Phytomedicine 10: 53–58 (2003)

http://www.urbanfischer.de/journals/phytomed

Phytomedicine

Ethanolic extracts of *Euphorbia* and other ethnobotanical species as inhibitors of human tumour cell growth

L. C. Whelan and M. F. Ryan

Department of Zoology, University College Dublin, Belfield, Dublin, Ireland

Summary

Ethanolic extracts of 20 plant species, selected from the ethnobotanical literature, were analysed for their pharmacological potential as antineoplastic agents against the HEp-2 cell line. *Psoralea corylifolia* and *E. grandidens* were the most efficacious species eliciting IC_{50} values of 22 µg/ml and 57 µg/ml respectively. *Psoralea corylifolia*, additionally tested against lung carcinoma (A549) cells gave an IC_{50} value of 68 µg/ml. Such data would justify a search for active compounds from this species.

Key words: antineoplastic activity, A549, HEp-2, plant extracts

Introduction

Plant families cited as sources of medicinal agents include Apocynaceae, Cephalotaxaceae, Compositae, Euphorbiaceae, Leguminosae, Papaveraceae, Phytolaccaceae, Ranunculaceae, Rubiaceae and Solanaceae (Gentry, 1993). We selected species from 11 families represented in ethnomedicine, especially the Euphorbiaceae that are rich in active compounds including terpenoids, alkaloids, phenolics and fatty acids, having ethnopharmaceutical uses (Rizk, 1987). However, some extracts from *Euphorbia* species exhibit mixed biological activity as some are highly caustic, irritants, deter feeding by herbivores, activate blood platelets and prostaglandin production; and some promote tumours (Rizk, 1987), but others exhibit antineoplastic activity (Wu et al. 1991).

E. kansui (Wu et al. 1991), *E. esula* and *Croton tiglium* (Kupchan et al. 1976) have demonstrated antileukemic activity against the P-388 lymphocytic leukemia in mice at a dosage of 0.1 mg/kg, 130 to 360 µg/kg and 60 to 250 µg/kg respectively. Moreover, *E. kansui* is also selectively toxic to leukemic, non-small cell lung cancer, colon cancer, melanoma and renal cancer, with IC_{50} values in the range 0.07–13 µg/ml (Wu et al. 1991). Other species from this family with anti-tumour activity include *E. poisonii* (Fatope, 1996), *E. pulcherrima* (Smith-Kielland, 1996) and *E. splendens* (Lee, 1993).

The latices of *Euphorbia grandidens*, *E. candelabrum*, *E. grandicorni* and *E. triangularis* elicit papillomas in mice pretreated with a subcarcinogenic dose of 7,12-dimethylbenz(a)anthracene (DMBA) (Roe et al. 1961). The latex of *E. candelabrum* elicited irritant activity in the mouse ear, attributable to ingenol esters. Furthermore, the latices of *E. coerulescens*, *E. pentagona*, *E. lactea* and *E. grandidens* are irritants, with *E. lactea* and *E. grandidens* possibly causing blindness (Watt et al. 1962). The biological activity of *E. trigona* and *E. istigy* has not been reported. To date the latices of a broad range of *Euphorbia* species have not been screened for antitumour activity and so the present investigation firstly, clarifies their antineoplastic status by direct comparison of their effects on HEp-2 cell line. Secondly, a context is provided for such data by also screening the following twenty ethnobotanical species, including some with established antineoplastic activity.

0944-7113/03/10/01-053 $ 15.00/0

Psoralea corylifolia (Leguminosae) and *Chelidonium majus* (Papaveraceae) are used in folk medicine for the treatment of breast, colon, ovarian, testicular and stomach cancer (Duke, 1985). In Russia, Asia and Latin America *Plantago major* (Plantaginaceae) has demonstrated antineoplastic activity against cancer of the breast, anus, stomach, eye, foot, intestine and liver, and against neuroblastoma cancer (Duke, 1985). *Cephalotaxus fortunei* (Cephalotaxaceae) was included on the basis that homoharringtonine, an alkaloid isolated from a related species, *Cephalotaxus harringtonia*, is undergoing clinical trials against leukemia (Cragg et al. 1993; Wang et al. 1992). *Sophora flavescens* (Leguminosae) is antineoplastic against leukemia and melanoma (Ko et al. 2000); and *Coptis chinensis* (Ranunculaceae) is cytostatic against HepG2 cells (Chi et al. 1994). *Terminalia chebula* (Combretaceae) has demonstrated anticaries activity against *Streptococcus mutans* (Jagtap et al. 1999) and serves as a tonic and astringent (Trease, 1989). *Atropa belladonna* (Solanaceae) was used for acute radiodermatitis. The remaining plant species, belonging to families used in ethnomedicine, include *Dianthus sinensis* (Caryophyllaceae), *Phytolacca polyandra* (Phytolaccaceae) and *Polygonathum odoratum* (Convallariaceae). This communication describes the effects of ethanolic extracts of all the forgoing plant species on proliferation of human tumour cells.

