

## Characterization and Antiglycation Activity of Phenolic Constituents from *Viscum album* (European Mistletoe)

M. Iqbal CHOUDHARY,<sup>\*,a</sup> Saima MAHER,<sup>a</sup> Afshan BEGUM,<sup>a</sup> Ahmed ABBASKHAN,<sup>a</sup> Sajjad ALI,<sup>a</sup> Ambreen KHAN,<sup>a</sup> Shafique-ur-REHMAN,<sup>b</sup> and Atta-ur-RAHMAN<sup>\*,a</sup>

<sup>a</sup>H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi; Karachi-75270, Pakistan; and <sup>b</sup>Department of Botany, University of Azad Jammu and Kashmir; Muzafarabad-13100, Pakistan. Received February 2, 2010; accepted February 4, 2010; published online April 2, 2010

4'-O-[ $\beta$ -D-Apioeryl(1 $\rightarrow$ 2)]- $\beta$ -D-glucosyl]-5-hydroxyl-7-O-sinapylflavanone (1), 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2E-propenyl- $\beta$ -D-glucopyranoside (2), 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2E-propenyl- $\beta$ -D-glucopyranoside (3), 5,7-dimethoxy-4'-O- $\beta$ -D-glucopyranoside flavanone (4), 4',5-dimethoxy-7-hydroxy flavanone (5), and 5,7-dimethoxy-4'-hydroxy flavanone (6), were isolated from the organic extracts of *Viscum album* L. (European Mistletoe). These compounds were studied for their anti-glycation and antioxidant properties. The structures of new compounds 1 and 2 were deduced on the basis of spectroscopic evidence.

**Key words** *Viscum album*; European Mistletoe; Loranthaceae; anti-glycation activity; antioxidant activity; phenylpropanoid

*Viscum album* L. (Loranthaceae), commonly known as European Mistletoe, is a bi-perennial shrub, widely distributed in tropical and sub-tropical regions of Africa, Asia and Europe.<sup>1,2)</sup> It is commonly found as a semi parasite on trees. Some species of the genus *Viscum* are used in folk medicines for the treatment of diabetes, jaundice, indigestion, common fever and asthma.<sup>3)</sup> In Pakistan, it is found in Neelam valley (Azad Kashmir) as a hemi-parasite on the trees of *Juglans regia* (Walnut).

A number of biological activities of mistletoe, including immunostimulatory and antitumor activity, have been reported.<sup>4)</sup> Previous investigation resulted in the isolation of lectins, viscotoxins, alkaloids, amines, flavonoids, acids and terpenoids.<sup>5,6)</sup> Anti-glycation potential of the methanolic extract of *V. album* was also reported earlier by Gray and Flat.<sup>7)</sup>

During the biological screening of medicinal herbs of Pakistani origin, the crude extract of *V. album* showed a significant anti-glycation activity (Table 1). Non-enzymatic glycation of proteins is a process that impairs the functioning of biomolecules and leads to a variety of endocrine disorders. Free radicals also contributed in the formation of advanced glycation end products (AGEs).<sup>8)</sup> Eventually this may lead to oxidative stress and a variety of degenerative diseases, such as cancer, diabetes, inflammation and aging.<sup>9)</sup> The elimination or inactivation of reactive oxygen species (ROS) is considered to be an instrumental approach to reduce the risk of these diseases.<sup>10)</sup>

The present work resulted in the isolation of compounds 1—6, which were found to possess anti-glycation activity, whereas compounds 2 and 5 exhibited anti-oxidant activities. Among the recently isolated compounds flavanone glycoside (1) and phenyl propanoid (2), were found to be new. The structures of the isolated compounds were elucidated mainly with the help of NMR spectroscopic techniques.

### Results and Discussion

The air dried powdered plant material was extracted with 80% aqueous methanol. The flavanone 1 and phenyl propanoid 2 were isolated from the *n*-BuOH and EtOAc extracts of the plant, respectively.

