

## Antiproliferative, cytotoxic and antitumour activity of coumarins isolated from *Calophyllum brasiliense*

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### Abstract

Among the eight *Calophyllum* species found on the American continent, *Calophyllum brasiliense* is the most widely distributed. Chemical analysis of this species has shown the presence of xanthenes with cancer chemopreventive properties and antifungal activity. Recently, three new coumarins with antineoplastic properties have been found. In this study, we have evaluated the biological effects of the antiproliferative activity of coumarins isolated from *C. brasiliense* on the survival, cell cycle and apoptosis of cells in-vitro and their antitumour effects in mice. The cytological study showed that coumarins from *C. brasiliense* reduce the survival of BMK cells (baby mouse kidney cells) by inducing apoptosis and, to a lesser degree, necrosis. The cell cycle was arrested in S-phase and the division of BMK cells was inhibited. Coumarins had caused a reduction of experimental tumours in 83% of animals by the end of the treatment. Therefore, coumarins have the potential to be used alone or in combination with other antineoplastic drugs, and they might increase the effectiveness of other treatments for cancer.

### Introduction

Coumarins comprise a group of natural compounds found in a variety of plant sources. They have extensive biological properties that promote health and help reduce the risk of disease (Kostova & Momekov 2006). The coumarins are extremely variable in structure, due to various substitutions in their basic structure, which can influence their biological activity. Coumarin, the parent molecule of dicumarol, and a variety of coumarin compounds have numerous antitumour and antiproliferative effects. Coumarin compounds inhibit the proliferation of particular human malignant cell lines in-vitro (Chaya et al 2004; Kostova & Momekov 2006; Riviere et al 2006), as well as affecting the activity of several tumour types in-vivo (Ishihara et al 2006; Kawase et al 2005; Yang et al 2005). In clinical trials, these compounds have shown some activity against prostate cancer, malignant melanoma, and metastatic renal cell carcinoma (Burgos et al 1999; Agata et al 2004; Lacy & O'Kennedy 2004).

*Calophyllum* species contain coumarins and have recently received considerable attention pharmacologically. Coumarins are a common family of plant secondary metabolites. Many studies on natural products have documented their biological effects and there have been many attempts to isolate and purify active compounds. It has been reported that coumarins have a broad spectrum of biological activity, including antitumour effects (Kashman et al 1992; Patil et al 1993; McKee et al 1998).

Among the eight *Calophyllum* species found on the American continent, *Calophyllum brasiliense* has the widest distribution, growing in the tropical Mexican and Brazilian rainforests (Stevens 1980). Numerous ethnomedical applications have been recorded for this species throughout Latin America (Sovak et al 2002; Reddy et al 2004; Tsuda et al 2004). Nevertheless, none appears related to its antiviral and antitumour effects. Previous chemical analysis of *C. brasiliense* collected in Brazil has reported the presence of xanthenes with cancer chemopreventive properties and antifungal activity. In addition, three new coumarins with antineoplastic properties have been found (Ito et al 2002, 2003). The organic extract from the leaves of *C. brasiliense* yields coumarins of the mammea type: mammea

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A/BA, A/BB, B/BB, C/OA, C/OB, B/BA cyclo F, B/BB cyclo F and isomammeigin (Reyes-Chilpa et al 2004). Recent studies have shown that mammea A/BA and A/BB have high cytotoxic activity against some tumour cell lines (Reyes-Chilpa et al 2004).

We have studied the biological effects of the antiproliferative activity of mammea A/BA and A/BB, isolated from *C. brasiliense*, on the survival, cell cycle and apoptosis of BMK cells. We attempted to define their antitumour effects in mice.

## Materials and Methods

### Chemicals

Vincristine was obtained from Lemery Laboratories (Mexico). DMEM, fetal calf serum, MTT ((3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide), trypsin, trypsin inhibitor, ribonuclease A (RNase), and propidium iodide were obtained from Sigma Chemical Co. (USA).

### Extraction and isolation of mammea A/BA and A/BB from *C. brasiliense*

*C. brasiliense* (Clusiaceae) was collected at Ejido Benigno Mendoza, Sierra de Santa Marta, Veracruz, Mexico. A voucher specimen was deposited in the Herbarium of the Instituto de Investigaciones Biológicas, Universidad Veracruzana at Xalapa, Mexico, labelled number 435. Dried leaves (2075 g) were extracted at room temperature over one week with hexane, acetone, and methanol, successively. Compounds were isolated after spontaneous crystallization or by column chromatography on Silica Gel-60, and individualized by their spectroscopy ( $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR, UV), EIMS and CIMS. The results of these experiments were compared with Reyes-Chilpa et al (2004).