Table 1. Efficacies of *Euphorbia* latices assayed against HEp-2 cells*.

Botanical name[a]	IC_{50} (µg/ml)[†]
E. grandidens[3]	57 ± 12
E. grandicorni[3]	89 ± 14
E. latea[3]	89 ± 5
E. coerulescens[3]	121 ± 22
E. trigona[1]	330 ± 17
E. istigy[3]	444 ± 22
E.candelabrum[1]	N/D
E. pentagona[1]	N/D
E. triangularis[3]	N/D

Plants originated from: [1]National Botanic Gardens, Glasnevin, Dublin, Ireland; [2]Taiwan, [3]Thornfield Greenhouses, University College Dublin, Ireland.
[*]assessed by the MTT assay; [†]IC_{50} values (concentrations eliciting 50% inhibition) were determined from linear regression analysis; N/D – could not be determined; [a]Dose range tested, 8.5–853 µg/ml; lower concentrations of *Euphorbia* latex (0.5–8.5 µg/ml) did not alter cell proliferation (data not shown). For comparison, the IC_{50} value for taxol was 0.01 µM ± 0.004.

Materials and Methods

Plant species

Latex samples from *Euphorbiaceae* (Table 1) were collected between June and August of 1997 from the National Botanic Gardens, Glasnevin, Dublin, Ireland and from Thornfield greenhouse, University College Dublin, Ireland. Plant species from the other families, were collected from the [1]National Botanic Gardens, Glasnevin, Dublin, Ireland and [2]Taiwan (Table 2). All plant material from Taiwan was authenticated by Mr. Nan-Un Chou, Graduate Institute of Pharmacy, Chinese Medical College, Taichung, Taiwan.

Extracts

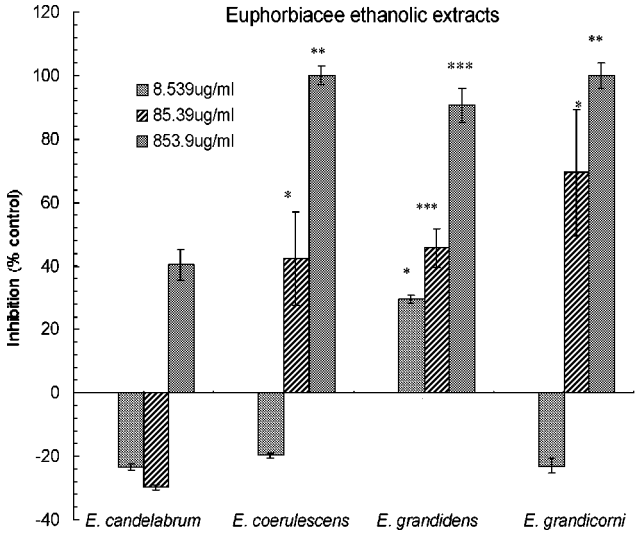
A deep incision in the stem of the Euphorbiaceae released latex, which was then extracted repeatedly with absolute ethanol and shaken for 24 h. After filtering the extract through Whatman no. 1 filter paper, the entire

Table 2. Efficacies of 11 ethnobotanical species assayed against HEp-2 cells*.

Botanical name[a]	Plant part tested	IC_{50} (µg/ml)[†]
Psoralea corylifolia[2] (Leguminosae)	Fruit	22 ± 6
Dianthus sinensis[1] (Caryophyllaceae)	Whole plant	111 ± 19
Phytolacca polyandra[1] (Phytolaccaceae)	Whole plant	129 ± 13
Sophora flavescens Ait[2] (Leguminosae)	Root	134 ± 17
Cephalotaxus fortunei[2] (Cephalotaxaceae)	Branch	134 ± 27
Polygonatum odoratum[1] (Convallariaceae)	Root	142 ± 11
Coptis chinensis French[2] (Ranunculaceae)	Whole plant	412 ± 32
Terminalia chebula Retz[2] (Combretaceae)	Fruit	201 ± 51
Atropa belladonna[1] (Solanaceae)	Fruit	N/D
Chelidonium majus[1] (Papaveraceae)	Whole plant	N/D
Plantago major[1] (Plantaginaceae)	Root	N/D