Compound 1 was obtained as a colorless powder. The IR

Table 1. <sup>1</sup>H- and <sup>13</sup>C-NMR Chemical Shift Data for Compounds 1 and 2

Compound 1		Compound 2	
$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1	—	1	—
2	5.23 (dd, $J=12.8, 3.1$ Hz)	2	6.74 (s)
3	2.64 (dd, $J=17.8, 3.1$ Hz)	3	—
	2.86 (dd, $J=17.8, 12.8$ Hz)		
4	—	4	—
5	—	5	—
6	6.01 (d, $J=2.3$ Hz)	6	6.75 (s)
7	—	7	6.57 (d, $J=15.0$ Hz)
8	5.97 (d, $J=2.3$ Hz)	8	6.36 (dt, $J=15.0, 5.54$ Hz)
9	—	9	4.22 ( $J=2.0$ Hz)
10	—	1'	4.86 (d, $J=7.2$ Hz)
1'	—	2'	3.38 (t, $J=7.2$ Hz)
2'	7.31 (d, $J=8.6$ Hz)	3'	3.38 (t, $J=7.8$ Hz)
3'	7.02 (d, $J=8.7$ Hz)	4'	3.41 (dd, $J=7.0, 4.3$ Hz)
4'	—	5'	3.44 (m)
5'	7.31 (d, $J=8.6$ Hz)	6'	3.82 ( $J=7.5, 11.5$ Hz)
6'	7.02 (d, $J=8.6$ Hz)	OAc	1.88 (s)
		(OC=OCH <sub>3</sub> )	30.7
1''	—		
2''	6.82 (s)		
3''	—		
4''	—		
5''	—		
6''	6.82 (S)		
7''	7.59 (d, $J=15.8$ Hz)		
8''	6.31 (d, $J=15.8$ Hz)		
9''	—		
OCH <sub>3</sub>	3.82 (s)		
<b>Glucose</b>			
C-1'''	4.93 (d, $J=7.9$ Hz)		100.5
C-2'''	3.64 (dd, $J=9.2, 7.5$ Hz)		78.1
C-3'''	3.99 (t, $J=9.2$ Hz)		77.7
C-4'''	3.84 (dd, $J=9.2, 6.8$ Hz)		71.6
C-5'''	3.40 (m)		71.4
C-6'''	3.67 (dd, $J=14.3, 7.3$ Hz)		62.3
<b>Apiose</b>			
C-1''''	5.54 (d, $J=1.4$ Hz)		110.3
C-2''''	3.91 (d, $J=1.4$ Hz)		75.8
C-3''''	—		79.0
C-4''''	4.24 (d, $J=11.5$ Hz)		78.8
C-5''''	4.39 (d, $J=11.3$ Hz)		67.5

<sup>1</sup>H-NMR (400 MHz in CD<sub>3</sub>OD), <sup>13</sup>C-NMR (100 MHz in CD<sub>3</sub>OD).

