In vitro Antioxidant and Antiproliferative Activities of Flavonoids from *Ailanthus excelsa* (Roxb.) (Simaroubaceae) Leaves

Ataa Said[a], Rosa Tundis[b], Usama W. Hawas[c], Salah M. El-Kousy[d], Khaled Rashed[a], Federica Menichini[b], Marco Bonesi[b], Antje Huefner[e], Monica Rosa Loizzo[b,*], and Francesco Menichini[b]

[a] Pharmacognosy Department, National Research Centre, Dokki, Giza, Egypt
[b] Pharmaceutical Sciences Department, Faculty of Pharmacy, Nutrition and Health Sciences, University of Calabria, Arcavacata Rende 87036 (CS), Italy. Fax: +3 99 84 49 32 98. E-mail: mr.loizzo@unical.it
[c] Phytochemistry and Plant Systematic Department, National Research Centre, Dokki, Giza, Egypt
[d] Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Khom, Egypt
[e] Pharmaceutical Sciences Institute, Pharmaceutical Chemistry Department, University of Graz, Schubertstr. 1, A-8010 Graz, Austria

* Author for correspondence and reprint requests

Z. Naturforsch. **65 c**, 180–186 (2010); received November 5/24, 2009

The present study aimed to investigate the chemical composition, and the antioxidant and antiproliferative activities of *Ailanthus excelsa*, a plant used in Egyptian traditional medicine. Chromatographic separation of a methanol extract of *A. excelsa* leaves yielded four flavones, namely apigenin (**1**), apigenin 7-*O*-*β*-glucoside (**2**), luteolin (**3**), and luteolin 7-*O*-*β*-glucoside (**4**), and seven flavonols, namely kaempferol (**5**), kaempferol 3-*O*-*α*-arabinoside (**6**), kaempferol 3-*O*-*β*-galactoside (**7**), quercetin (**8**), quercetin 3-*O*-*α*-arabinoside (**9**), quercetin 3-*O*-*β*-galactoside (**10**), and quercetin 3-*O*-rutinoside (**11**). The *A. excelsa* extract tested in different *in vitro* systems (DPPH and FRAP assays) showed significant antioxidant activity. The potential antiproliferative activity of the *A. excelsa* extract and isolated flavonoids against five human cancer cell lines such as ACHN, COR-L23, A375, C32, and A549 was investigated *in vitro* by the SRB assay in comparison with one normal cell line, 142BR. The extract exhibited the highest inhibitory activity against C32 cells with an IC_{50} value of 36.5 μg ml^{-1}. Interesting activity against COR-L23 was found with **10** (IC_{50} value of 3.2 μg ml^{-1}). Compounds **1** and **3** inhibited cell growth in both amelanotic melanoma and malignant melanoma cells.

Key words: Ailanthus excelsa Flavonoids, Antioxidant, Antiproliferative

Introduction

Free radicals and other reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, are an entire class of highly reactive molecules derived from the normal metabolism of oxygen or from exogenous factors and agents (Halliwell and Gutteridge, 1990). ROS are not only strongly associated with lipid peroxidation resulting in deterioration of food materials, but are also involved in the development of pathology of numerous diseases, including cancer (Panayiotidis, 2008).

Cancer is the second largest single cause of death claiming over six million lives every year worldwide. It is well established that natural products are an excellent source of chemical structures with a wide variety of biological activities, including anticancer properties (Cragg and Newman, 2005). This has opened up new fields of investigation of potential antitumour compounds, some of which are already widely used in cancer chemotherapy (Arcamone *et al.*, 1980). Cytotoxic screening models provide important preliminary data to select plant extracts and natural products with potential anticancer properties.

Ailanthus excelsa is a deciduous tree from the Simaroubaceae family and widely distributed in Asia and North Australia. Its native origin is China and it is known as "tree of heaven" (Adamik and Brauns, 1957). In traditional medicine *A. excelsa* is used to cure wounds and skin eruption, and in the indigenous system of medicine it is used in febrifuge, bronchitis, asthma and in conditions of diarrhea and dysentery (British Pharmacopoeia, 1988). Previous phytochemical studies on *A. ex-*

celsa have demonstrated the presence of quassinoids, flavonoids, alkaloids, terpenoids, and proteins (Ogura *et al.*, 1977; Loizzo *et al.*, 2007; Joshi *et al.*, 2003a; Sherman *et al.*, 1980; Nag and Matai, 1994). *A. excelsa* extracts and some of its isolated compounds have demonstrated medicinal properties such as significant antileukemic, antibacterial, antifungal, and antifertility activities (Ogura *et al.*, 1977; Dhanasekaran *et al.*, 1993; Shrimali *et al.*, 2001; Joshi *et al.*, 2003b). As part of a screening program searching for natural products with anticancer properties, we have conducted an *in vitro* screening to evaluate the antioxidant and antiproliferative activity of the methanol extract and flavonoids isolated from the leaves of *Ailanthus excelsa*.