Plants originated from: [1]National Botanic Gardens, Glasnevin, Dublin,[2] Taiwan.
[*]assessed by the MTT assay; [†]IC_{50} values (concentrations eliciting 50% inhibition) were determined from linear regression analysis; N/D – could not be determined. [a]Dose range tested, 8.5–853 µg/ml. For comparison, the IC_{50} value for taxol was 0.01 ± 0.004.

extract was roto-evaporated at 40 °C resulting in a dried mass that was stored in the dark at –20 °C until usage. The dried mass was dissolved in ethanol for assays. The air-dried plants of Chinese origin were crushed in a grinder, and extracted exhaustively with absolute ethanol (10 mg/ml) following the above procedure. Treatment doses of all extracts were 8.53 µg/ml, 85.3 µg/ml and 853.9 µg/ml.



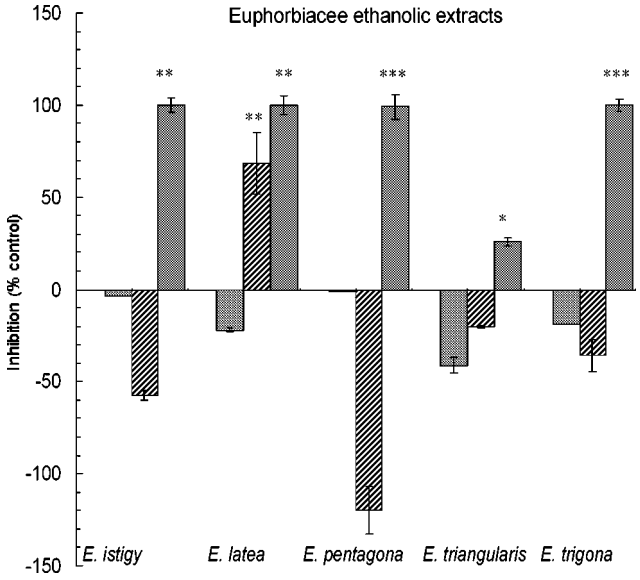
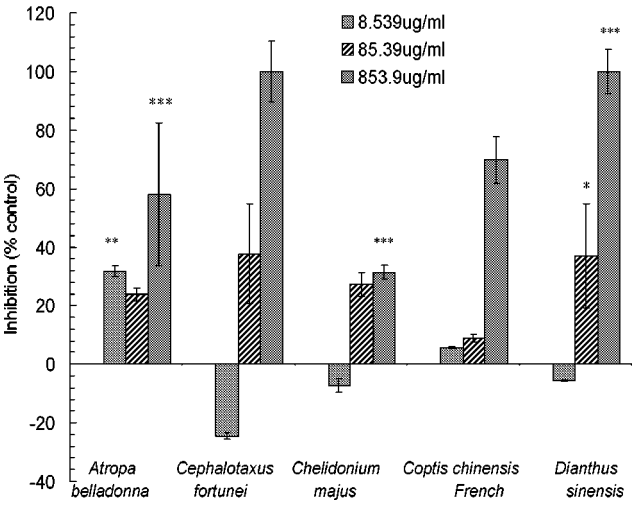


Fig. 1. a+b. Inhibition of cellular growth by ethanolic extracts of various Euphorbiaceae, as assessed by the MTT assay in HEp-2 cells and expressed as a percentage of control values. Cells were seeded on day 1, exposed to extracts on day 3 and assayed for cellular viability on day 4. Results are expressed as % control ± S.E.M. of four replicates.
* indicates statistically significant ($p < 0.05$) differences from control
** indicates statistically highly significant ($p < 0.01$) differences from control
*** indicates statistically very highly significant ($p < 0.001$) differences from control



