SHORT REPORT

A C G publications

Rec. Nat. Prod. 10:3 (2016) 385-391

records of natural products

Cytotoxic Activity of New Tropinene Glycoside Isolated from *Solandra grandiflora* Sw.

Magdy M. D. Mohammed[1,2,*], Michael Spiteller[1], Nabaweya A. Ibrahim[2] and Khaled M. Mohamed[2]

[1] *Institut für Umweltforschung (INFU), Technische Universität Dortmund, Otto-Hahn-Strasse 6, D-44221 Dortmund, Germany*

[2] *Department of Pharmacognosy, National Research Centre, Dokki-12622, Cairo, Egypt*

(Received January 11, 2015; Revised September 08, 2015; Accepted September 10, 2015)

Abstract: The first phytochemical investigation of *Solandra grandiflora* Sw. leaves using LC/MS resulted in the identification without isolation of eight known and one unknown compounds from the neutral and the alkaloidal fractions. Further chromatographic fractionation and isolation of both fractions using RP-HPLC resulted in the isolation of chlorogenic acid, *neo*chlorogenic acid, protocatechuic acid, scopoletin and *iso*scopoletin from the neutral fraction. Moreover, a new tropinene glycoside identified as 3-*O*-*β*-D-glucosyl 6-tropinene, together with atropine, scopolamine and hyoscyamine were isolated from the alkaloidal fraction. The structures of all isolated compounds were established and confirmed by ^{1}H-, ^{13}C-NMR, COSY, HSQC & HMBC spectroscopy, while the exact masses were confirmed by HRESIMS. The cytotoxic activity of all isolates was evaluated against HepG-2, HCT-116, A-549 and MCF-7, and the obtained results suggested selective antiproliferative and cytotoxic effect.

Keywords: *Solandra grandiflora* Sw.; Solanaceae; tropane alkaloids; cytotoxicity; SAR.

1. Plant Source

Plants of the Solanaceae (Nightshade Family) produce a variety of alkaloids. One such group of alkaloids possesses a tropane nucleus. The effects and uses of solanaceous plants in medicinal practice derived primarily from one of three groups of toxic alkaloids: the tropane-, steroid- or pyridine-types. However, the compounds which play a role as therapeutic or toxic agents are all various tropane alkaloids. Even today, Solanaceae is one of the top ranking families of drug-yielding plants used not only in modern medicine but also in traditional and herbal medicine for the treatment of a wide range of ailments [1]. *Solandra grandiflora* Sw. (Showy Chalice Vine, Cup-of-Gold) belongs to the family Solanaceae, genus *Solandra*, which is indigenous to Mexico [2], most of the species occur in central Mexico, the genus was represented to the South as far as the rain forests of Chiapas. Several species have spread into the Caribbean and to South America (Peru) [3].

Solandra grandiflora Sw. leaves were collected from the garden of the National Research Centre, Cairo, Egypt, in May 2006. It was kindly identified by Mrs. Teresa Labib, Head of the

* Corresponding author: E-mail: melhenawy111@gmail.com; Phone:+2-0233371718, *Fax:+2-0233370931.*

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 10/12/2015 EISSN:1307-6167

Taxonomy specialists at El-Orman Botanical Garden, Giza, Egypt. A voucher specimen (No. 000274Sc/07/02/04/24) has been deposited at the Herbarium of El-Orman Botanical Garden, Giza, Egypt. The plant material was dried, finely powdered, and used for the successive extraction.

The aims of the presented study were to investigate the phytochemical constituents (alkaloids and phenolics) of *S. grandiflora* Sw. leaves, followed by an evaluation of their cytotoxicity against HepG-2, HCT-116, A-549 and MCF-7 cell lines. Structure activity relationship of the biologically active metabolites was conducted in order to highlight their potentials as candidates for new drugs that may be of value in the treatment and/or prevention of human and livestock diseases.