Material and Methods

General experimental procedures

Nuclear magnetic resonance (NMR) experiments were performed using a Bruker Avance 300 MHz spectrometer (solvent CD_3OD). The signals in the ^1H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques. Mass spectra were performed using an electrospray ionization mass spectrometer ZAB2-SEQ. Infrared spectra were recorded on a Perkin-Elmer FT 1600 spectrometer. UV spectra were recorded on a Beckman DU 640 apparatus. TLC analyses were carried out on silica gel 60 F_{254} precoated plates (VWR International, Milan, Italy). Chemicals and reagents used for the study of antioxidant and cytotoxic activities were purchased from Sigma-Aldrich Co. (Milan, Italy) while other chemicals, solvents and reagents used in chromatography were purchased from Merck (Cairo, Egypt).

Plant material

A. excelsa (Roxb.) leaves were collected from Zoo Garden, Giza, Egypt. The plant material was identified by Dr. Kamal El-Batanony, Professor of Taxonomy and Botany, Faculty of Science, Cairo University, Egypt. A voucher specimen was deposited in the NRC herbarium.

Extraction and isolation

Powdered air-dried leaves of *A. excelsa* (1 kg) were extracted with methanol (70%) in a Soxhlet apparatus at 50 °C. The extract was concentrated under reduced pressure to dryness to give 260 g (yield 26%). The crude extract was dissolved in 500 ml of distilled water and extracted with *n*-hexane. The residue (215 g) was subjected to silica gel column chromatography and eluted with *n*-hexane/methylene chloride/ethyl acetate/methanol using gradually increasing polarity of solvents. 130 fractions were collected. Fractions showing similar TLC profiles were pooled to provide combined fractions. The fractions were further purified by Sephadex LH-20 column chromatography to afford eleven pure compounds, **1**–**11**.

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The radical scavenging capacity was determined according to the technique reported by Loizzo *et al.* (2009a). Aliquots of 1.5 ml of 0.25 mM DPPH solution in ethanol and 1.5 ml of *A. excelsa* MeOH extract at different concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature within 30 min. Decolourization of DPPH was determined by measuring the absorbance at λ = 517 nm with a Varian spectrophotometer. The DPPH radicals' scavenging activity was calculated according to the following equation: scavenging activity = $[(A_0 - A_1/A_0) \cdot 100]$, where A_0 is the absorbance of the control (blank, without extract) and A_1 is the absorbance in the presence of the extract.

Ferric reducing ability power (FRAP) assay

The FRAP method measures the absorbance change that appears when the Fe^{3+}-TPTZ (2,4,6-tripyridyl-*s*-triazine) complex is reduced to the Fe^{2+}-TPTZ form in the presence of antioxidant compounds. An intense blue colour, with absorbance maximum at λ = 595 nm, develops (Benzie and Strains, 1996). Briefly, the FRAP reagent contained 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$, and 25 ml of 0.3 M acetate buffer (pH 3.6) freshly prepared. *A. excelsa* (0.2 ml) was mixed with 1.8 ml of FRAP reagent, and the absorbance of the reaction mixture was measured at λ = 595 nm. Ethanol solutions of known Fe^{2+} concentration, in the range of 50–500 μM $FeSO_4$, were used for obtaining the calibration curve. The FRAP value represents the ratio between the slope of the linear plot for reducing Fe^{3+}-TPTZ reagent by *A. excelsa* extract compared to the slope of the plot for $FeSO_4$.