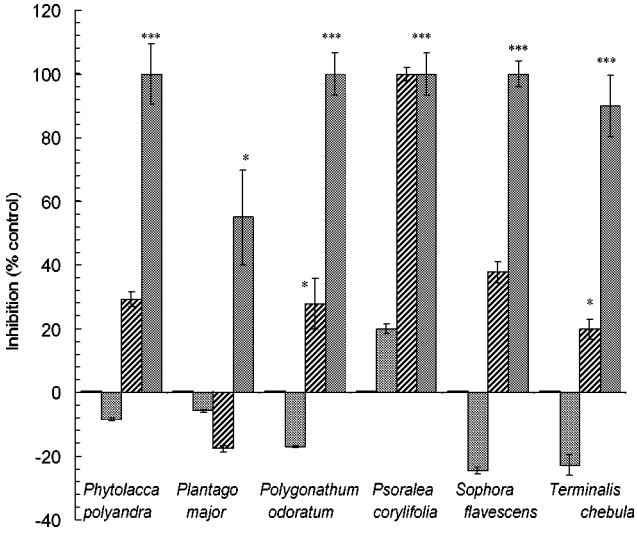


Fig. 2. a+b. Inhibition of cellular growth of various plant ethanolic extracts, as assessed by the MTT assay in HEp-2 cells and expressed as a percentage of control values. Cells were seeded on day 1, exposed to extracts on day 3 and assayed for cellular viability on day 4. Results are expressed as % control ± S.E.M. of four replicates.
* indicates statistically significant ($p < 0.05$) differences from control
** indicates statistically highly significant ($p < 0.01$) differences from control
*** indicates statistically very highly significant ($p < 0.001$) differences from control

Cell cultures

Cell lines from human epidermoid carcinoma of the larynx HEp-2 and human lung carcinoma A549 were obtained from the National Cell and Tissue Culture Centre, Dublin City University, Dublin 9. Cell lines were maintained at 37 °C in 5% CO_2 as subconfluent monolayers in 75 cm^2 culture flasks (Costar-Corning). HEp-2 cells were grown in Dulbeccos Modified Eagle's Medium (DMEM) supplemented with 5% FBS, 2% penicillin-streptomycin and 1% L-glutamine. The extract most efficacious against HEp-2 cell line was also assayed against the A549 cells that were maintained in nutrient mixture F-12 HAM HEPES modification supplemented with 5% foetal bovine serum (FBS), 2% penicillin-streptomycin and 0.5% L-glutamine.

MTT colorimetric assay

Cell proliferation/cytotoxicity was evaluated using the MTT (3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide) assay in which viable but not dead cells cleave the yellow tetrazolium salt (MTT) to a blue formazan product (Mosmann, 1983). This assay is widely used as a preliminary screen to quantify cell proliferation, viability, cytoxicity and sensitivity (Mosmann, 1983; Fokkema et al. 2002; Itamochi et al. 2002). Taxol (Sigma-Aldrich) served as a positive control. The solvent control contained ethanol and DMSO at a concentration less than 0.01%. After incubating cells with plant extracts (8.5 µg/ml–853 µg/ml) for 24 h, the cytotoxicity was expressed from the mean of four replicates (± S.E) as a percentage of the control. Significant differences were detected by the Student's *t*-test. The IC_{50} values (drug concentration eliciting 50% inhibition) were determined by linear regression analysis.

Results

Effect of *Euphorbia* extracts on HEp-2 cellular proliferation

Extracts of four out of nine *Euphorbia* species tested, at concentrations of 85.39 µg/ml and 853.9 µg/ml for 24 h, decreased cellular viability as determined by the MTT assay (Fig. 1a+b, Table 1); this effect was dose-dependent and *E. grandidens* was the most efficacious (IC_{50} of 57 µg/ml). However, lower drug concentrations (8.539 µg/ml) increased cellular proliferation in seven species, *E. candelabrum, E. coerulescens, E. grandicorni, E. istigy, E. latea, E. triangularis* and *E. trigona*. *E. candelabrum, E. pentagona* and *E. triangularis* did not elicit 50% inhibition at any concentration tested. All nine species tested stimulated cellular proliferation at doses less than 50 µg/ml (data not shown).

Effect of extracts from 10 other families on HEp-2 cellular proliferation

Of all the 11 plant species tested, *Psoralea corylifolia* was the most potent with an estimated IC_{50} of 22 µg/ml (Table 2). The IC_{50} could not be determined for *Atropa belladonna*, *Plantago major* and *Chelidonium majus* as their extracts did not elicit 50% inhibition (Fig. 2a+b; Table 2). Weak inhibition was elicited by *Chelidonium majus*.

Effect of *Psoralea corylifolia* extract on A549 cellular proliferation

Subjecting A549 cells to the extract of *Psoralea corylifolia* confirmed its cytotoxic activity, with an IC_{50} of 68 µg/ml.

