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Bioactive polymethoxylated flavonoids from *Chiliadenus montanus*

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ABSTRACT

*Phytochemical investigations of the CH_2Cl_2/MeOH (1:1) extract of air-dried aerial parts of Chiliadenus montanus afforded seven methoxylated flavonoids (**1-7**), three of them were isolated for the first time from the genus (**1-3**). Structures were established by spectroscopic methods, including HREIMS, ^1H, ^{13}C, DEPT, ^1H-^1H COSY, HMQC and HMBC NMR analyses. In the present study, three of the isolated compounds were tested for the ability to induce NAD(P)H:quinoneoxidoreductase 1 (NQO1) using a quantitative bioassay in a murine hepatoma cell line. Compound **1** revealed dose-dependent NQO1 inducing properties with a concentration that doubled the specific enzyme activity by 2-fold (CD value) of 7.0 μM, and a magnitude of induction of 3.3-fold at the highest concentration tested (100 μM).*

INTRODUCTION

South Sinai is an epicenter of medicinal plants in the Arabian Desert with active plant constituents serving as a focal point for ecologists, taxonomists and phytochemists alike from around the globe [1-6]. *Chiliadenus montanus* (Vahl.) Brullo[=*Jasonia montana*, *Varthemia montana* (Vahl.) Boiss.], an herbendogenous to the Sinai region of Egypt, is a member of the Asteraceae [7], popularly known as Haneida, is common in the Sinai Peninsula. This medicinal plant is traditionally used for chest diseases, diarrhea, renal troubles and stomachache [8]. Moreover, evidences for their hypoglycemic, antioxidant and anticholestatic activities have been recently investigated [9,10]. Previous phytochemical researchers have identified the presence of active constituents in the aerial parts, including phenolic compounds that give *C.montanus* their medicinal values [8, 11].

C. montanus is used as a herbal tea for the treatment of renal troubles and select chemical components have been shown to exhibit antimicrobial, anti-diabetic, antioxidant, antiatherogenic, antibacterial, antifungal and anti-obesity activities [12-14].

Flavonoids are well known as antioxidant and chelating properties raised for many factors such as: (a) multiple hydroxyl groups which confer substantial antioxidant, chelating and prooxidant activity, (b) methoxy groups which increase lipophilicity and membrane partitioning, and (c) occurance of a double bond and carbonyl function

suggested to increases activity by affording a more stable flavonoid radical through conjugation and electron delocalization. Many structure-activity relationships studies for natural metabolites support these functions [15-21].

NAD(P)H: quinone oxidoreductase1 (NQO1) is a chemoprotective enzyme catalyzing the reduction and detoxification of exogenous quinones, and may also be involved in scavenging superoxide in cells and prevention of oxidative recycling. Herein, phytochemical investigations of the CH_2Cl_2/MeOH (1:1) metabolites of *C. montans* air-dried aerial parts afforded seven polymethoxylated flavonoids (**1**-**7**), three of them are isolated for the first time from the genus (**1**-**3**). Structures were established by spectroscopic methods, and three of the isolated compounds (compounds **1**, **5** and **7**) were tested for NQO1 inducer activity.

EXPERIMENTAL SECTION

General procedures: Instrumentation included JEOL JMS-GCMATE mass spectrometer for EI-MS and HR-EI-MS; a JEOL JNM-ECA 600 spectrometer with tetramethylsilane as an internal standard for 1H (600 MHz) and 13C (150 MHz) NMR spectra. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography, silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); TLC, pre-coated TLC plates with silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254} (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF_{254} (Merck, 0.25 mm); and detection was achieved by spraying with (1:9) H_2SO_4-MeOH followed by heating.

Plant material

Air-dried aerial parts of *Chiliadenus montanus* (Vahl.) Brullo. were collected in 2013, from Wadi Gebal, North Sinai, Egypt. Plant material was identified by Dr. El-Bialy E. Hatab, Egyptian Environmental Affairs Agency, Nature Conservation Sector, Siwa Protected Area, Siwa, Egypt. A voucher specimen SK-1001 has been deposited in the Herbarium of Saint Catherine Protectorate, Egypt.

