>d</i> , $J= 2.2$ Hz 4.56 <i>m</i>
30	19.22	CH_3	1.69 <i>s</i>
9'	167.81	C	---
8'	116.01	CH	6.30 <i>d</i> (15.6)
7'	144.42	CH	7.58 <i>d</i> (15.6)
1'	127.23	C	---
2'	115.24	CH	7.18 <i>d</i> (1.2)
3'	143.91	C	---
4'	146.44	C	---
5'	114.11	CH	6.86 <i>d</i> (8.2)
6'	122.41	CH	6.96 <i>dd</i> (8 ,1.6)

The presence of a caffeoyl ester was shown by the carbon signals at δ 116.01, 144.42, 115.24, 114.11 and 122.21, as well as the quaternary carbons at δ 127.23, 143.91, and 146.44, and carbonyl carbon at δ 167.81. in the ^{13}C -NMR spectrum. Also, two olefinic signals that are characteristic of *trans* double bond at δ 6.30 [1H, *d*, $J=15.6$ Hz.] and δ 7.58 [1H, *d*, $J=15.6$ Hz.]. The position of *O*-caffeoyl group confirmed by HMBC spectrum, which showed long-range correlation between oxymethine proton at δ 4.64 and C-9. On the basis of the above evidence, and comparative ^1H -NMR, ^{13}C -NMR data, of (2) with related compounds [30-31], compound (2) was elucidated to be lupane-3*E*-*O*-caffeoyl.

Compound (3) (Fig 3.) isolated as a pale yellow amorphous powder. The IR spectrum of (3) showed absorption band for hydroxyl groups 3400 cm^{-1} , carbonyl group 1650 cm^{-1} ($\text{C}=\text{O}$ α,β -unsaturated ester), aromatic double bond 1520 cm^{-1} , 3070 cm^{-1} for aromatic C-H, and 1175 for C-O functionalities. Compound (3) gave a molecular ion peak in its EI-mass spectrum at m/z 484, in agreement with the molecular formula $\text{C}_{21}\text{H}_{20}\text{O}_{11}$. The other prominent peaks were found to occur at m/z 286 $[\text{M-glucose}]^+$, 134, 164, 121, 85, 71, 57. In the ^1H -NMR spectrum of (3) (Table 3.), 2 meta coupled aromatic protons at δ 6.12 and 6.58 (2 doublets) were clearly attributed to H-6 and H-8 protons. The signals at δ 6.90 and 8.04 belonged to H-3' (H-5') and H-2' (H-6').

Fig 3. Kaempferol-3-*O*-glucoside

Table 3. ^{13}C -NMR and ^1H -NMR Data of Compound (3), (400 MHz, $\text{DMSO}-d_6$)

No	C	DEPT	H	No	C	DEPT	H
2	157.20	C	--	3'	115.32	CH	6.90, <i>d</i> , $J = 8.4$
3	134.40	C	--	4'	160.22	C	--
4	177.68	C	--	5'	115.32	CH	6.90, <i>d</i> , $J = 8.4$
5	160.84	C	--	6'	130.40	CH	8.04, <i>d</i> , $J = 8.8$
6	98.80	CH	6.12, <i>d</i> , $J = 2.0$	1''	103.14	CH	5.20, <i>d</i> , $J = 7.2$
7	164.42	C	--	2''	74.68	CH	3.36, <i>m</i>
8	93.66	CH	6.58, <i>d</i> , $J = 2.0$	3''	77.10	CH	3.47, <i>m</i>
9	156.28	C	--	4''	70.88	CH	3.28, <i>m</i>
10	103.38	C	--	5''	77.94	CH	3.19, <i>m</i>
1'	120.64	C	--	6''	61.96	CH_2	3.67, <i>dd</i> , $J = 12.0, 2.4$ 3.52, <i>dd</i> , $J = 12.0, 4.8$
2'	130.40	CH	8.04, <i>d</i> , $J = 8.8$		OH		11.80, <i>s</i>

The ^1H -NMR spectrum (Table 3.) of compound (3) showed an anomeric proton signal at [δ 5.20 (*d*, $J = 7.2$ Hz, H-1'')], indicate to β -*D*-glucopyranosyl moiety. The glycoside linkages of (3) were constructed on the basis of HMBC spectrum. In the HMBC experiment, correlations were observed between the 1''-H and the C-3 carbon. By comparative ^1H -NMR, ^{13}C -NMR data, of (3) with literatures [31-31], compound (3) was elucidated to be kaempferol-3-*O*-glucose.