2. Previous Studies

Ten to twelve vine-like species are currently botanically recognized as belonging to the genus *Solandra* [2], which has a long folkloric-history in the treatment of tumors and carcinomatous ulcers [4]. In Mexico, cup of gold was used primarily as a love drink and aphrodisiac, the Huastec people used the rainwater or dew that has collected in the buds of the *S. grandiflora* as eye drops to improve sight and reduce the irritation caused by eye [5]. Many Mesoamerican Indian tribes liken the aphrodisiac effects produced by smoking the dried leaves and flowers of *S. grandiflora* Sw. which are said to be very similar to the effect produced when smoking other Nightshade family plants: *Brugmansia*, *Datura* and *Latua pubiflora* [6]. A tea made from the flowers of the cup-of-gold was drunk to reduce the severity of coughs [7]. In colonial Mexico, Indians used the cup of gold to add zest to their cacao drinks [8]. All the Mexican species of *Solandra* contain potently parasympatholytic tropane alkaloid, most *Solandra* species contain approximately 0.15% alkaloids, the highest concentration of alkaloids (calculated as atropine) was (0.64%) and found in the roots of *S. grandiflora* [9, 10]. The roots generally exhibited the highest alkaloid concentrations, the primary alkaloids are atropine, noratropine, and (-)-hyoscyamine; the secondary alkaloids are norhyoscyamine, tropine, nortropine, etc., and cuscohygrine [2, 10].

3. Present Study

The MeOH extract of *Solandra grandiflora* leaves was defatted with hexane and then subjected to acid-base shakeout method to obtain the alkaloidal and phenolic fractions, further chromatographic separation and isolation using RP-HPLC resulted in the isolation of atropine (**1**), scopolamine (**2**) and hyoscyamine (**3**) [10, 11], together with a new tropinene glycoside (**4**) from the alkaloidal fraction. Moreover, chlorogenic acid (**5**), *neo*chlorogenic acid (**6**) [12], protocatechuic acid (**7**) [13], scopoletin (**8**) and *iso*scopoletin (**9**) [14], were isolated from the neutral fraction. The structure elucidation of the known compounds was confirmed by 1D-, 2D-NMR and HR-ESIMS, then by comparison with the literature.

Compound **4**, was obtained as an amorphous white powder, with the molecular formula $C_{14}H_{23}NO_6$ as determined by a high resolution ESIMS (*m/z* 302.16000 for $[M + H]^+$ calcd 302.16036 for $C_{14}H_{24}NO_6$). CID of the molecular ion peak at *m/z* 302 produced a base peak at *m/z* 163 with the molecular formula $C_6H_{11}O_5$ suggested the possible presence of a hexose moiety, identified as glucose based on ^{13}C-NMR data which exhibited six carbon resonances of a sugar moiety (Table 1). ^{1}H-NMR spectroscopic data of compound **4** in CH_3OH-d_4 (Table 1) displayed resonance signals nearly the same as tropane derivative [15], which can be characterized as follow; a broad olefinic signal appeared at δ_H 5.79 (2H, brs, H-6 & H-7), the bridgehead protons (H-1, H-5) appeared at δ_H 4.05 as a broad multiplet, the presence of the characteristic 3H singlet for the *N*-methyl protons in the spectrum at δ_H 1.91 indicated a tropane nucleus. Two broad multiplet signals appeared at δ_H 1.32 – 1.38 (4H, *m*) corresponding to H-2 and H-4, a broad triplet signal appeared at δ_H 4.33 (1H, *t*, $J_{3eq,2ax} = J_{3eq,4ax} = 4$ Hz) was assigned to the C-3 equatorial (β) proton [15]. The large vicinal *J* values of the glycone H-2` appeared at $\delta_H$ 2.93 (1H, br*t*, $J = 7.2$ Hz) and coupling constant of the anomeric proton H-1`at δ_H 4.66 (1H, *d*, $J = 8$ Hz) indicated that the glycone part of this glycoside is the pyranose form of β-glucose [16].

The ^{13}C-NMR chemical shift assignments were found to be consistent with the structure proposed. Thus, the bridgehead carbons (C-1 and C-5) appeared at δ_C 68.95, while the C-3 signal

resonated at δ_C 68.62. The signal at δ_C 37.27 was attributed to the C-2 and C-4 methylene carbons. The olefinic C-6 and C-7 resonances appeared at δ_C 136.93. The equatorial orientation of the *N*-methyl group was demonstrated by a signal at δ_C 50.74. The anomeric carbon for the glycone was assigned at δ_C 101.90 and the methylene hydroxy group was assigned at δ_C 63.14. The attachment position of the glucose moiety on the aglycone part was assigned (based on HMBC) to be at C-3 of the aglycone due to the cross-peak correlation between H-1`at δ_H 4.66 and C-3 at δ_C 68.62. Thus, the above data established the structure of the new alkaloid as formulated in (Figure 1) and assigned to 3-*O*-*β*-D-glucopyranosyl-6-tropinene.