\* To whom correspondence should be addressed. e-mail: hej@cyber.net.pk

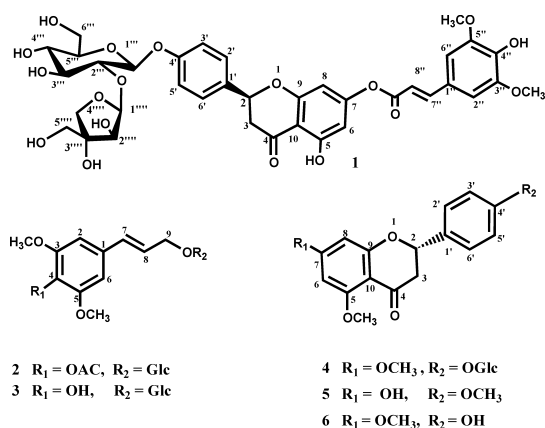


Fig. 1. Structures of the Compounds 1—6

spectrum showed absorptions at 3412 (OH), 1668 ( $\alpha,\beta$ -unsaturated carbonyl), and 1606, 1520  $\text{cm}^{-1}$  (C=C). The UV spectrum showed absorptions at 326 and 283 nm, characteristic of a flavanone skeleton and sinapyl group, respectively. The HR-fast bombardment (FAB)-MS (+ve) showed the  $[M+H]^+$  at  $m/z$  773.1073, corresponding to the formula  $C_{37}H_{41}O_{18}$  (Calcd 773.1079). The electron ionization-mass spectra (EI-MS) showed the presence of a sinapyl moiety as inferred from the fragment ion  $C_{11}H_{11}O_4^+$  at  $m/z$  207. The presence of sugar moieties (apiose and glucose) was inferred by the of  $[M-462]^+$  at  $m/z$  311 in HR-FAB-MS (+ve). The  $^1\text{H-NMR}$  spectrum of **1** showed the presence of three mutually coupled protons double doublet at  $\delta_{\text{H}}$  2.64 (1H,  $J=17.8$ , 3.1 Hz) and 5.23 (1H,  $J=12.8$ , 3.1 Hz), corresponding to the C-3 methylene and C-2 methine proton of ring C of flavanone, respectively. A 2H doublet at  $\delta_{\text{H}}$  7.31 with the coupling constant 8.6 Hz was assigned to C-3' and C-5' aromatic protons. Similarly, another 2H doublet at  $\delta_{\text{H}}$  7.02 with the coupling constant 8.6 Hz was assigned to C-2' and C-6' magnetically equivalent aromatic protons. Two *meta* coupled protons of ring A appeared at  $\delta_{\text{H}}$  5.97 (1H,  $J=2.3$  Hz) and 6.01 (1H,  $J=2.3$  Hz), corresponding to the C-8 and C-6 protons, respectively. The presence of a sinapyl moiety in compound **1** was inferred from an AB doublets of the *trans* olefinic protons at  $\delta_{\text{H}}$  7.59 (1H,  $J=15.8$  Hz) and 6.31 (1H,  $J=15.8$  Hz) and a two proton singlet at  $\delta_{\text{H}}$  6.82, assigned to the C-2'' and C-6'' protons of the phenyl ring. Two methoxy groups were inferred from a singlet at  $\delta_{\text{H}}$  3.82 (s, 6H). The  $^1\text{H-NMR}$  spectrum also showed signals ( $\delta_{\text{H}}$  3.40—5.54) corresponding to sugar moieties. Two anomeric protons appeared as doublets at  $\delta_{\text{H}}$  5.54 ( $J=1.4$  Hz) and 4.93 ( $J=7.9$  Hz), were in agreement with those of apiose and glucose moieties.<sup>11</sup> The carbon signals for sugars were also characteristic of  $\beta$ -D-glucopyranosyl and  $\beta$ -D-apiofuranosyl sugars.<sup>1</sup>

The structure of compound **1** was finally deduced by detailed interpretation of 2-D NMR data. In the heteronuclear multiple bond correlation (HMBC) spectrum, the signal at  $\delta_{\text{H}}$  4.93 (C-1'') was  $^3J$  correlated with  $\delta_{\text{C}}$  158.9 (C-4'), whereas the apiose anomeric proton ( $\delta_{\text{H}}$  5.54) showed a  $^3J$  connectivity with C-2''' ( $\delta_{\text{C}}$  78.1), suggesting the attachment of apiose at C-2''' of glucose. This was further inferred from the downfield shift of C-2 of the glucose ( $\delta_{\text{C}}$  79.1). The NMR data of sugar was in agreement with those of apiose and glucose moieties.<sup>12</sup> Characteristic signals for  $\alpha,\beta$ -unsat-

urated carbonyl ( $\delta_{\text{C}}$  162.8), and two methine carbons ( $\delta_{\text{C}}$  147.9, 115.5) indicated the presence of a sinapyl moiety. A bathochromic shift of band II (*ca.* 24 nm) in the presence of shift reagent ( $\text{AlCl}_3/\text{HCl}$ ) indicated C-5 hydroxyl functionality.<sup>13</sup> The only possible position of sinapyl moiety in ring A at OH of C-7 was deduced from the downfield shift of C-7 ( $\delta_{\text{C}}$  164.3).<sup>14</sup>