Cell culture and in vitro cytotoxicity assay

Cytotoxic screening models provide important preliminary data to select plants with potential anticancer compounds. Therefore the sulforhodamine B (SRB) assay, used in this study, is commonly employed. Five human cancer cell lines, including lung large cell carcinoma COR-L23 (ECACC No. 92031919), amelanotic melanoma C32 (ATCC No. CRL-1585), renal cell adenocarcinoma ACHN (ATCC No. CRL-1611), malignant melanoma A375 (ECACC No. 88113005), lung carcinoma A549 (ECACC No. 86012804), and one normal cell line, namely skin fibroblasts 142BR (ECACC No. 90011806), were used in our experiments. The COR-L23, C32, and ACHN cells were cultured in RPMI 1640 medium, while 142BR, A549, and A375 cells were cultured in DMEM. Both media were supplemented with 10% foetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. The cell lines were maintained at 37 °C in a 5% CO_2 atmosphere with 95% humidity.

The protein-staining SRB assay was used for measuring of the cell proliferation (Loizzo *et al.*, 2005). Briefly, cells were placed in 96-well plates in the range $5 \cdot 10^4$ to $15 \cdot 10^4$ cells, to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at $\lambda = 490$ nm and cell number, and incubated to allow for cell attachment. After 24 h the cells were treated with serial dilutions of the *A. excelsa* methanol extract and isolated flavonoids. Each sample was initially dissolved in DMSO and further diluted in medium to produce different concentrations. 100 μl well^{-1} of each dilution was added to the plates in six replicates. The final mixture used for treating the cells contained not more than 0.5% of the solvent (DMSO), the same as in the solvent-control wells. After 48 h of exposure 100 μl of ice-cold 40% trichloroacetic acid (TCA) were added to each well, left for 1 h at 4 °C, and washed with distilled water. The TCA-fixed cells were stained for 30 min with 50 μl of 0.4% (w/v) SRB in 1% acetic acid. The plates were washed with 1% acetic acid and air-dried overnight. For reading plate, the bound dye was solubilized with 100 μl of 10 mM Tris [tris(hydroxymethyl)aminomethane]. The absorbance of each well was read on a Molecular Devices SpectraMax Plus Plate Reader (Molecular Devices, CELBIO, Milan, Italy) at $\lambda = 490$ nm. Cell survival was measured as the percentage absorbance compared to the untreated control. Vinblastine sulfate was used as positive control. The antiproliferative activity of the *A. excelsa* extract and isolated flavonoids was expressed in terms of their IC_{50} values.

Statistical analysis

All experiments were carried out in triplicate. Data were expressed as mean ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Dunnett's test. Differences were considered significant at $^{**}p < 0.01$. The 50% inhibitory concentration (IC_{50}) was calculated by a nonlinear regression curve with the use of Prism Graphpad Prism version 4.0 for Windows [GraphPad Software, San Diego, CA, USA (www.graphpad.com)]. The dose-response curve was obtained by plotting the percentage of inhibition versus the concentrations.

Results and Discussion

The methanol extract of *A. excelsa* leaves was chromatographed on a silica gel column followed by successive separation on Sephadex LH-20 affording eleven pure known flavonoids identified as four flavones, namely apigenin (**1**), apigenin 7-*O*-β-glucoside (**2**), luteolin (**3**), and luteolin 7-*O*-β-glucoside (**4**), and seven flavonols, namely kaempferol (**5**), kaempferol 3-*O*-α-arabinoside (**6**), kaempferol 3-*O*-β-galactoside (**7**), quercetin (**8**), quercetin 3-*O*-α-arabinoside (**9**), quercetin 3-*O*-β-galactoside (**10**), and quercetin 3-*O*-rutinoside (**11**) (Fig. 1). Compounds **2**, **5**, **8**, **10**, **11** were isolated here for the first time from *A. excelsa*. All the structures were determined from UV, MS and NMR spectral data, which were identical with those previously reported (Nakasugi and Komai, 1998; Sanbongi *et al.*, 1998; Foo *et al.*, 2000; Flamini *et al.*, 2001).

The *A. excelsa* antioxidant activity was screened employing different established *in vitro* systems. A rapid, simple and inexpensive method to measure the antioxidant capacity involves the use of the free DPPH radical. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity. It has also been used to quantify antioxidants in complex biological systems in recent years. The IC_{50} value for DPPH scavenging by an *A. excelsa* extract is 85.71 μg ml^{-1}.