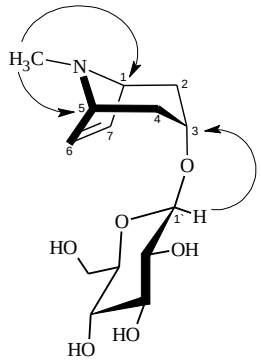

Figure 1. Selected correlations of ^{1}H-^{1}H COSY indicated by bold bonds (▬) and HMBC indicated by arrows (→) of 3-*O*-*β*-D-glucosyl 6-tropinene.

Table 1. The NMR spectral data of compound **4** (in CD_3OD).

No.	Compound 4		
	δ_H	δ_C	HMBC(H→C)
1, 5	4.05 (2H, *m*)	68.95 [b]	2,3,7 & 4,3,6
2, 4	1.32 – 1.38 (4H, *m*)	37.27 [c]	1,3 & 3,5
3	4.33 (1H, *t*, *J* = 4 Hz)	68.62 [b]	1`,2,4
6, 7	5.79 (2H, br*s*)	136.93 [b]	1,5
N-CH_3	1.91 (3H, *s*)	50.74 [d]	1,5
1`	4.66 (1H, *d*, *J* = 8 Hz)	101.90 [b]	3,2`
2`	2.93 (1H, br*t*, *J* = 7.2 Hz)	72.36 [b]	1`,3`
3`	3.75 (1H, *m*)	72.89 [b]	2`,4`
4`	3.66 (1H, *m*)	71.65 [b]	3`,5`
5`	3.49 (1H, *m*)	75.46 [b]	4`,6`
6`	3.84 (2H, *m*)	63.14 [c]	5`

a: quaternary (C), b: tertiary (CH), c: secondary (CH_2), d: primary (CH_3)

3. Biological investigation

The cytotoxicity (Table 2) of the isolated compounds was evaluated against HepG-2, HCT-116, A-549 and MCF-7, and the results revealed that all the isolated compounds possessed cytotoxic activity with different percentages against different cell lines which reflects some selectivity. In brief, scopoletin was cytotoxic against MCF-7 with 23.8% at 100 ppm, and it was inactive against other cell lines, while *iso*scopoletin showed cytotoxicity with 10.1% at 100 ppm against HCT-116 and it was inactive against other cells. Moreover, at 100 ppm protocatechuic acid showed cytotoxicity only against HepG-2 with 26.6%, chlorogenic acid caused 55.8% cytotoxicity against MCF-7 only, while its isomer revealed higher cytotoxicity with 78.6% against MCF-7 and with 23.5% against A-549. On the other hand, the cytotoxicity of the isolated alkaloids was of some interest as follow, atropine showed cytotoxicity at 100 ppm against HCT-116 with 2.6%, HepG-2 with 9.7% and MCF-7 with 49.8%, while its levorotary isomer hyoscyamine revealed cytotoxicity with 35.8% against MCF-7 and was inactive against other cells, scopolamine was cytotoxic against A-549 and MCF-7 with 3.2% and 66.5% respectively at 100 ppm. Finally, the new alkaloid 3-*O*-*β*-D-glucosyl 6-tropinene showed weak

cytotoxicity against HCT-116 and HepG-2 with 2.6% and 24.8% respectively, and was inactive against A-549 and MCF-7.

The cytotoxic activity of the isolated compounds may be attributed mainly to the presence of some structural features (Figure 2) e.g., the presence of an epoxide group which exhibit growth inhibitory property [17] as in scopolamine increased its cytotoxicity against MCF-7 compared with both of atropine and hyoscyamine which have nearly the same %, while the compound (**4**) was inactive against MCF-7. The presence of ortho di-hydroxy (phenolic acids) and/or hydroxy-methoxy groups (coumarins) should have electron donating properties and can be attributed to their cytotoxic activity [18].

Table 2. The cytotoxic activity of the isolated compounds.

Sample	**Cytotoxicity % at 100 ppm**				**IC_{50} (μg/mL) MCF-7**
	HCT-116	**A-549**	**HepG-2**	**MCF-7**	
3β-glucosyl 6-tropinene	2.6	0	24.8	0	ND
Atropine	2.6	0	9.7	49.8	94.4
Scopolamine	0	3.2	0	66.5	81.5
Hyoscyamine	0	0	0	35.8	ND
Scopoletin	0	0	0	23.8	ND
*Iso*scopoletin	10.1	0	0	0	ND
Protocatechuic acid	0	0	26.6	0	ND
Chlorogenic acid	0	0	0	55.8	89.2
*Neo*chlorogenic acid	0	23.5	0	78.6	64.4
Doxorubicin					26.1