Extraction and isolation

Aerial parts (2.0 kg) of *C. montanus* were powdered and was percolated with CH_2Cl_2:MeOH (1:1) (10 L, 3 days x 2) at room temperature. The supernatants were combined and concentrated *in vacuo* to give a concrete (175 g), which was fractionated on a silica gel column (6 x 120 cm) eluting with n-hexane (3 L) followed by a gradient of *n*-hexane-$CHCl_3$ up to 100% $CHCl_3$ and then a $CHCl_3$–MeOH studies at up to 15% MeOH (3 L each of the solvent mixture) to yield 10 fractions.

Fraction **4**, (4.5 g) eluted with n-hexane–EtOAc (4:1) was subjected to a second silica gel column separation (120 cm x 3 cm) to afford compounds **2** (4 mg), **5** (50 mg),**6** (7 mg) and **7** (400 mg).Fraction **5**, (5.6 g) eluted with *n*-hexane–EtOAc (4:1) was subjected to a second silica gel column separation (120 cm x 3 cm) to afford compounds **1** (80 mg), **3** (6 mg) and **4** (5 mg).

5,7-Dihydroxy-3,3',4'-trimethoxyflavone (**1**): $C_{I8}H_{16}O_7$, ^{1}H NMR (600 MHz, $CDCl_3$): 7.69 (1H, d, *J* = 2.0 Hz, H-2'), 7.67 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 7.00 (1H, d, *J* = 8.2 Hz, H-5'), 6.44 (1H, d, H-8), 6.35 (1H, d, H-6) 3.97, 3.87 and 3.85 (9H, singlets, 3 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$):156.0 (C-2), 138.9 (C-3), 178.8 (C-4), 162.1 (C-5), 97.9 (C-6), 165.5 (C-7), 92.3 (C-8), 156.8 (C-9), 106.1 (C-10), 122.8 (C-l`), 111.0 (C-2`), 148.4 (C-3`), 146.4 (C-4`), 114.7 (C-5`), 122.5 (C-6`), 60.3 (3-OCH_3), 56.2, 55.9 (3`-$OCH_3$ and 4`-OCH_3).

5,4`-Dihydroxy-3,6,7,3`-tetramethoxyflavone (Chrysosplenetin) (**2**): $C_{I9}H_{18}O_8$, ^{1}H NMR (600 MHz, $CDCl_3$):7.72 (1H, dd, *J* = 8.4, 2.0, H-6`), 7.67 (1H, d, *J* = 2.0, H-2`), 6.99 (1H, d, *J* = 8.4, H-5`), 6.55 (1H, s, H-8), 4.00, 3.96, 3.95 and 3.84 (12H, singlets, 4 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$): 156.0 (C-2), 138.9 (C-3), 178.9 (C-4), 153.0 (C-5), 132.3 (C-6), 158.9 (C-7), 92.4 (C-8), 152.5 (C-9), 109.3 (C-10), 122.5 (C-1`), 110.9 (C-2`), 146.4 (C-3`), 148.5 (C-4`), 115.7 (C-5`), 122.9 (C-6`), 60.1 (OCH_3), 56.3 (OCH_3), 56.1 (OCH_3), 50.9 (OCH_3).

5,4`-Dihydroxy-3,7-dimethoxyflavone **(3)**: $C_{I7}H_{14}O_6$, $^1$H NMR (600 MHz, $CDCl_3$):7.99 (1H, d, *J* = 8.80, H-2`, 6`), 6.88 (1H, d, *J* = 8.90, H-3`, 5`), 6.60 (1H, s, H-8), 6.31 (1H, s, H-6), 3.87 and 3.77 (6H, singlets, 2 OCH_3).