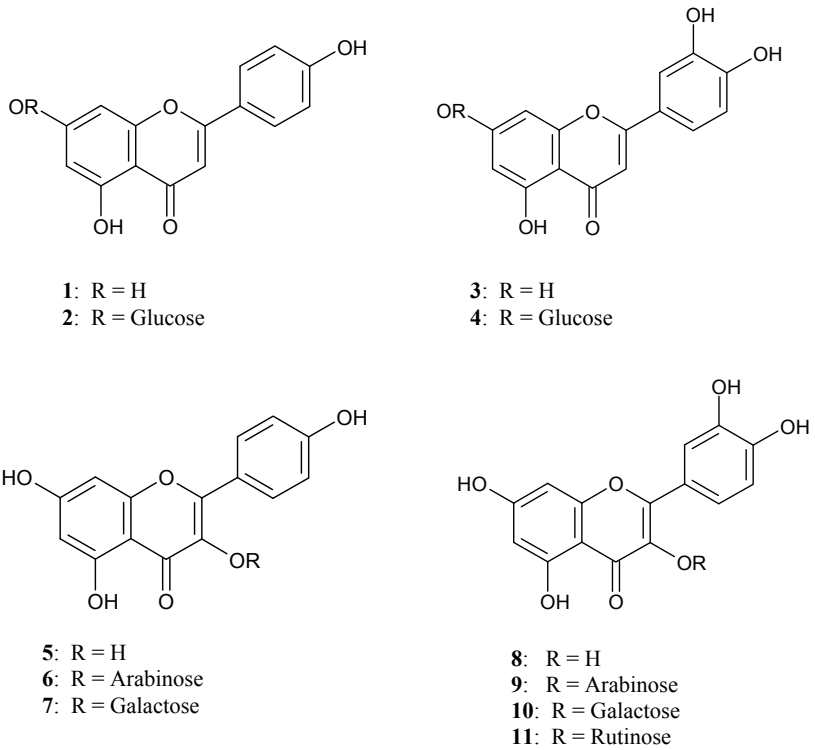

Fig. 1. Chemical structures of the flavonoids apigenin (**1**), apigenin 7-*O*-*β*-glucoside (**2**), luteolin (**3**), luteolin 7-*O*-*β*-glucoside (**4**), kaempferol (**5**), kaempferol 3-*O*-*α*-arabinoside (**6**), kaempferol 3-*O*-*β*-galactoside (**7**), quercetin (**8**), quercetin 3-*O*-*α*-arabinoside (**9**), quercetin 3-*O*-*β*-galactoside (**10**), and quercetin 3-*O*-rutinoside (**11**) isolated from *Ailanthus excelsa* leaves.

Table I. Antioxidant activity of *A. excelsa* extract in DPPH and FRAP assays.

Sample	DPPH (IC_{50}) [μg ml^{-1}]	FRAP [μM Fe(II) g^{-1}]
A. excelsa	85.71 ± 1.2	1.2 ± 0.05
Ascorbic acid[a]	–	0.9 ± 0.05
BHT[a]	18.21 ± 0.47	–

Data are expressed as mean ± S.D. (n = 3).
[a] Positive control.

The FRAP assay measures the reducing ability of an antioxidant that reacts with the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) complex (Benzie and Strains, 1996). Using this assay, the FRAP value of the extract was found to be 1.2 compared to ascorbic acid (0.9) (Table I). Some researchers reported that phenolic compounds exhibited redox properties (*i.e.* acted as reducing agents, hydrogen donators, and singlet oxygen quenchers). The redox potential of phenolic phytochemicals plays a crucial role in determining the antioxidant properties (Rice-Evans *et al.*, 1997).

The antiproliferative activity of the *A. excelsa* extract and isolated flavonoids on inhibition of cell proliferation in the human cancer cell lines COR-L23, C32, ACHN, A375 and A549 was evaluated in comparison with one normal cell line, 142BR. A dose-response relationship was observed for all tested samples. The IC_{50} values are reported in Table II. The *A. excelsa* extract exhibited the highest inhibitory activity against C32 cells with an IC_{50} value of 36.5 μg ml^{-1}. Among the identified flavonoids quercetin (**8**) evidenced strong antiproliferative activity against amelanotic melanoma, malignant melanoma and lung large cell carcinoma cells with IC_{50} values ranging from 0.3 to 0.7 μg ml^{-1}, respectively, as previously reported (Loizzo *et al.*, 2009b). Quercetin 3-*O*-*β*-galactoside (**10**)