The nature of glycosides was deduced by the hydrolysis (see Experimental). The stereochemistry at C-2 position was assigned as *S* on the basis of the circular dichroism (CD) spectrum, in which compound **1** exhibited a positive Cotton effect at 330 nm and a negative Cotton effect at 286 nm.<sup>11</sup> Based on above mentioned evidences, the new compound was deduced as 4'-O- $[\beta$ -D-apiosyl(1 $\rightarrow$ 2)]- $\beta$ -D-glucopyranosyl]-5-hydroxyl-7-O-sinapylflavanone (**1**).

Compound **2** was isolated as a white amorphous powder. The IR spectrum showed absorptions at 3385, 1735, 1660 and 1593  $\text{cm}^{-1}$ , indicating the presence of hydroxyl group, carbonyl ester and an aromatic ring, respectively. UV spectrum showed absorption indicative of the presence of 261 (3.45), 242 (3.77), 203 (4.05), 189 (4.07). The HR-FAB-MS (+ve) showed  $[M+H]^+$  at  $m/z$  415.1522 corresponded to the formula  $C_{19}H_{27}O_{10}$  (Calcd 415.1526). The overall spectral data of compound **2** closely resembled to a known compound, 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2*E*-propenyl- $\beta$ -D-glucopyranoside (**3**),<sup>15</sup> the only difference between the two compounds being the appearance of signals for acetate (O-C=OCH<sub>3</sub>) moiety in the NMR spectra of **2**. A downfield methyl singlet at  $\delta_{\text{H}}$  1.88 indicated the presence of an acetate moiety, whereas a downfield signal in  $^{13}\text{C-NMR}$  spectrum at  $\delta_{\text{C}}$  180.0, along with a methyl signal at  $\delta_{\text{C}}$  30.7, further supported the presence of acetate functionality.

The substitution of the acetate moiety at C-4 was deduced from the downfield shift of C-4 ( $\delta$  135.3). The above spectral evidence and comparison of data supported the structure of compound **2** as 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2*E*-propenyl- $\beta$ -D-glucopyranoside.

The known compounds, isolated from *V. album*, were identified as 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2*E*-propenyl- $\beta$ -D-glucopyranoside (**3**),<sup>15</sup> 5,7-dimethoxy-4'-O- $\beta$ -D-glucopyranoside flavanone (**4**),<sup>12</sup> 4',5-dimethoxy-7-hydroxy flavanone (**5**),<sup>16</sup> and 5,7-dimethoxy-4'-hydroxy flavanone (**6**).<sup>17</sup> The structures of these compounds were determined by comparing the EI-MS,  $^1\text{H}$ - and  $^{13}\text{C-NMR}$  data with the reported data.

**Biological Activities** The methanolic extract of *V. album* showed a potent anti-glycation activity, *i.e.* 72.5% ( $\text{IC}_{50}=199.85\pm 0.067\ \mu\text{M}$ ). Compounds **1—6** showed activities against the formation of AGEs (Table 2) the antioxidant activity of these compounds was evaluated by using a superoxide anion scavenging assay. Compounds **2** ( $\text{IC}_{50}=211.69\pm 7.11\ \mu\text{M}$ ) and **5** ( $\text{IC}_{50}=58.36\pm 2.9\ \mu\text{M}$ ) were found to be active in this assay. The antioxidant potential of compound **5** was determined to be greater than that of standard (Table 3). *n*-Propylgallate was used as a standard reference ( $\text{IC}_{50}=67.5\pm 0.9\ \mu\text{M}$ ). In conclusion, we report here for the first time, anti-glycation properties of the plant phenolics, obtained from *V. album*.