#### Hexane extracts (70.3 g)

While in solution, the extract showed spontaneous precipitation, giving a white powder (19.8 g). Part of this material (5.1 g) was treated with  $\text{CH}_2\text{Cl}_2$ . The insoluble part was a mixture (583 mg) of friedelin and canophyllol, and the soluble part afforded a mixture (4 g) of coumarins of two mammea types: mammea A/BA and mammea A/BB. The extract was then concentrated in-vacuo (35.3 g), and 9.4 g subjected to column chromatography (200 g). Elution with hexane first yielded friedelin (45.9 mg), then a mixture of coumarins (5 mg; mammea A/BA and mammea A/BB).

### Identification and chemical structure of mammea A/BA and mammea A/BB

The compounds isolated after spontaneous crystallization or column chromatography were individually identified. The purification of compounds (mp 122–124°C) was obtained by HPLC using an ODS-column and elution with 90% MeOH. Chemical structures are shown in Figure 1. The identification of the mammeas was as follows:

$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3/\text{TMS}$ ): 7.56 m, 3H (Ar); 7.41 m, 2H (Ar); 6.0 s, 1H (H-3); 5.95 s, 1H (OH-5). Isoprenyl on C-6: 5.09 tm,  $J=6.9$  Hz, 1H (CH); 3.29 d,  $J=6.9$  Hz, 2H ( $\text{CH}_2$ ); 1.65 and 1.70, both s and 3H, ( $2\text{CH}_3$ ).

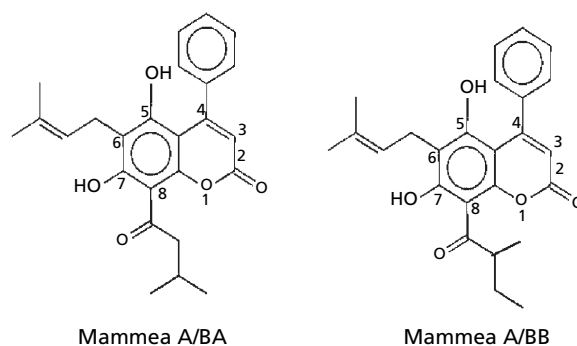


Figure 1 Coumarins isolated from *Calophyllum brasiliense* leaves.

1: 14.61 s, 1H (OH-7). R=3-methylbutyryl: 3.19 d,  $J=6.7$  Hz, 2H ( $\text{CH}_2$ ); 2.32 m,  $J=6.7$  Hz, 1H (CH); 1.06 d,  $J=6.6$  Hz, 6H, ( $2\text{CH}_3$ ).

2: 14.57 s, 1H (OH-7). R=2-methylbutyryl (A/BB): 3.95 m,  $J=6.6$  Hz, 1H (CH- $\text{CH}_3$ ); 1.29 d,  $J=6.7$  Hz, 3H (CH- $\text{CH}_3$ ); 1.94 m, 2H ( $\text{CH}_2$ ); 1.01 t,  $J=7.2$  Hz, 3H ( $\text{CH}_3$ ).

EMIE: 70 eV m/z (%): 406  $\text{M}^+$  (96.4%) [ $\text{C}_{25}\text{H}_{26}\text{O}_5$ ] $^+$ ; 363 (55.6%); 351 (76.0%); 293 (100%). IR  $\nu$  max (KBr) 3985 (OH); 2964, 2932, 2872 (C-H); 1729 (C=O); 1724 (C=O); 1614 (C=C); 1556 (C=C); 1390 (C-O).

For the pharmacological studies, a mixture of coumarins, mammea A/BA and mammea A/BB, at a ratio of 2:1 was used. The mix of mammeas ( $5\text{ mg mL}^{-1}$ ) was prepared in 0.1% dimethyl sulfoxide (DMSO) (Aldrich, USA) at room temperature.