Discussion

Of nine *Euphorbia* species tested, *E. grandidens, E. grandicorni*, and *E. latea* exhibited the greatest potency against HEp-2 cells by eliciting IC_{50} at concentrations of 57 µg/ml, 89 µg/ml, and 89 µg/ml respectively. Much higher concentrations of *E. coerulesecens* (121 µg/ml), *E. trigona* (330 µg/ml) and *E. istigy* (444 µg/ml) were required to elicit the same degree of inhibition (Fig.1). Cell proliferation was increased by 3–24% in HEp-2 cells treated with *E. candelabrum, E. coerulescens, E. grandicorni, E. istigy, E. latea, E. triangularis and E. trigona* at a concentration of 8.539 µg/ml. Increased cell growth was also elicited by *E. candelabrum, E. istigy, E. pentagona, E. triangularis* and *E. trigona* at a concentration of 85.89 µg/ml.

Some Euphorbiaceae are both tumour-promoting and cytotoxic. *Croton tiglium* elicits both antileukemic activity (Kupchan et al. 1976) and tumour promotion *in vivo* and *in vitro* (Rizk, 1987). The present study confirms this dual, concentration-dependent effect in eight species. Several Euphorbiaceae contain phorbol esters, which may interact with the cell membrane to alter permeability characteristics; these could affect the entry or exit of amino acids and nucleotides known to regulate cellular metabolism (Rizk, 1987; Van Duuren et al. 1968).

Of all plant species tested, *Psoralea corylifolia* was the most efficacious with an IC_{50} value of 22 µg/ml against HEp-2 cells, and 68 µg/ml against A549 cells. This 3.1-fold difference may be due to cell-turnover rates; HEp-2 cells double every 3.5 days compared with one day for A549 cells. *P. corylifolia* contains as major constituents DNA polymerase and topoisomerase II inhibitors, that inhibit DNA replication enzymes (Sung et al. 1998). Isobavachalcone (chemical structure not

available), isolated from *P. corylifolia* is antineoplastic against bone-muscle tumour, lung tumour and intestinal tumour at a dosage of 15–25 g a day in China (Wang, 1991). As this species has not been previously screened against the HEp-2 and A549 cell lines deployed in the present study, the present data are novel.

Much higher concentrations of *Dianthus sinensis* (5-fold), *Phytolacca polyandra* (5.8-fold), *Sophora flavescens* Ait (6.1-fold), *Cephalotaxus fortunei* (6.1-fold), *Polygonatum odoratum* (6.5-fold), *Terminalia chebula* Retz (9-fold) and *Coptis chinensis* French (18.7-fold) were required to elicit the same degree of inhibition in HEp-2 cells. *Sophora flavescens* roots have shown anti-tumour activity against sarcoma 180, lymphoid leukemia 1210 and melanotic melanoma. Four flavonoids, sophoraflavanone, kurarinone, norkurarinol, kurarinol and kushk isolated from *Sophora flavescens* induce apoptosis (Ko et al. 2000) and inhibit cell proliferation in HL60 and HEpG2 cells at IC_{50} values of 11.3–8.5 µM and 13.3–3 µM respectively (Choi et al. 1999). In the present study, the ethanolic extract of *Sophora flavescens* exhibited an IC_{50} value of 134 µg/ml.

This screening study establishes for the first time in a direct comparison antineoplastic and cell proliferation effects on HEp-2 cells by relatively high and low concentrations respectively, of eight species of *Euphorbia*. This dual effect is consistent with observations on other *Euphorbia* species. More importantly this study highlights *Psoralea corylifolia* as the most effacious of twenty species tested against HEp-2 cell line. This might justify the search for active compounds.

Acknowledgements

We thank Dr. M. Jebb of the National Botanic Gardens, Glasnevin, Dublin for donating plant material. We also thank Nan-Un Chou, Graduate Institute of Pharmacy, Chinese Medical College, Taichung, Taiwan for plant collection and identification.

References

Chi CW, Chang YF, Chao TW, Chiang SH, P'eng FF, Lui WY, Liu TY (1994) Flow cytometric analysis of the effect of berberine on the expression of glucocorticoid receptors in human hepatoma HepG2 cells. Life Science 54(26): 2099–2107

Choi SU, Kim KH, Choi EJ, Park SH, Lee CO, Jung NP, Yoon SK, Ryu SY (1999) P-glycoprotein (Pgp) does not affect the cytoxicity of flavonoids from *Sophora flavescens*, which also have no effects on Pgp action. Anticancer Research 19(13A): 2035–2040

Cragg GM, Boyd MR, Cardellina H, Grever MR, Schepartz SA, Snader KM, Suffness M (1993) Role of plants in the National Cancer Institute drug discovery and development program. In: Kinghorn AD, Balandrin MF, editors. Human medicinal agents from plants. Symposium Series No. 534, American Chemical Society, Washington, DC, pp 80–95

Duke JA (1985) CRC Handbook of Medicinal Herbs. CRC Press Inc., Boca Raton, Florida.