#### Experimental

**General Methods** Unless otherwise stated, the following procedures were adopted. UV spectra were measured on a Shimadzu UV240 machine in

Table 2. Anti-glycation Activity of Compounds of *V. album*

Compounds/Extracts	Inhibition (%)	IC <sub>50</sub> (μM) ± S.E.M. <sup>b)</sup>
Methanolic extract	72.5	199.8 ± 0.1
Ethyl acetate extract	66.5	270.7 ± 0.4
Butanolic extract	61.45	689.4 ± 0.5
1	74.5	264.5 ± 0.9
2	71.36	255.4 ± 0.5
3	72.92	413.9 ± 0.5
4	71.4	345.6 ± 0.8
5	74.62	264.5 ± 0.7
6	73.8	405.8 ± 0.8
Rutin <sup>a)</sup>	85.9	67.5 ± 0.9

a) Standard. b) Results are reported in ± standard error of mean of three experiments.

Table 3. Superoxide Anion Scavenging Activity of Compounds of *V. album*

Compounds	Inhibition (%)	IC <sub>50</sub> (μM) ± S.E.M. <sup>b)</sup>
2	74.75	211.7 ± 7.0
5	95.40	58.5 ± 3.0

a) Standard. b) Results are reported in ± standard error of mean of three experiments.

MeOH solutions as  $\lambda_{\max}$  nm (log  $\epsilon$ ). IR spectra were recorded as KBr discs on a JASCO A-302 spectrometer and presented in  $\text{cm}^{-1}$ . <sup>1</sup>H- and <sup>13</sup>C-NMR, HMQC and HMBC spectra were recorded on a Bruker AV-400 spectrometer, operating at 400 (<sup>1</sup>H) and 100 (<sup>13</sup>C) MHz in CD<sub>3</sub>OD. The chemical shifts values were reported in  $\delta$  (ppm), referenced with respect to the residual solvent signal of CD<sub>3</sub>OD and coupling constants (*J*) were measured in Hz. EI-MS were taken at 70 eV on a Finnigan MAT-112 or MAT-312 instrument and major ions are presented as *m/z* (%). FAB-MS were measured as a glycerol matrix on a JEOL HX110 Mass spectrometer. TLC purification was carried out on pre-coated silica gel cards (E. Merck) and the spots were observed first under UV (254 nm) and then sprayed with cerium(IV) sulfate reagent and heated until coloration developed. Recycling preparative HPLC (RPH-PLC) was used for final purification (JAI LC-908W, Japan Analytical Industry Co., Ltd.) with a column YMC ODS H-80 or L-80 (YMC, Japan).

**Plant Material** The whole plant of *Viscum album* L. was collected from walnut (*Juglans regia*) trees from Kundershah, Neelum Valley, Azad Kashmir, on the 15th of August 2002, and identified by Prof. Shafiq-ur-Rehman. A voucher specimen (No. Azbuherb 231) was deposited in the Herbarium of the University of Azad Jammu and Kashmir, Muzaffarabad.