### Cell culture and pharmacological treatments

For in-vitro studies BMK cells were used, derived from baby mouse kidney. These cells were kindly donated by Professor Sophie Hallez from Brussels University, Belgium. Cells were cultured in minimal essential medium (Gibco-BRL Inc., Grand Island, NY), supplemented with nonessential amino acids (Gibco-BRL Inc., Grand Island, NY), 10% fetal calf serum (Gibco-BRL Inc., Grand Island, NY),  $2\text{ mol L}^{-1}$  L-glutamine and antibiotics. Cells were plated in 100-mm culture dishes ( $10^6$  cells/dish), and maintained at 37°C in an atmosphere of 5%  $\text{CO}_2$  in humidified air. Subcultures were obtained by trypsinization (0.025% trypsin solution containing 0.01% *N,N*-diethyldithiocarbamic acid, sodium salt, and EDTA).

For cytological investigation,  $10^5$  cells were plated and allowed to adhere overnight. The medium was then aspirated and replaced with medium containing vehicle alone or medium containing the mixture of mammeas (3, 5, 8, 10, 16, 20 and  $24\text{ }\mu\text{g mL}^{-1}$ ), and the cells were then incubated for 24 h. After incubation, the cells were collected for further cytological investigation.

### Assessment of cell viability

BMK cells were incubated in 96-well plates. After 24 h, the medium was removed; the cells were washed twice with phosphate-buffered saline (PBS) and then incubated with the mixture of coumarins (3, 5, 8, 10, 16, 20 and  $24\text{ }\mu\text{g mL}^{-1}$ ).

After 24 h, both cells and conditioned media were collected and processed. Cell viability was measured by the MTT assay (Wang et al 1996). Briefly, 20  $\mu\text{L}$  MTT (5  $\text{g L}^{-1}$ ) was added to each well and incubated for 4 h at 37°C, 5%  $\text{CO}_2$ . The culture media was then discarded and 200  $\mu\text{L}$  DMSO with 25  $\mu\text{L}$  Sorensen's buffer (glycine 0.1 M, NaCl 0.1 M, pH 10.5) was added to each well. Once the blue crystals had dissolved, the optical density was determined on a microreader (Bio-Rad Co.) at 450 nm.

### Mitotic activity

BMK cells were fixed in 3% paraformaldehyde with 0.25 M mannitol (45.54  $\text{g L}^{-1}$ ) for 2 h, rinsed in PBS and stained with DAPI (4',6-diamidino-2-phenylindol, 480 nm). After rinsing in PBS, the cells were embedded in Citifluor mounting medium. The mitotic index was counted with a fluorescent microscope (Optiphot 2 Nikon). For each experiment, the indices were determined per 1000 cells and with four replicates.

### Survival of cells and detection of percentage of apoptosis and necrosis

Survival of cells was evaluated based on propidium iodide (PI; Sigma P4170) staining (Koopman et al 1994). PI (final concentration 5  $\mu\text{g mL}^{-1}$ ) was added to each sample for 10 min (room temperature, in the dark). The samples ( $10^5$ ) were analysed using a FACScalibur flow cytometer at 488 nm (Becton Dickinson, USA, equipped with an argon laser). Ten thousand cells were analysed using four replicates. The results were analysed using the CELLQuest program. Based on the penetration of PI and the size of the cells, the percentage of apoptotic and necrotic cells was determined.

### Analysis of cell cycle

Cells ( $10^5$ ) were fixed in 75% ethanol for 24 h and then washed in PBS and resuspended in 0.1% Nonidet P40 (Biochemica Fluka) and DNase-free RNase (10  $\mu\text{g mL}^{-1}$ ) for 20 min at room temperature (Darzynkiewicz et al 2001). PI was then added (final concentration 5  $\mu\text{g mL}^{-1}$ ) and incubated for 12 h at 4°C in the dark. Samples were analysed using a FACScalibur flow cytometer (Becton Dickinson). For each sample, 10 000 cells were analysed using four replicates. The results were analysed using the CELLQuest program.

### Tumour formation in mice

Female BALB/c mice (6–8-weeks-old; purchased from Harlan Mexico, Mexico) were fed using a standard basal diet (24% protein) and exposed to a daily cycle of alternating 12-h light periods. The animals were housed in a temperature-and-humidity-controlled environment and food and water were freely available. The experiments were conducted according to the principles set forth in "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication Number 86-23; Committee on Care and Use of Laboratory Animals of the Institute for Laboratory Animal Resources 1986) and the Animal Welfare Act of 1966, as amended.