4. Conclusion

In this study, A new eudesmane sesquiterpene glycoside (**1**) were isolated and identified from the aerial parts of *Calendula officinalis*. Besides to 2 known compounds (**2**, **3**), theis compounds are first time isolated from *Calendula officinalis*.

Acknowledgments.

We thank Prof. Dr. Anwer Al-khateeb (Botany Department, Faculty of Science, Damascus University) for plant taxonomic identification.

References

- [1] Paul Mouterde, Nouvelle Flore du Liban et de la Syrie, Vol. III, Editions de l'Impr. catholique, Beirut, 1966.
- [2] T. Shipochliev, A. Dimitrov, E.Aleksandrova, Anti-inflammatory Action of a Group of Plant Extracts, Vet. Med. Nauki. 18 (1981) 87-94.
- [3] A. Tubaro, R. Della Loggia, S. Sosa, H. Becker, S. Saar, O. Isaac, The Role of Triterpenoids in the Topical Anti-inflammatory Activity of *Calendula officinalis* Flowers, Planta Med. 60 (1994) 516-520.
- [4] M. Ukiya, T. Akihisa, K. Yasukawa, H. Tokuda, T. Suzuki, Y. Kimura, Anti-inflammatory, Anti-tumor-promoting, and Cytotoxic Activities of Constituents of Marigold (*Calendula officinalis*) Flowers, J. Nat. Prod. 69 (2006) 1692-1696.
- [5] K. C. Preethi, G. Kuttan, R. Kuttan, Anti-inflammatory Activity of Flower Extract of *Calendula officinalis* Linn. and Its Possible Mechanism of Action, Indian J. Exp. Biol. 47 (2009) 113-120.
- [6] H. Wagner, A. Proksch, I. Riess-Maurer, A. Vollmar, S. Odenthal, H. Stuppner, K. Jurcic, M. Le Turdu, Y.H. Heur, Immunostimulant Action of Polysaccharides (Heteroglycans) from Higher Plants, Arzneimittelforschung. 34 (1984) 659-661.
- [7] Z. Amirghofran, M. Azadbakht, M.H. Karimi, Evaluation of the Immunomodulatory Effects of Five Herbal Plants, J. Ethnopharmacol. 72 (2000) 167-172.
- [8] M.A Rusu, M. Tamas, C. Puica, I. Roman, M. Sabadas, The Hepatoprotective Action of Ten Herbal Extracts in CCl₄ Intoxicated Liver, Phytother. Res. 19 (2005) 744-749.
- [9] K.C. Preethi, R. Kuttan, Wound Healing Activity of Flower Extract of *Calendula officinalis*, J. Basic Clin. Physiol. Pharmacol. 20 (2009) 73-79.
- [10] C.A.S. Cordova, I.R. Siqueira, C.A. Netto, R.A. Yunes, A.M. Volpato, V.C. Filho, R. Curi-Pedrosa, T.B. Creczynski-Pasa, Protective Properties of Butanolic Extract of the *Calendula officinalis* L. (Marigold) Against Lipid Peroxidation of Rat Liver Microsomes and Action as Free radical Scavenger, Redox Rep. 7 (2002) 95-102.
- [11] K.C. Preethi, G. Kuttan, R. Kuttan, Antioxidant Potential of an Extract of *Calendula officinalis*. Flowers in Vitro and in Vivo, Pharmaceutical Biology. 44 (2006) 691-697.
- [12] P.C Braga, M. Dal Sasso, M. Culici, A. Spallino, M. Falchi, A. Bertelli, R. Morelli, R. Lo Scalzo, Antioxidant Activity of *Calendula officinalis* Extract: Inhibitory Effects on Chemiluminescence of Human Neutrophil Bursts and Electron Paramagnetic Resonance Spectroscopy, Pharmacology. 83 (2009) 348-355.
- [13] G. Dumenil, R. Chemli, C. Balansard, H. Guiraud, M. Lallemand, Evaluation of Antibacterial Properties of Marigold Flowers (*Calendula officinalis* L.) and Mother Homeopathic Tinctures of *C. officinalis* L. and *C. arvensis* L., Ann. Pharm. Fr. 38 (1980) 493-499.