**Extraction and Isolation** The methanolic crude extract (45 g) was suspended in dist. H<sub>2</sub>O and partitioned with EtOAc (31×3) and *n*-BuOH (31×3) successively, yielding EtOAc (1.2 g), and *n*-BuOH extracts (19.4 g). The EtOAc extract was subjected to column chromatography over silica gel and eluted with CHCl<sub>3</sub> and MeOH in a gradient manner (mention different proportions of solvents) to afford eight fractions (E1—E8). Repeated column chromatography of fraction E-2 (100 mg) over silica gel using the solvent system CHCl<sub>3</sub> and MeOH (10%, 600 ml) yielded compounds 6 (5 mg, 1.6×10<sup>-4</sup>%) and 4 (6 mg, 2.0×10<sup>-4</sup>%). The third fraction (E-3, 60 mg) was again chromatographed using a silica gel and eluted with 20% MeOH-CHCl<sub>3</sub> (600 ml) to yield 5 (3 mg, 1.0×10<sup>-4</sup>%). Fractions E-4 (23 mg) and E-5 (45 mg) were combined and subjected to polyamide column chromatography and eluted with 100% CHCl<sub>3</sub>, followed by gradual increase of polarity with MeOH. This led to five sub-fractions (1—5). Among these, sub-fraction 4 was subjected to silica gel column chromatography using 30% MeOH in CHCl<sub>3</sub> (300 ml) as eluent to obtain compounds 3 (10 mg, 3.33×10<sup>-4</sup>%) and 2 (5.5 mg, 2.5×10<sup>-4</sup>%).

A part of *n*-BuOH fraction (10 g) was passed through a Diaion HP-20 column and eluted with 100% H<sub>2</sub>O (2.7 g), H<sub>2</sub>O-MeOH (1 : 1, 3.5 g), and 100% MeOH (2.0 g). The fraction eluted with H<sub>2</sub>O-MeOH (1 : 1) was subjected to

polyamide column chromatography and eluted with 15% MeOH-CHCl<sub>3</sub> which yielded two major fractions (P1, 1 g and P2, 575 mg). The sub-fraction P1 was further subjected to Sephadex LH-20 column chromatography and eluted with H<sub>2</sub>O-MeOH (1 : 1), to afford three fractions (7—10). Fraction 7 (15 mg) was loaded onto RHPLC (L-80, H<sub>2</sub>O : MeOH, 1 : 1, 4 ml/min) to yield compound 1 (3 mg, 1.0×10<sup>-4</sup>%, *t<sub>R</sub>* 25 min).

4'-*O*-[ $\beta$ -D-Apioosyl(1→2)]- $\beta$ -D-glucosyl]-5-hydroxyl-7-*O*-sinapylflavanone (1): Colorless powder [ $\alpha$ ]<sub>D</sub><sup>25</sup> +8.4 (*c*=3.5, MeOH). UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 326 (2.75), 283 (3.04), 214 (3.33), 206 (3.35). IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3412 (OH), 1668, 1606, 1520, <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 400 MHz): see Table 1; <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz): see Table 1; EI-MS at *m/z* (C<sub>11</sub>H<sub>11</sub>O<sub>4</sub><sup>+</sup>) 207, [M-462]<sup>+</sup> at *m/z* 311 sugar moieties (apiose and glucose) HR-FAB-MS (+ve). HR-FAB-MS *m/z*: (Calcd for C<sub>37</sub>H<sub>41</sub>O<sub>19</sub>; 773.1079). [M+H]<sup>+</sup> 773.1073.

3-(4-Acetoxy-3,5-dimethoxy)-phenyl-2*E*-propenyl- $\beta$ -D-glucopyranoside (2): White amorphous powder [ $\alpha$ ]<sub>D</sub><sup>25</sup> +65 (*c*=8.5, MeOH). UV  $\lambda_{\max}$  (MeOH) nm (log  $\epsilon$ ): 261 (3.45), 242 (3.77), 203 (4.05), 189 (4.07). IR (KBr)  $\text{cm}^{-1}$ : 3385, 1735, 1660, 1593. <sup>1</sup>H-NMR (400 MHz, CD<sub>3</sub>OD): see Table 1; <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 100 MHz) see Table 1; HR-FAB-MS *m/z*: (Calcd for C<sub>19</sub>H<sub>27</sub>O<sub>10</sub>, 415.1526). 415.1522 [M+H]<sup>+</sup> C<sub>19</sub>H<sub>27</sub>O<sub>10</sub>.