Centaureidin (**4**): $C_{I8}H_{16}O_8$, ^{1}H NMR (600 MHz, $CDCl_3$): 7.67 (1H, d, *J* = 2.0 Hz, H-2'), 7.66 (1H, d, *J* = 2.0 Hz, H-6'), 7.04 (1H, d, *J* = 8.2 Hz, H-5'), 6.54 (1H, s, H-8), 3.83, 3.96 and 4.02 (9H, singlets, 3 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$):152.4 (C-2), 137.9 (C-3), 178.9 (C-4), 152.4 (C-5), 131.3 (C-6), 157.4 (C-7). 93.7 (C-8), 156.5 (C-9), 105.5 (C-10).122.3 (C-1`). 115.1 (C-2`), 147.5 (C-3`), 149.7 (C-4`), 111.5 (C-5`), 121.5 (C-6`), 59.3, 59.7 (3-OCH_3 and 6-OCH_3), 55.2 (7- OCH_3).

5,7-Dihydroxy-3,6,3',4'-tetramethoxyflavone (bonanzin) (**5**): $C_{I9}H_{18}O_8$, ^{1}H NMR (600 MHz, $CDCl_3$): 7.69 (1H, d, *J* = 2.0 Hz, H-2'), 7.65 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 7.04 (1H, d, *J* = 8.2 Hz, H-5'), 6.49 (1H, s, H-8), 3.97, 3.95, 3.91 and 3.85 (12H, singlets, 4 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$): 152.9 (C-2), 138.0 (C-3), 178.9 (C-4), 152.4 (C-5). 132.4 (C-6), 158.9 (C-7), 90.4 (C-8), 156.1(C-9), 106.7 (C-10), 122.7(C-l`), 114.7 (C-2`), 146.4 (C-3`), 148.4 (C-4`), 111.0 (C-5`), 122.5 (C-6`) 60.9, 60.3 (3-OCH_3 and 6-OCH_3), 56.4, 56.2 (7-OCH_3 and 4`-OCH_3).

5,3`,4`-Trihydroxy-3,6,7-trimethoxyflavone(chrysosplenol-D) (**6**): $C_{I8}H_{16}O_8$, ^{1}H NMR (600 MHz, $CDCl_3$): 7.60 (1H, d, *J* = 2.0 Hz, H-2'), 7.53 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 6.92 (1H, d, *J* = 8.2 Hz, H-5'), 6.54 (1H, s, H-8), 3.82, 3.76 and 3.71 (9H, singlets, 3 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$): 152.9 (C-2), 137.9 (C-3), 178.7 (C-4), 152.1 (C-5). 131.7 (C-6), 157.8 (C-7), 94.6 (C-8), 156.1 (C-9), 105.1 (C-10), 122.7(C-l`), 116.2 (C-2`), 148.0 (C-3`), 150.3(C-4`), 112.6 (C-5`), 121.3 (C-6`) 60.5, 60.2 (3-OCH_3 and 6-OCH_3) and 56.2 (4`-OCH_3).

5-Hydroxy-3,6,7,3`,4`-pentamethoxy flavone(artemetin) **(7)**. $C_{20}H_{20}O_8$, ^{1}H NMR (600 MHz, $CDCl_3$): 7.69 (1H, d, *J* = 2.0 Hz, H-2'), 7.66 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 7.0 (1H, d, *J* = 8.2 Hz, H-5'), 6.49 (1H, s, H-8), 3.97, 3.95, 3.92, 3.91 and 3.85 (15H, singlets, 5 OCH_3).
^{13}C NMR (125 MHz, in $CDCl_3$): 152.8 (C-2), 138.7 (C-3), 178.9 (C-4), 152.4 (C-5), 130.5 (C-6), 158.8 (C 7), 90.4 (C-8), 156.2 (C-9), 106.7 (C-10), 122.8 (C-l`), 115.8 (C-2`), 146.5 (C-3`), 148.5 (C-4), 114.7 (C-5`), 122.7 (C-6`), 60.3, 60.9 (3-$OCH_3$ and 6-$OCH_3$) 56.4, 56.4, 56.2 (7-OCH3, $_3$`-OCH_3 and 4`-OCH_3).