- [14] L. Iauk, A.M. Lo Bue, I. Milazzo, A. Rapisarda, G. Blandino, Antibacterial Activity of Medicinal Plant Extracts Against Periodontopathic Bacteria, *Phytother. Res.* 17 (2003) 599-604.
- [15] A. Szakiel, D. Ruszkowski, A. Grudniak, A. Kurek, K.I. Wolska, M. Doligalska, W. Janiszowska, Antibacterial and Antiparasitic Activity of Oleanolic Acid and its Glycosides Isolated from Marigold (*Calendula officinalis*), *Planta Med.* 74 (2008) 1709-1715.
- [16] R.B. Malabadi, S.V. Kumar, Assessment of Anti-dermatophytic Activity of Some Medicinal Plants, *Journal of Phytological Research.* 18 (2005) 103-106.
- [17] J.C. Chalchat, R.P. Garry, A. Michet, Chemical Composition of Essential Oil of *Calendula officinalis* L. (Pot Marigold), *Flavour And Fragrance Journal.* 6 (1991) 189-192.
- [18] O.O. Okoh, A.A. Sadimenko, A.J. Afolayan, The Effects of Age on the Yield and Composition of the Essential Oils of *Calendula officinalis*, *J. Appl. Sci.* 7 (2007) 3806-3810.
- [19] N. Crabas, B. Marongiu, A. Piras, T. Pivetta, S. Porcedda, Extraction, Separation and Isolation of Volatiles and dDyes from *Calendula officinalis* L. And *Aloysia triphylla* (L'Her.) Britton by Supercritical CO₂. *J. Essent. Oil Res.* 15 (2003) 350-355.
- [20] L. Petrović, Ž Lepojević, V. Sovilj, D. Adamović, V. Tešević, An Investigation of CO₂ Extraction of Marigold (*Calendula officinalis* L.), *J. Serb. Chem. Soc.* 72 (2007) 407-413.
- [21] Z.C. Gazim, C.M. Rezende, S.R. Fraga, B.P. Dias Filho, C.V. Nakamura, E. Garcia, D.A. Cortez, Analysis of the Essential Oils from *Calendula officinalis* Growing in Brazil Using Three Different Extraction Procedures, *Rev. Bras. Cienc. Farm.* 44 (2008) 391-395.
- [22] Z.C. Gazim, C.M. Rezende, S.R. Fraga, T.I. Svidzinski, E. Garcia, D.A. Cortez, Antifungal Activity of the Essential Oil from *Calendula officinalis* L. (Asteraceae) Growing in Brazil, *Braz. J. Microbiol.* 39 (2008) 61-63.
- [23] M. Toshiyuki, K. Akinobu, Y. Masayuki, Structures of New Ionone and Sesquiterpene Glycosides from Egyptian *Calendula officinalis*, *Chem. Pharm. Bull.* 49 (2001) 974-978.
- [24] H.I. El-Askary, M.R. Meselhy, A.M. Galal, Sesquiterpenes from *Cymbopogon proximus*, *Molecules.* 8 (2003) 670-677.
- [25] M. Stavri, K. Thomas Mathew, T. Gibson, R. Thomas Williamson, S. Gibbons, New Constituents of *Artemisia monosperma*, *J. Nat. Prod.* 67 (2004) 892-894.
- [26] G.T. Maatooq, Microbial Transformation of a Beta- and Gamma-eudesmols Mixture, *Z. Naturforsch C.* 57 (2002) 654-659.
- [27] F.A. Badria, B.R. Mikhaeil, G.T. Maatooq, M.A. Amer, Immunomodulatory Triterpenoids from the Oleogum Resin of *Boswellia carterii* Birdwood, *Z. Naturforsch C.* 58 (2003) 505-516.
- [28] P. Van Kiem, N. Tien Dat, C. Van Minh, J.J. Lee, Y. Ho Kim, Lupane-triterpenes from the Leaves of *Brassaiopsis glomerulata*, *Arch. Pharm. Res.* 26 (2003) 594-596.
- [29] M. Sholichin, K. Yamasaki, R. Kasai, O. Tanaka, ¹³C Nuclear Magnetic Resonance of Lupane-Type Triterpenes, Lupeol, Betulin and Betulinic Acid, *Chem. Pharm. Bull.* 28 (1980) 1006-1008.
- [30] H. Fuchino, T. Satoh, N. Tanaka, Chemical Evaluation of *Betula* Species in Japan. I. Constituents of *Betula ermanii*, *Chem. Pharm. Bull.* 43 (1995) 1937-1942.
- [31] P. Chumkaew, S. Kato, K. Chantrapromma, A New Triterpenoid Ester from the Fruits of *Bruguiera parviflora*, *Chem. Pharm. Bull.* 53 (2005) 95-96.
- [32] J.D. Su, T. Osawa, S. Kawakishi, M. Namiki, Antioxidative Flavonoids Isolated from *Osbeckia chinensis* L., *Agric. Biol. Chem.* 51 (1987) 2801-2803.
- [33] S.Y. Lim, J.Y. Hwang, H.R. Yoon, Y.S. Paik, Antioxidative Phenolics from the Petals of *Carthamus tinctorius*, *J. Appl. Biol. Chem.* 50 (2007) 304-307.