exhibited the highest inhibitory effect against COR-L23 cells with an IC_{50} value of 3.2 μg ml^{-1}. Luteolin (**3**) showed the highest antiproliferative activity against the amelanotic melanoma C32 cell line with an IC_{50} value of 2.4 μg ml^{-1}. An interesting activity was found with apigenin (**1**); it was applied to both amelanotic melanoma and malignant melanoma cell cultures with IC_{50} values of 4.7 and 5.5 μg ml^{-1}, respectively. Both, quercetin 3-*O*-*β*-galactoside (**10**) and quercetin 3-*O*-*α*-arabinoside (**9**) are able to inhibit lung large cell carcinoma proliferation with IC_{50} values of 3.2 and 7.5 μg ml^{-1}, respectively. Several studies have been done on flavonoids' antiproliferative effects against human ovarian, leukemic, intestinal, lung, breast and bladder cancer cells (Scambia *et al*., 1994; Agullo *et al*., 1997; Narayanan *et al*., 1999; Uddin and Choudhry, 1995).

The ability of flavonoids to exert specific cytotoxic activity towards cancer cells has generated large interest in developing flavonoid-based cytostatics for anticancer therapy (Galati and O'Brien, 2004). There are several suggested mechanism by which flavonoids could exert anticancer activity including proteasome inhibition (Chen *et al.*, 2005), inhibition of fatty acid synthesis (Brusselmans *et al.*, 2005), topoisomerase inhibition (Constantinou *et al.*, 1995), inhibition of phosphatidylinositol 3-kinase (Agullo *et al.*, 1997), induction of cell cycle arrest (Lepley *et al.*, 1996), induction of p53 (Plaumann *et al.*, 1996) or enhanced expression of c-fos and c-myc proto-oncogenes (Chen *et al.*, 1998).

Plants are an excellent source of chemical compounds with a wide variety of biological activities, including anticancer properties. Fundamental phytochemical investigations should be encouraged, especially in view of the urgent need to discover new bioactive molecules with greater efficacy and less side effects than existing drugs. The *A. excelsa* extract and isolated flavonoids evidenced significant antioxidant and antiproliferative activities. An increasing number of research studies have established a positive correlation between the consumption of flavonoids and reduced risk of cancer. This is supported by a multitude of *in vitro* and *in vivo* studies, which show that flavonoids may inhibit various stages in the carcinogenesis process, namely tumour initiation, promotion, and progression. Based on this studies many mechanisms of action may be involved. These include carcinogen inactivation, antiproliferation, cell cy-

Table II. Antiproliferative activity of *A. excelsa* extract and isolated constituents (IC_{50} in μg ml^{-1}).

Cell line	*A. excelsa*	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	Vinblastine
ACHN	>100	20.7 ± 2.1**	38.6 ± 2.1**	26.6 ± 3.3**	34.2 ± 0.9**	42.8 ± 2.9**	13.6 ± 0.06**	34.9 ± 0.06**	12.6 ± 1.1**	>50	11.5 ± 0.35**	35.5 ± 0.35**	22.7 ± 1.6
COR-L23	95.2 ± 3.1**	8.9 ± 0.012**	36.6 ± 4.8**	5.3 ± 0.07**	13.5 ± 0.04**	2.7 ± 0.08**	36.1 ± 0.09**	44.9 ± 0.11°	0.3 ± 0.002**	7.5 ± 0.07**	3.2 ± 0.02**	14.2 ± 0.02**	45.5 ± 0.7
A375	78.4 ± 3.7**	5.5 ± 0.042°	42.2 ± 2.5**	5.2 ± 0.05°	14.0 ± 0.09**	36.8 ± 2.3**	>50	>50	0.7 ± 0.006**	>50	>50	>50	7.2 ± 0.7
C32	36.5 ± 2.9**	4.7 ± 0.021**	>50	2.4 ± 0.06**	12.5 ± 0.08**	41.7 ± 2.6**	>50	>50	0.5 ± 0.007**	>50	>50	>50	3.0 ± 0.08
A549	81.8 ± 4.2**	>50	>50	>50	>50	>50	>50	>50	22.1 ± 1.4**	>50	>50	>50	67.3 ± 2.0
142BR	>100	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	29.3 ± 0.9