Tumour formation in mice was performed as described by Dehenhardt et al (2002). Briefly,  $2.5 \times 10^7$  BMK cells were injected into each mouse and tumours were measured regularly.

### Pharmacological evaluation of coumarins

Mice were randomly divided into four groups, each group containing 10 animals. Group I, control animals that received no treatment; Group II, mice with BMK-cell tumours; Group III, mice with BMK-cell tumours and treated with coumarins (20  $\text{mg kg}^{-1}$ , i.p., three times per week, for three weeks); Group IV, mice with BMK-cell tumours and treated with vincristine (1  $\text{mg m}^{-2}$ , i.p., twice per week, for two weeks). Coumarins and vincristine treatments started two weeks after the BMK cells were injected, when the tumour size was 5 mm.

To assess the pharmacological effect of coumarins on tumour growth, the size of the tumour was measured every third day during the treatment. After completion of the treatment period, animals were anaesthetized with diethyl ether and killed by exsanguination. A complete post-mortem examination was performed on each animal. Samples of liver, kidney, thymus, spleen and lungs were weighed and sliced, and several sections were fixed by immersion in alcohol and embedded in paraffin for histological analysis to identify the presence of metastasis.

### Statistical analysis

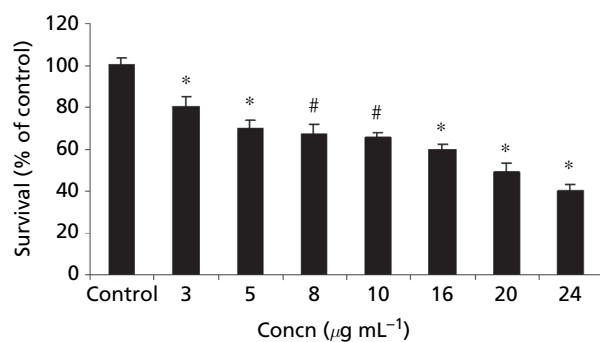
Data were reported as means  $\pm$  s.d. of three independent experiments conducted in quadruplicate. Statistical analysis was performed using a nonparametric analysis of variance and by Chi-squared testing. Individual differences between treatments were analysed using Tukey's test. Significant differences were established at  $P < 0.001$ .

## Results

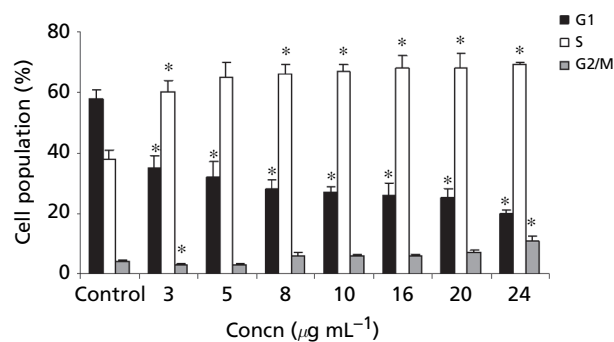
### Cell survival and induction of apoptosis and necrosis

The coumarin extract caused a significant decrease in the survival of BMK cells (Figure 2). The coumarins (mammea A/BA and mammea A/BB) showed a concentration-dependent inhibitory effect on the growth of BMK cells (Figure 2) ( $P < 0.001$ ). The lowest concentration produced a 20% reduction in cell survival ( $P < 0.001$ ). Concentrations of 5, 8 and 10  $\mu\text{g mL}^{-1}$  reduced the survival by approximately 30% ( $P < 0.001$ ). A reduction in cell survival greater than 50% was observed with concentrations higher than 20  $\mu\text{g mL}^{-1}$  ( $P < 0.001$ ).

During incubation with all concentrations of coumarins, the decrease in the percentage of live cells was accompanied by a proportional increase in the percentage of apoptotic and necrotic cells (Table 1). Treatment with the lowest concentration did not produce any important change in the relationship between live and dead cells. Treatment of cells with concentrations higher than 5  $\mu\text{g mL}^{-1}$  gave a higher increase in the percentage of apoptotic cells than the increase in necrotic cells (Table 1). All concentrations gave a higher percentage of apoptotic cells than necrotic cells ( $P < 0.001$ ).