Fatope MO, Zeng L, Ohayaga JE, Shi G, McLaughlin JL (1996) Selectively cytotoxic diterpenes from *Euphorbia poisonii*. J Med Chem 39(4):1005–1008

Fokkema E, Groen HJ, Helder MN, de Vries EG, Meijer C (2002) JM216-, JM118-, and cisplatin-induced cytoxicity in relation to platinum-DNA adduct formation, glutathione levels and p53 status in human tumour cell lines with differential sensitivities to cisplatin. Biochem Pharmacol 63(11): 1989–1996

Gentry AH (1993) Tropical forest biodiversity and the potential for new medicinal plants. In: Kinghorn AD, Balandrin MF, editors. Human medicinal agents from plants. Symposium Series No. 534, American Chemical Society, Washington, DC, pp 13–24

Itamochi H, Kigawa J, Sultana H, Iba T, Akeshima R, Kamazawa S, Kanamori Y, Terakawa N (2002) Sensitivity to anticancer agents and resistance mechanisms in clear cell carcinoma of the ovary. Jpn J Cancer Res 93(6): 723–728

Jagtap AG, Karkera SG (1999) Potential of the aqueous extract of *Terminalia chebula* Retz as an anticaries agent. Journal of Ethnopharmacology 68: 299–306

Ko WG, Kang TH, Kim NY, Lee SJ, Kim YC, Ko GI, Ryu SY, Lee BH (2000) Lavandulylflavonoids: a new class of *in vitro* apoptogenic agents from *Sophora flavescens*. Toxicology *In vitro* 14(5): 429–433

Kupchan SM, Uchida I, Branfman AR, Dailey RG, Yu Fei B (1976) Antileukemic principles isolated from *Euphorbiaceae* plants. Science 19: 1571–1572

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytoxicity assays. J Immunol Methods 95: 55–63

Rizk AFM (1987) The chemical constituents and economic plants of the Euphorbiaceae. In: Jury SL, Reynolds T, Cutler TDF, Evans FJ, editors. Euphorbiales. Chemistry, taxonomy and economic botany. Academic Press Inc. London, pp 293–326

Roe FJC, Pierce EH (1961) Tumour promotion by Euphorbia latices. Cancer Research 21: 338–344

Smith-Kielland I, Dornish JM, Malterud KE, Hvistendahl G, Romming C, Bockman OC, Kolsaker P, Stenstrom Y, Nordal A (1996) Cytotoxic triterpenoids from the leaves of *Euphorbia pulcherrima*. Planta Med 62(4): 322–325

Sung NJ, Woo SH, Cassady JM, Snapka RM (1998) DNA polymerase and topoisomerase II inhibitors from *Psoralea corylifolia*. Journal of Natural Products 61: 362–366

Trease, GE (1989) Trease and Evans' pharmacognosy. 13th ed. London: Bailliere Tindall

Van Duuren BL, Sivak A. (1968) Tumour promoting agents from *Croton tiglium L.* and their mode of action. Cancer Research 28: 2349–2356

Wang LU (1991) Chinese medicine for cancer treatment. 2nd ed. Sunny Books (in Chinese)

Wang DZ, MA, GE, Xu RS (1992) Studies on the alkaloids of *Cephalotaxus*. VII. Structures and semi-synthesis of two

anticancer cephalotaxine esters. Yao Xue Xue Bao 27(3): 173–177

Watt JM, Breyer-Brandwijk MG (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological effects and Toxicology in Man and Animal. 2nd Edn. Livingstone, Edinburgh and London

Wu TS, Lin YM, Haruna M, Pan DJ, Shingu T (1991) Antitumor agents, 119. Kansuiphorins A and B, two novel antileukemic diterpene esters from *Euphorbia kansui*. Journal of Natural Products 54: 823–829

Address

M. F. Ryan, Department of Zoology, University College Dublin, Belfield, Dublin 4, Ireland
Tel.: ++353-1-7062345; Fax: ++353-1-7061152;
e-mail: MFRyan@ucd.ie