COR-L23, lung large cell carcinoma (ECACC No. 92031919); C32, amelanotic melanoma (ATCC No. CRL-1585); ACHN, renal cell adenocarcinoma (ATCC No. CRL-1611); A375, malignant melanoma (ECACC No. 88113005); A549, lung carcinoma (ECACC No. 86012804); 142BR, skin fibroblasts (ECACC No. 90011806). Data are given as the mean of at least three independent experiments ± S.D. ACHN cell line: One-way ANOVA, *** $p < 0.0001$ ($F = 116.3$, $r^2 = 0.98$); Dunnett's multiple comparison test, ** $p < 0.01$ except ° $p > 0.05$ [vinblastine *vs* (**1**), vinblastine *vs* (**3**)]. CORL-23 cell line: One-way ANOVA, *** $p < 0.0001$ ($F = 341.1$, $r^2 = 0.99$); Dunnett's multiple comparison test, ** $p < 0.01$ except ° $p > 0.05$ [vinblastine *vs* (**7**)]. A375 cell line: One-way ANOVA, *** $p < 0.0001$ ($F = 843.9$, $r^2 = 0.99$); Dunnett's multiple comparison test, ** $p < 0.01$ except ° $p > 0.05$ [vinblastine *vs* (**1**), vinblastine *vs* (**3**)]. C32 cell line: One-way ANOVA, *** $p < 0.0001$ ($F = 467.3$, $r^2 = 0.99$); Dunnett's multiple comparison test, ** $p < 0.01$ except ° $p > 0.05$ [vinblastine *vs* (**1**), vinblastine *vs* (**3**), vinblastine *vs* (**8**)]. A549 cell line: One-way ANOVA, *** $p < 0.0001$ ($F = 502.3$, $r^2 = 0.99$); Dunnett's multiple comparison test, ** $p < 0.01$.

cle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation, and reversal of multidrug resistance or a combination of these mechanisms. Furthermore, the intriguing results from laboratory and epidemiological studies have stimulated the development of flavonoids in human clinical trials.

Adamik K. and Brauns F. E. (1957), *Ailanthus glendulosa* (tree-of-heaven) as a pulpwood. TAPPI **40**, 522–527.

Agullo G., Gamet-Payrastre L., Manenti S., Viala C., Remesy C., Chap H., and Payrastre B. (1997), Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. Biochem. Pharmacol. **53**, 1649–1657.

Arcamone F., Cassinelli G., and Casazza A. M. (1980), New antitumor drugs from plants. J. Ethnopharmacol. **2**, 149–160.

Benzie I. F. and Strains J. J. (1996), The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. Anal. Biochem. **239**, 70–76.

British Pharmacopoeia (1988), II. H. M. Stationary Office, London, p. 704.

Brusselmans K., Vrolix R., Verhoeven G., and Swinnen J. V. (2005), Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. J. Biol. Chem. **280**, 5636–5645.

Chen Z. P., Schell J. B., Ho C. T., and Chen K. Y. (1998), Green tea epigallocatechin gallate shows a pronounced growth inhibitory effect on cancerous cells but not on their normal counterparts. Cancer Lett. **129**, 173–179.

Chen D., Daniel K. G., Chen M. S., Kuhn D. J., Landis-Piwowar K. R., and Dou Q. P. (2005), Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. Biochem. Pharmacol. **69**, 1421–1432.

Constantinou A., Mehta R., Runyan C., Rao K., Vaughan A., and Moon R. (1995), Flavonoids as DNA topoisomerase antagonists and poisons: structure-activity relationships. J. Nat. Prod. **58**, 217–225.

Cragg G. M. and Newman D. J. (2005), Plants as source of anticancer agents. J. Ethnopharmacol. **100**, 72–79.

Dhanasekaran S., Suresh B., Sethuraman M., Rajan S., and Dubey R. (1993), Antifertility activity of *Ailanthus excelsa* Linn. in female albino rats. Indian J. Exp. Biol. **31**, 384–385.

Flamini G., Antognoli E., and Morelli I. (2001), Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. Phytochemistry **57**, 559–564.

Foo L. Y., Lu Y., Molan A. L., Woodfield D. R., and McNabb W. C. (2000), The phenols and prodelphinidins of white clover flowers. Phytochemistry **54**, 539–548.

Galati G. and O'Brien P. J. (2004), Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radic. Biol. Med. **37**, 287–303.

Halliwell B. and Gutteridge J. M. C. (1990), Role of free radicals and catalytic metal ions in human disease: an overview. Meth. Enzymol. **186**, 1–85.