NQO1 inducer activity
We employed a robust quantitative NQO1 microtiter plate bioassay that is based on previously published method [22]. Murine hepatoma Hepa1c1c7 cells were grown as monolayer in α-MEM supplemented with 10% (v/v) of heat- and charcoal-inactivated fetal bovine serum. Cells were routinely maintained in a humidified incubator at 37 °C, 5% CO_2. For each experiment, cells (10,000 per well) were seeded onto 96-well plates and incubated for 24 h to form a sub-confluent monolayers. After 24 h, cell monolayers were treated with either vehicle (DMSO at 0.1%, v/v) or serial dilutions (0.78-100 µM) of the compounds in octuplet wells. Treated cells were incubated for a further 48 h. At the end of the 48 h exposure period, cells were lysed for 30 min at 25 °C in digitonin (0.8 g/L, pH 7.8). The specific activity of NQO1 was evaluated in cell lysates using menadione as a substrate. Protein concentrations were determined in each well by the BCA protein assay (Thermo Scientific). Sulforaphane, a potent NQO1 inducer was used as a positive control.

RESULTS AND DISCUSSION

Seven known methylated flavonols were identified by comparing their spectral data with published data as 5,7-dihydroxy-3,3',4'-trimethoxyflavone (**1**) [23], 5,4`-dihydroxy-3,6,7,3`-tetramethoxyflavone (Chrysosplenetin) (**2**) [24-26], 5,4`-dihydroxy-3,7-dimethoxyflavone (**3**) [27], Centaureidin (**4**) [28-30], 5,7-dihydroxy-3,6,3',4'-tetramethoxyflavone (bonanzin) **(5)** [31], 5,3`,4`-trihydroxy-3,6,7-trimethoxyflavone (chrysosplenol-D) (**6**) [32, 33], and 5-Hydroxy-3,6,7,3`,4`,-pentamethoxy flavones (artemetin) (**7**) [34, 35]. It is note worthy that three of them are isolated for the first time from this genus (**1-3**). The structure formulae of the isolated flavonoid are displayed in Fig.1. The yield of the isolated compounds permits only the biological testing of three compounds **1**, **5** and **7**.

Flavonoids are comprising a wide class of phytochemicals with diverse health benefits against several diseases such as cronary heart disease and cancer [22, 36]. In the present study we tested the potential of three of the isolated flavones for their potential to induce the activity of the chemopreventive enzyme NQO1. Compound **1** showed a pronounced dose-dependent induction of NQO1 with a CD value of 7.0 µM and a magnitude of induction of 3.3 fold over that of vehicle control at the highest tested concentration (100 µM). However, both compound **5** and compound **7** were unable to induce NQO1 enzymatic activity at the tested concentration range (Fig.2).