**Acid Hydrolysis of Compound 1** Compound 1 (1.3 mg) was dissolved in 5% HCl/H<sub>2</sub>O (2 ml) and refluxed for 1 h. The solution was extracted with ethyl acetate (1 ml×3) to afford aglycone. While the aqueous part was neutralized by NaHCO<sub>3</sub> (pH 6) and hydrolyzed sugars were identified by paper chromatography using standard sugars in the solvent system EtOAc/AcOH/H<sub>2</sub>O (5 : 3 : 2). The results indicated the presence of apiose and glucose.<sup>1)</sup>

**Assay of Anti-glycation and Superoxides Anion** The anti-glycation and superoxide Anion assays on various secondary metabolites of *Viscum album* were carried out according to established protocols.<sup>18,19)</sup>

**Acknowledgments** We are grateful to the IDB (The Islamic Development Bank, Jeddah and KSA) for financial support to M.I.C. for the research project entitled, "Phytochemical, Pharmacological and Toxicological Studies on Medicinal Plants of Sudan and Pakistan."

## References

- 1) Leu L. Y., Kuo M. S., Hwang, T. L., Chiu S. T., *Chem. Pharm. Bull.*, **52**, 858—860 (2004).
- 2) EMEA/MRL, "The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation Unit," Final August, 680—699 (1999).
- 3) Arndt B., "The Genus *Viscum* Medicinal and Aromatic Plant, Industrial Profiles," Harwood Academic, Amsterdam, 2000, p. 45.
- 4) Hajto T., Hostanska K., Berki T., Palinkas L., Boldizsar F., Nemeth, P., *eCam.*, **2**, 59—67 (2005).
- 5) Renata J., Piotrowski A., *Can. J. Plant Pathol.*, **24**, 21—28 (2002).
- 6) Stein G. M., Pfuller U., Schietzel M., Bussing A., *Anticancer Res.*, **20**, 2987—2994 (2002).
- 7) Gray A. M., Flatt P. R., *Endocrinology*, **160**, 409—414 (1999).
- 8) Halliwell B., *Drugs Aging*, **18**, 685—716 (2001).
- 9) Hyder N., Bouhleh I., Skandrani I., Kadri M., Steiman R., Guiraud P., Mariotte M. A., Ghedira K., Dijoux-Franca M. G., Chekir-Ghedira L., *Toxicol. In Vitro*, **22**, 567—581 (2008).
- 10) Pietta P. G., *J. Nat. Prod.*, **63**, 1035—1042 (2000).
- 11) Hosoya T., Yun Y. S., Kunugi A., *Tetrahedron*, **61**, 7037—7044 (2005).
- 12) Fukunaga T., Kajikawa I., Nishiya K., Watanabe Y., Takeya I. H., *Chem. Pharm. Bull.*, **35**, 3292—3297 (1987).
- 13) Mebry T. J., Markham K. R., Thomas, M. B., "The Systematic Identification of Flavonoids," Springer-Verlag, New York, 1975, pp. 33—35.
- 14) Wei L., Koike K., Tatsuzaki M., Koide, A., Nikaido T., Cucurbitosides F.-M., *J. Nat. Prod.*, **68**, 1754—1757 (2005).
- 15) Greca M. D., Ferrara M., Fiorentino A., Monaco L., Previtera L., *Phytochemistry*, **49**, 1299—1304 (1988).
- 16) Tanaka R., Matsunaga S., Sasaki T., *Planta Med.*, **55**, 570—571 (1989).
- 17) Lam J., Wrang P., *Phytochemistry*, **14**, 1621—1623 (1975).
- 18) Kim H. Y., Kim K. J., *J. Agric. Food Chem.*, **51**, 1586—1591 (2003).
- 19) Candan F., *J. Enzyme Inhib. Med. Chem.*, **18**, 59—62 (2003).