Tropane alkaloids e.g., atropine, hyoscyamine and scopolamine, are of great interest for the pharmaceutical industry, they are widely used for their mydriatic, antispasmodic, anesthetic, bronchodilators and antiasthmatic properties [19]. Scopolamine is medicinally the most important, mainly because it is used as the starting material for the semi-synthesis of several important drugs, scopolamine hydrobromide has been used for the treatment of Parkinsonism, for the prevention of motion sickness (Transderm Scop®), to dilate pupils for eyes examination (Isopto® Hyoscine), and as an ingredient of analgesic medication [20]. Both of scopolamine and hyoscyamine possess strong parasympatholytic activity, blocking parasympathic action by binding to the muscarinic acetylcholine receptors in synapses, without exerting any intrinsic activity. It is worthy of note that Solanaceae tissue cultures often grow vigorously and regenerate more easily than do those of many other medicinal plants. Almost all tissue culture systems known for plants have been realized with Solanaceae; these include root cultures, shoot cultures and de-differentiated cells as callus or cell suspension cultures [21]. The most interesting natural product MDR reversing agents that have been discovered were tropane alkaloid aromatic esters, e.g., those isolated from the root of *Erythroxylum perillei* Baillon [22].

Protocatechuic acid (PCA) has been reported to induce apoptosis of human leukemia cells, as well as malignant HSG1 cells taken from human oral cavities [23], but PCA was found to have mixed effects on TPA-induced mouse skin tumours. Depending on the amount of PCA and the time before application, it could reduce or enhance tumour growth. Similarly, PCA was reported to increase proliferation and inhibit apoptosis of neural stem cells [24]. In an *in vitro* model using HL-60 leukemia cells, protocatechuic acid showed an antigenotoxic effect and tumoricidal activity [25]. Jiang and co-workers [26] studied the cytotoxicity of chlorogenic acid (CGA) against human oral squamous cell carcinoma (HSC-2) and salivary gland tumor (HSG) cell lines, and suggested that CGA induces cytotoxicity in oral tumor cell lines, possibly by hydrogen peroxide-mediated oxidation mechanism. Furthermore, CGA was reported by Jin and co-workers [27] to involve tumor cell invasion and metastasis, it is strongly inhibit the effect of matrix metalloproteinase (MMP)-9 activity in a concentration-dependent manner on zymography.

Research involving coumarins and their antiproliferative effect on malignant melanoma, leukaemia, renal cell carcinoma, prostate and breast cancer are discussed [28]. e.g., scopoletin and

*iso*scopoletin showed considerable inhibition of CCRF-CEM leukaemia cell proliferation, with IC_{50} values of 2.6 and 4.0 μM [29]. The insensitivity of many human tumour cell lines, previously tested to growth inhibition by coumarin, seems to confirm the generally held belief that coumarin is not responsible for the observed *in vivo* effects, but is a pro-drug for other active metabolites [27]. The most evident trend from the results was that a dihydroxy-function in either an ortho- or meta-format, was an extremely potent chemical structure for toxicity in human tumour cell lines. Since this potency was not evident in either of the single hydroxycoumarin compounds, the added potency may be due to the existence of a double hydroxy-function on the coumarin ring. It is evident from the research described that coumarin and coumarin-related compounds are a plentiful source of potential anti-cancer drugs deserving further study [30].