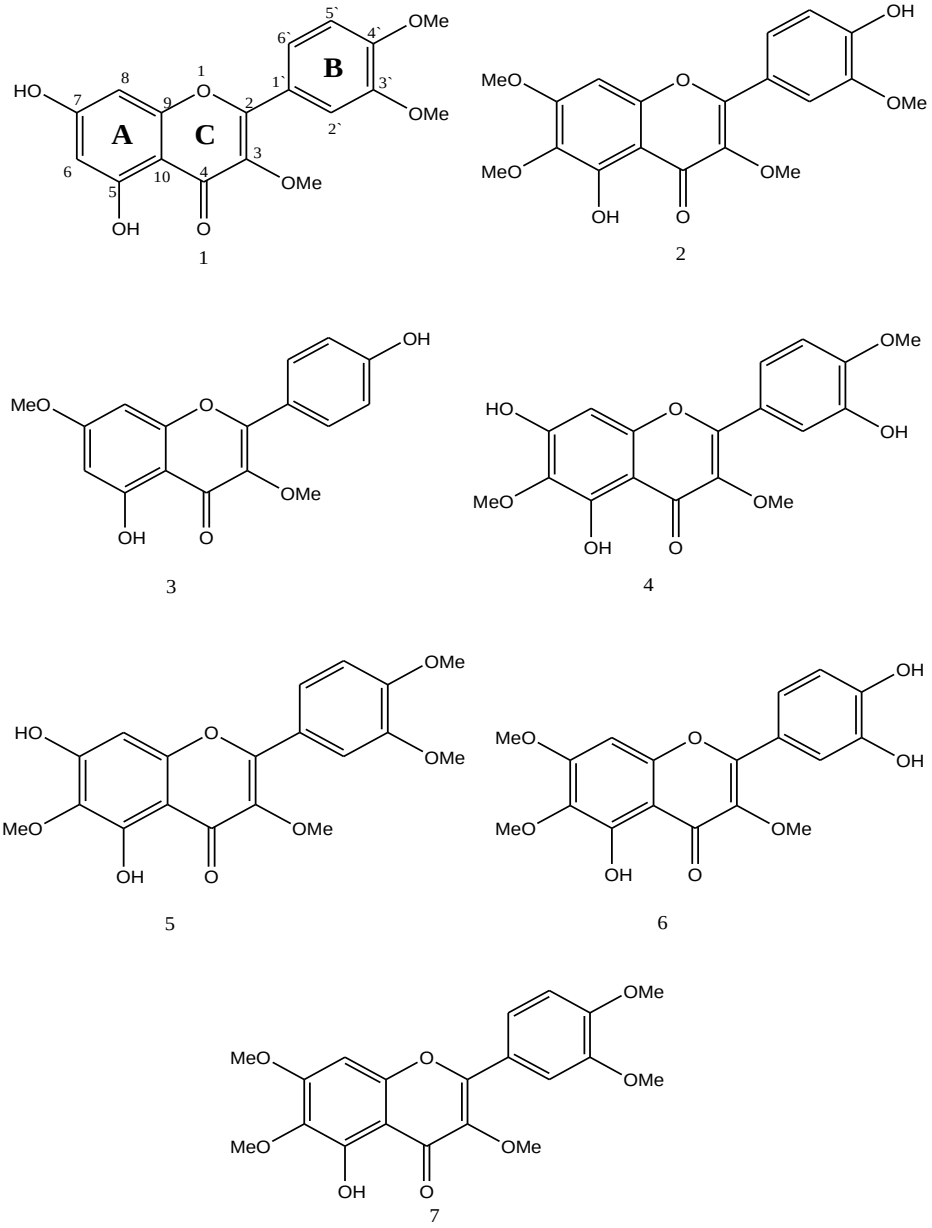

Fig. 1 Chemical structure of the isolated flavonoid compounds 1-7

Compound	CD (μM)
1	7.00
5	>100
7	>100

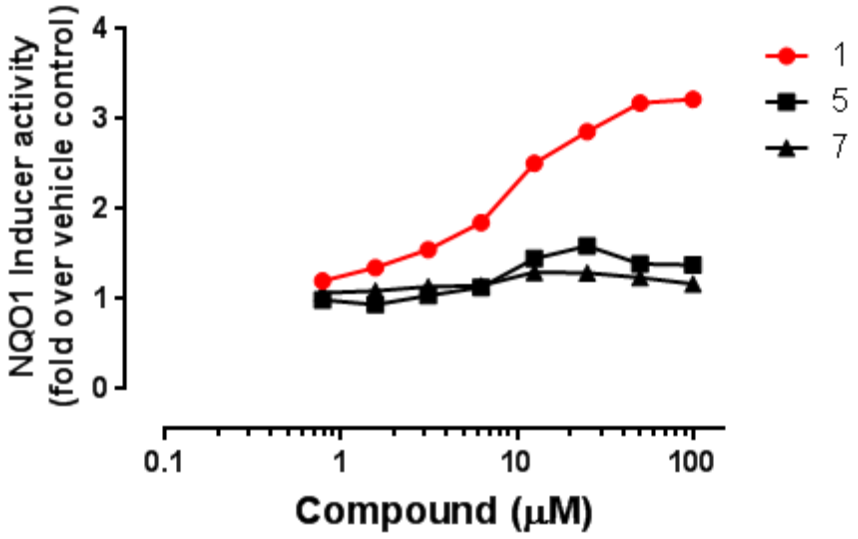

Fig. 2 NQO1 inducer activity of compounds 1, 5 and 7. Monolayers of Hepa1c1c7 cells were treated with increasing concentrations as indicated of compound 1, 5 or 7 for 48 h and analysed for NQO1 inducer activity as described in the Experimental Section. Data are means of octuplet measurement. CD value is the concentration needed to double the NQO1 activity of the vehicle control (0.1% DMSO)

Comparing the structures of the three tested compounds (Fig.1), the inducer activity of compound **1** could be largely correlated with the free hydroxyl groups at C-5 and C-7 of the A-ring of its flavones structure. However, the inducer activity is diminished by methoxylation of C-6 (compound **5**) and additional methylation of the hydroxyl group at C-7 (compound **7**). It is also obvious that substitutions at B-ring of the flavones structure of these compounds have no effect on the NQO1 inducer potency of the flavones. These structure–activity relationships are in agreement with previous reports that concluded that substitutions at B-ring of a flavone