**Figure 2** Changes in cell survival after incubation for 24 h in different concentrations of coumarins from *Calophyllum brasiliense* (control = 100% survival). The results are presented as means  $\pm$  s.d. of three independent experiments, \* $P < 0.001$  as compared with all groups; # $P < 0.001$  as compared with control, 3, 16, 20 and 24  $\mu\text{g mL}^{-1}$



**Figure 3** Percentage of cells in G1, S, G2/M phases of the cell cycle after incubation for 24 h in different concentrations of coumarins from *Calophyllum brasiliense*. The results are presented as means  $\pm$  s.d. of three independent experiments. \* $P < 0.001$  vs control values.

**Table 1** Changes in the percentage of normal, apoptotic and necrotic BMK cells after incubation for 24 h in different concentrations of coumarins from *Calophyllum brasiliense* extract

Concn extracted coumarins ( $\mu\text{g mL}^{-1}$ )	Normal cells (%)	Apoptotic cells (%)	Necrotic cells (%)
Control	87 $\pm$ 6.5	8.5 $\pm$ 1.2	4.5 $\pm$ 1.2
3	86 $\pm$ 3.2	10 $\pm$ 2.1	4.3 $\pm$ 1.4
5	79 $\pm$ 3.3	16 $\pm$ 2.1	5.5 $\pm$ 1.2
8	74 $\pm$ 3.7	18 $\pm$ 2.8	8.7 $\pm$ 3.0
10	70 $\pm$ 3.4	21 $\pm$ 2.9	9.0 $\pm$ 2.1
16	64 $\pm$ 6.5	24 $\pm$ 2.5	10.5 $\pm$ 2.6
20	62 $\pm$ 4.1	28 $\pm$ 3.3	9.7 $\pm$ 3.8
24	52 $\pm$ 5.3	30 $\pm$ 4.0	20.1 $\pm$ 2.0

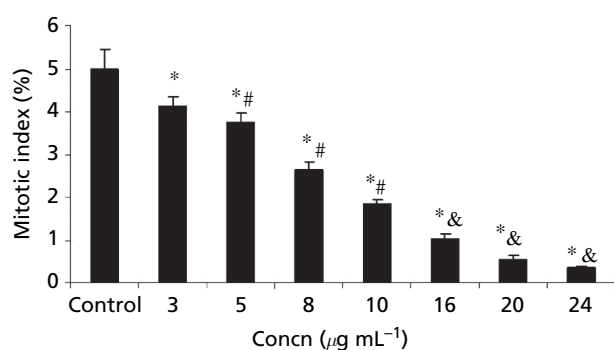
### Cell cycle analysis

Cell cycle analysis following treatment with coumarins (mammea A/BA and mammea A/BB) showed that, during incubation with the extract, the proportion of the BMK cell population in each of G1 and S phases varied with the concentration of the extract (Figure 3). The proportion of G1 phase cells reduced, while the percentage of cells at S phase of the cell cycle increased (Figure 3). This change was dependent on the concentration of the coumarins, and was accompanied by the appearance of apoptotic cells at the sub-phase G1 (data not shown).

Treatment with the lower concentrations of coumarins (3 and 5  $\mu\text{g mL}^{-1}$ ) caused a reduction in G1 and G2/M phases after 24 h incubation (Figure 3;  $P < 0.05$ ). Concentrations of coumarins higher than 5  $\mu\text{g mL}^{-1}$  caused an almost 50% reduction of G1 phase cells ( $P < 0.05$ ). In contrast, a significant increase of the percentage in S phase cells was observed with all concentrations,  $P < 0.001$ .

### Mitotic index after treatment with coumarins

The coumarins (mammea A/BA and mammea A/BB), at all chosen concentrations, reduced the percentage of cell divisions,



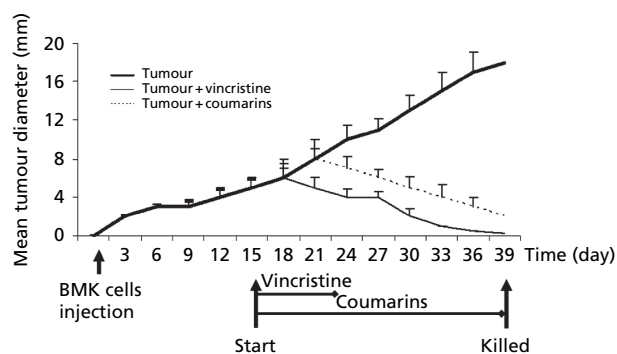
**Figure 4** Changes in the mitotic index of cells after incubation for 24 h in different concentrations of coumarins from *Calophyllum brasiliense*. The results are presented as means  $\pm$  s.d. of three independent experiments. \* $P < 0.001$  as compared with all groups; # $P < 0.001$  as compared with 3, 16, 20 and 24  $\text{mg mL}^{-1}$ ; & $P < 0.001$  as compared with 3, 5, 8 and 10  $\text{mg mL}^{-1}$ .