Joshi B. C., Pandey A., Sharma R. P., and Khare A. (2003a), Quassinoids from *Ailanthus excelsa*. Phytochemistry **62**, 579–584.

Joshi B. C., Pandey A., Chaurasia L., Pal M., Sharma R. P., and Khare A. (2003b). Antifungal activity of the stem bark of *Ailanthus excelsa*. Fitoterapia **74**, 689–691.

Lepley D. M., Li B., Birt D. F., and Pelling J. C. (1996), The chemopreventive flavonoid apigenin induces G2/M arrest in keratinocytes. Carcinogenesis **17**, 2367–2375.

Loizzo M. R., Tundis R., Statti G. A., Menichini F., and Houghton P. J. (2005), *In vitro* antiproliferative effects on human tumour cell lines of extracts and jacaranone from *Senecio leucanthemifolius* Poiret. J. Pharm. Pharmacol. **57**, 897–902.

Loizzo M. R., Said A., Tundis R., Rashed K., Statti G. A., Hufner A., and Menichini F. (2007), Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from *Ailanthus excelsa* (Roxb.) (Simaroubaceae). Phytother. Res. **21**, 32–36.

Loizzo M. R., Menichini F., Conforti F., Tundis R., Bonesi M., Saab A. M., Statti G. A., de Cindio B., Houghton P. J., Menichini F., and Frega N. G. (2009a), Chemical analysis, antioxidant, antiinflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss. and *Origanum syriacum* L. essential oils. Food Chem. **117**, 174–180.

Loizzo M. R., Said A., Tundis R., Hawas U. W., Rashed K., Menichini F., Frega N. G., and Menichini F. (2009b), Antioxidant and antiproliferative activity of *Diospyros lotus* L. extract and isolated compounds. Plant Foods Hum. Nutr. **64**, 264–270.

Nag A. and Matai S. (1994), *Ailanthus excelsa* Roxb. (Simaroubacae), a promising source of leaf protein. J. Agric. Food Chem. **42**, 1115–1117.

Nakasugi T. and Komai K. (1998), Antimutagens in the Brazilian folk medicinal plant carqueja (*Baccharis trimera* Less.). J. Agric. Food Chem. **46**, 2560–2564.

Narayanan B. A., Geoffroy O., Willingham M. C., Re G. G., and Nixon D. W. (1999), p53/p21 (WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. Cancer Lett. **136**, 215–221.

Ogura M., Cordell G. A., Kinghorn A. D., and Farnsworth N. R. (1977), Potential anticancer agents VI. Constituents of *Ailanthus excelsa* (Simaroubaceae). Lloydia **40**, 579–584.

Panayiotidis M. (2008), Reactive oxygen species (ROS) in multistage carcinogenesis. Cancer Lett. **266**, 3–6.

Plaumann B., Fritsche M., Rimpler H., Brandner G., and Hess R. D. (1996), Flavonoids activate wild-type p53. Oncogene **13**, 1605–1614.

Rice-Evans C. A., Miller N. T., and Paganga G. (1997), Antioxidant properties of phenolic compounds. Trends Plant Sci. **4**, 304–309.

Sanbongi C., Osakabe N., Natsume M., Takizawa T., Gomi S., and Osawa T. (1998), Antioxidative polyphenols isolated from *Theobroma cacao*. J. Agric. Food Chem. **46**, 454–457.

Scambia G., Panici P. B., Ranelletti F. O., Ferrandina G., De Vincenzo R., Piantelli M., Masciullo V., Bonanno G., Isola G., and Mancuso S. (1994), Quercetin enhances transforming growth factor beta 1 secretion by human ovarian cancer cells. Int. J. Cancer **57**, 211–215.

Sherman M. M., Borris R. P., Ogura M., Cordell G. A., and Farnsworth N. R. (1980), 3*S*,24*S*,25-Trihydroxytirucall-7-ene from *Ailanthus excelsa*. Phytochemistry **19**, 1499–1501.

Shrimali M., Jain D. C., Darokar M. P., and Sharma R. P. (2001), Antibacterial activity of *Ailanthus excelsa* (Roxb.). Phytother. Res. **15**, 165–166.

Uddin S. and Choudhry M. A. (1995), Quercetin, a bioflavonoid, inhibits the DNA synthesis of human leukemia cells. Biochem. Mol. Biol. Int. **36**, 545–550