by hydroxylation and/or methoxylation have no effect on the activity of the compound as NQO1 inducer [37, 38]. The muted NQO1 inducion (compound **5** and compound **7)** due to the methoxylation at C-6 or both C-6 and C-7 is supported with the previous report of Tsuji and coworkers who analysed the structure-activity relationship of 37 flavonoids as indirect antioxidants in the same *in vitro* murine model of NQO1 induction [38].

CONCLUSION

The fractionation of CH_2Cl_2/MeOH (1:1) extract of *C. montanus* afforded 7 methoxylated flavonoids (**1-7**), three of which are isolated for the first time from the genus. One of three tested compounds induced the chemopreventive enzyme NQO1 in Hepa1c1c7 cells. The study confirmed the structure prerequisite of the substitutions at the flavonoid A-ring for the NQO1 inducer activity.

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REFERENCES

[1] RH Abd El-Wahab, MS Zaghloul, AA Moustafa. Proceedings of First International Conference on Strategy of Egyptian Herbaria, *Giza, Egypt,* **2004,** pp. 231–251.
[2] C Bailey, A Danin. *Econ Bot,* **1981**, 35, 145–162.
[3] L Boulos, Medicinal Plants of North Africa, References Publication, Inc., Algonac, Michigan, **1983,** pp. 286.