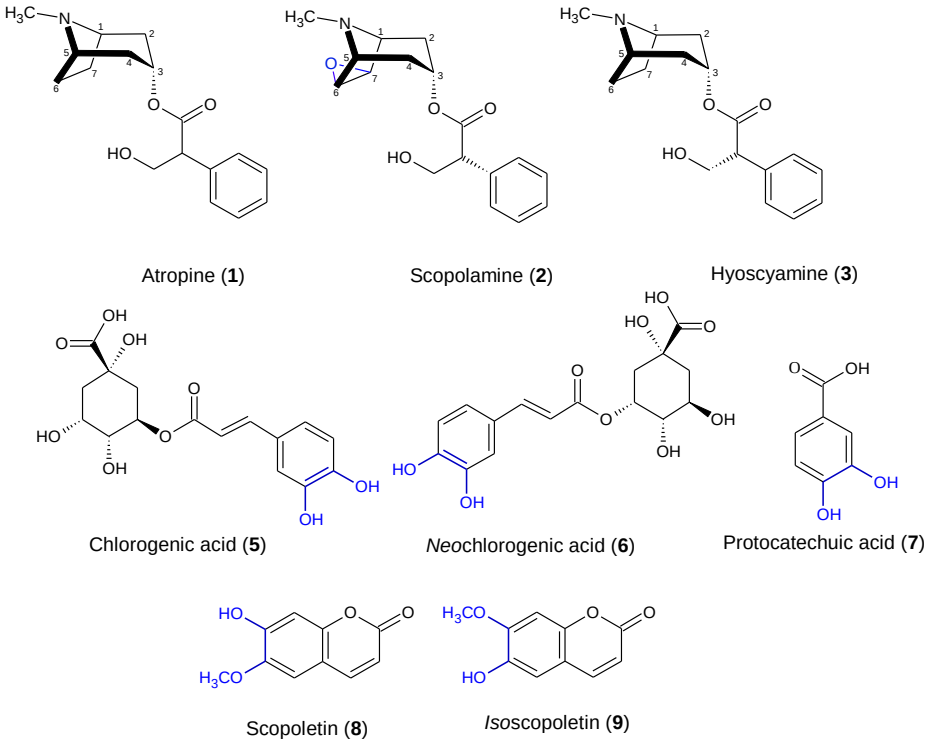

Figure 2. The structural features of the isolated known compounds with high multifunctional activities

Acknowledgements

This work was financially supported by the Deutscher Akademischer Austauschdienst (DAAD) and Ministry of Higher Education and Scientific Research (MOHESR) initiative ‘‘German Egyptian Research Short-term Scholarship (GERSS)’’ for postdoctoral research granted by Dr. Magdy M. D. Mohammed.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

Conflict of Interest

The authors have declared that there is no conflict of interest among them.

References

[1] P. Christen (2000). Tropane Alkaloids: Old Drugs Used in Modern Medicine. In: Atta-Ur-Rahman, editor. Studies in Natural Products Chemistry. Elsevier. New York. pp. 717–749.

[2] R. E. Schultes and N. R. Farnsworth (1980). Ethnomedical, botanical and phytochemical aspects of natural hallucinogens, *Botanical Museum Leaflets, Harvard University* **28**, 123–214.

[3] P. T. Furst (1995). The drunkard kiéri: New observations of an old problem in Huichol psychotropic ethnobotany, *Integration* **5**, 51–62.

[4] J. L. Hartwell (1982). Plants Used Against Cancer. A Survey. Quarterman. Lawrence MA. pp. 583.

[5] J. B. Alcorn (1984). Huastec Mayan ethnobotany. University of Texas Press. Austin. pp. 793.

[6] C. Rätsch (2005). The Encyclopaedia of Psychoactive Plants–Ethnopharmacology and Its Applications. Inner Traditions. Vermont, USA.

[7] M. Yasumoto (1996). The Psychotropic Kieri in Huichol Culture. In: People of The Peyote, edn. S. Stacy and T. F. Peter. University of New Mexico Press. Albuquerque. pp. 235–263.

[8] R. Heffern (1974). Secrets of Mind-Altering Plants of Mexico. Pyramid. New York. pp. 101.

[9] R. E. Schultes (1979). Solanaceous Hallucinogens and Their Role in the Development of New World Cultures. In: The Biology and Taxonomy of the Solanaceae. Linn Soc Symp Ser, Vol. 7. Hawkes, Lester, Skelding (Eds). Linnean Soc & Academic Press. London. pp. 137–160.

[10] W. C. Evans, A. Ghani and V. A. Woolley (1972). Alkaloids of *Solandra* species, *Phytochemistry* **11**, 470–472.

[11] M. Humam, T. Shoul, D. Jeannerat, O. Muñoz and P. Christen (2011). Chirality and numbering of substituted tropane alkaloids, *Molecules* **16**, 7199–7209.

[12] E. W. C. Chan, Y. Y. Lim, S. K. Ling, S. P. Tan, K. K. Lim and M. G. Khoo (2009). Caffeoylquinic acids from leaves of *Etlingera* species (Zingiberaceae), *LWT-Food Sci. Tech.* **42**, 1026–1030.

[13] D. Gutzeit, V. Wray, P. Winterhalter and G. Jerz (2007). Preparative isolation and purification of flavonoids and protocatechuic acid from sea buckthorn juice concentrate (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) by high-speed counter-current chromatography, *Chromatographia* **65**, 1–7.