[4] D Bown, Encyclopedia of Herbs and Their Uses, Dorling Kindersley, London, **1995.**
[5] KH Batanouny, E Aboutabl, M Shabana, F Soliman. Wild Medicinal Plants in Egypt. An Inventory to Support Conservation and Sustainable Use, Palm Press, Cairo, Egypt, **1999**, pp. 207.
[6] Y Hanafi, M Abdel-Waha. Wild medicinal Plants in Sinai. Arabian Gulf of Est. Egypt, **2000**, pp. 337.
[7] L Boulos. Flora of Egypt, volume 3, Al hadara publishing, Cairo, Egypt, **2002**, pp. 373.
[8] V Tâckholm. Students Flora of Egypt. Cooperative printing company Beirut. Cairo University, Cairo, **1974**.
[9] MA Hussein. *Saudi Pharm J*, **2008**, 16, 214–221.
[10] MA Hussein, SM Abdel-Gawad. *Saudi Pharm J,* **2010**, 18, 27–33.
[11] L Boulos, DJN Hind. Compositae, Flora of Egypt. (Verbenaceae - Compositae). El Cairo, Al Hadara, **2002**.
[12] TA Al-Howiriny, AJ Al-Rehaily, JR Pols, JR Porter, JS Mossa, B Ahmed. *Nat Prod Res,* **2005**, 9, 253–265.
[13] MA Hussein. *Free Radicals Antioxid***, 2011**, 1, 49–60.
[14] MF Hegazy , H Matsuda, S Nakamura, TA Hussein, M Yoshikawa, PW Paré. *Phytochemistry*, **2014,** 103, 154–161.
[15] O Farkas, J Jakus, K Héberger. *Molecules*, **2004**, 9, 1079-88.
[16] CA Rice-Evans, NJ Miller, G Paganga. *Free Rad Bio Med*, **1996**, 20, 933-956.
[17] KE Heim, AR Tagliaferro, DJ Bobilya. *J Nut Biochem*, **2002**, 13, 572-584.
[18] JB Harborne, CA Williams. *Phytochemistry*, **2000**, 55, 481-504.
[19] EJ Lien, SJ Ren, HYH Bui, RB Wang. *Free Rad Biol Med*, **1999**, 26, 285-297.
[20] D Amić, D Davidovic-Amić, D Bešlo, N Trinajstić. *Croat Chem Acta*, **2003**, 76, 55-61.
[21] JG Yang, BG Liu, GZ Liang, ZX Ning. *Molecules*, **2008** 23, 14, 46-52.
[22] JW Fahey, AT Dinkova-Kostova, KK Stephenson, P Talalay. *Methods Enzymol*, **2004**, 382, 243–58.
[23] JF Stevens, E Wollenweber, M Ivancic, VL Hsu, S Sundberg, ML Deinzer. *Phytochemistry*, **1999**, 51, 771-780.
[24] M Shimizu, N Morita. *Yakugaku Zasshi*, **1969**, 89, 702.
[25] H Saglam, T Gozler, B Gozler. *Fitoterapia*, **2003**, 74, 564–569.
[26] M-Y Lu, Z-X Liao, L-J, H-F Sun. *Fitoterapia*, **2013**, 85, 119–124.
[27] H Dong, Y-L Gou, S-G Cao, S-X Chen, K-Y Sim, S-H Goh. *Phytochemistry*, **1999**, 50, 899-902.
[28] MJ Abad, P Bermejo, A Villar, S Valverde. *J Nat Prod*, **1993**, 56, 1164–1167.
[29] JA Beutler, JH Cardellina, CM Lin, E Hamel, GM Cragg, MR Boyd. *BioMed Chem Lett*, **1993**, 3, 581–584.
[30] C Long, P Sauleau, B David, C Lavaud, V Cassabois, F Ausseil, G Massiot. *Phytochemistry*, **2003**, 64, 567–569.
[31] EA Kul'magambetova, LN Pribytkova, SM Adekenov. *Chem Nat Compd*, **2000**, 36, 95.
[32] M Ono, C Masuoka, Y Odake, S Ikegashira, Y Ito, T Nohara. *Food Sci Technol Res*, **2000**, 6, 106–114.
[33] BR Michael, SR. Gedara, MM. Amer, L Stevenson, AF Ahmed. *Nat Prod Res*, **2014**, 28, 909–913.
[34] H-Y Gao, H-Y Wang, G-Y Li, X-w Du, X-T Zhang, Y Han, J Huang, X-x Li, J-h Wang. *Phytochem Lett*, **2014**, 7, 150–155.
[35] A-U Rahman, D Ahmed, M Iqbal, Choudhary, S Turkoz, B Sener, *Planta Med*, **1988**, 54, 173.
[36] C Rice-Evans. *Curr Med Chem*, **2001**, 8, 797-807.
[37] Y Uda, KR Price, G Williamson, MJC Rhodes. *Cancer Lett*, **1997**, 120, 213–216.
[38] PA Tsuji, KK Stephenson, KL Wade, H Liu, JW Fahey. *Nutr Cancer*, **2013**, 65, 1014-25.