[14] M. Adams, T. Efferth and R. Bauer (2006). Activity-guide isolation of scopoletin and isoscopoletin, the inhibitory active principles towards CCRF-CEM leukaemia cells and multi-drug resistant CEM/ADR5000 cells, from *Artemisia argyi***,** *Planta Med.* **72**, 862–864.

[15] K. F. Khattak, Atta-ur-Rahman, M. I. Choudhary, K. D. Hemalal and L. M. Tillekeratne (2002). New tropane alkaloids from *Erythroxylum moonii*, *J. Nat. Prod.* **65**, 929–931.

[16] M. U. Roslund, P. Tähtinen, M. Niemitz and R. Sjöholm. (2008). Complete assignments of the ^{1}H and ^{13}C chemical shifts and $J_{H,H}$ coupling constants in NMR spectra of D-glucopyranose and all D-glucopyranosyl-D-glucopyranosides, *Carbohydr. Res.* **343**, 101–112.

[17] M. M. D. Mohammed, L. P. Christensen and P. L. Colla (2013). Anti-HIV-1 of Sesquiterpene Lactones from *Calocephalus brownii* F. Muell, *Nat. Prod. Res.* **28**(4), 221–229.

[18] O. V. Jesús and P. L. Leonardo (2002). Structure-activity relationships for the anti-HIV activity of flavonoids, *J. Chem. Inform. Computer Sci.* **42**, 1241–1246.

[19] G. Grynkiewicz and M. Gadzikowska (2008). Tropane alkaloids as medicinally useful natural products and their synthetic derivatives as new drugs, *Pharmacol. Rep.* **60**, 439–63.

[20] W. Christophe (2006). Medicinal Plants of the Asia-Pacific: Drugs for the Future? World scientific publishing Co. Pte. Ltd. 5 toh tuck link, Singapore.

[21] B. Dräger (2007). Biotechnology of Solanaceae Alkaloids: a Model or an Industrial Perspective? In: O. Kayser and W. J. Quax (eds) Medicinal Plant Biotechnology, From Basic Research to Industrial Applications, Vol 1. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. pp. 237–265.

[22] M. I. Qiuwen, C. Baoliang, L. S. Gloria, L. Daniel, L. Eula, C. Heebyung, G. H. Melinda, G. M. Joseph, K. A. Douglas and M. P. John (2002). Pervilleines B and C, new tropane alkaloid aromatic esters that reverse the multidrug-resistance in the hollow fiber assay, *Cancer Lett.* **184**, 13–20.

[23] H. Babich, A. Sedletcaia and B. Kenigsberg (2002). In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress, *Pharm. Toxicol.* **91**, 245–253.

[24] S. Guan, D. Ge, T. Q. Liu, X. H. Ma and Z. F. Cui (2009). Protocatechuic acid promotes cell proliferation and reduces basal apoptosis in cultured neural stem cells, *Toxicol. In Vitro* **23**, 201–208.

[25] J. Anter, M. Romero-Jiménez, Z. Fernández-Bedmar, M. Villatoro-Pulido, M. Analla, A. Alonso-Moraga and A. Muñoz-Serrano (2011). Antigenotoxicity, cytotoxicity, and apoptosis induction by apigenin, bisabolol, and protocatechuic acid, *J. Med. Food* **14**, 276–283.

[26] Y. Jiang, K. Kusama, K. Satoh, F. Takayama, S. Watanabe and H. Sakagami (2000). Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines, *Phytomedicine* **7,** 483–491.

[27] U-.H. Jin, J-.Y. Lee, S-.K. Kang, J-.K. Kim, W-.H. Park, J-.G. Kim, S-.K. Moon and C-.H. Kim (2005). A phenolic compound, 5-caffeoylquinic acid is a new type and strong matrix metalloproteinase-9 inhibitor: Isolation and identification from methanol extract of *Euonymus alatus*, *Life Sci.* **77**, 2760–2769.

[28] A. Lacy and R. O'Kennedy (2004). Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer, *Curr. Pharm. Des.* **10**, 3797–3811.

[29] A. Michael, E. Thomas and B. Rudolf (2006). Activity-guided isolation of scopoletin and isoscopoletin, the inhibitory active principles towards CCRF-CEM leukaemia cells and multi-drug resistant CEM/ADR5000 cells, from *Artemisia argyi*, *Planta Med.* **72**, 862–864.

[30] U. S. Weber, B. Steffen and C. P. Siegers (1998). Antitumour-activities of coumarin, 7-hydroxycoumarin and its glucuronide in several human tumour cell lines, *Res. Commun. Mol. Pathol. Pharm.* **99**, 193–206.