or led to their almost total inhibition, in a concentration-dependent manner (Figure 4). The lowest concentrations of coumarins (3 and 5  $\mu\text{g mL}^{-1}$ ) lowered the mitotic activity of the BMK cells by 18% and 30%, respectively, after incubation for 24 h. However, concentrations higher than 8  $\mu\text{g mL}^{-1}$  produced a reduction of cell division in a dose-dependent manner. The two highest concentrations used (20 and 24  $\mu\text{g mL}^{-1}$ ) caused almost total inhibition of cell division ( $P < 0.001$ ).

### Tumour formation in mice

The subcutaneous injection of viable BMK cells led to the development of tumours in mice. Tumour growth did not produce any significant change in body weight compared with the control group. Similarly, it did not lead to the death of any animals.

Treatment with coumarins caused a reduction in the development of tumours; this was evident seven days after the treatment started (Figure 5). Tumours reached an average size



**Figure 5** Effect of coumarins on tumour size. Viable BMK cells were injected subcutaneously into mice. The size of the tumours was measured every third day during the treatment. Mean tumour diameter (mm) was expressed relative to time after cell injection. The results are presented as means  $\pm$  s.d.,  $n = 10$  animals.

of 8 mm in animals treated with coumarins. Compared with animals with no treatment, a reduction of 83% was observed in tumour size at the end of treatment with coumarins and the tumour size was approximately 2 mm when the animals were killed. In contrast, vincristine stopped the development of tumours, which was evident after the first dose (Figure 5). The tumours reached a size of 6 mm (average) during the treatment with vincristine and they continued to reduce in size after the treatment. A reduction of 100% was observed in tumour size at the end of four weeks.

Histopathological analysis revealed the presence of metastatic cells in hepatic tissue and, to a lesser degree, in kidney tissue in tumour-bearing mice receiving no treatment (data not shown). Animals with tumours and treated with coumarins did not show any evidence of metastasis to any of the tissues analysed, including the liver. Animals with tumour and treated with vincristine had livers showing large areas of metastatic cells (data not shown).

## Discussion

Cancer is one of the main causes of death in the world. Two related fields are being explored through basic and clinical research: the identification of the molecular mechanisms involved in the illness, and the discovery of drugs for prevention or treatment (Kirsner 2003). In the area of drug discovery, there is increasing interest in cancer-prevention agents of plant origin (Newman et al 2003).

Coumarins comprise a vast array of biologically active compounds ubiquitous in plants, many of which have been used in traditional medicine for thousands of years. The coumarins constitute an important class of compounds with several types of pharmacological actions, possessing anticancer, anti-HIV, anticoagulant, spasmolytic and antibacterial activity among others. However, their most prominent actions are their antioxidant and antiproliferative effects (Mattern et al 1999; Kostova & Momekov 2006).

Antineoplastic drugs are designed either to inhibit abnormal cell proliferation or to cause the death of abnormal cells.

Due to the complex biochemical pathways and the specific phases of cellular life cycles, there are numerous opportunities for these drugs to exert a beneficial effect. Some types of coumarin show antitumour activity in-vivo and in-vitro (Guilet et al 2000; Kawaii et al 2001; Finn et al 2002); however, there have been no investigations regarding the effect of coumarins from *C. brasiliense* on the mitotic index, survival, apoptosis induction or cell cycle of cells in-vitro. The results of our investigation have shown that the coumarins isolated from *C. brasiliense*—a mixture of mammea A/BA and A/BB—were capable of producing antiproliferative and cytotoxic effects in-vitro, and reduced tumour development in mice.

Our results showed that coumarins at concentrations of 5–10  $\mu\text{g mL}^{-1}$  had a cytotoxic effect on BMK cells (30%). However, with higher concentrations (15–24  $\mu\text{g mL}^{-1}$ ) the cytotoxic effect was the greatest. The cytotoxicity produced by other types of coumarin have been reported using a non-small-cell bronchial carcinoma line (Kofinas et al 1998; López-González et al 2000), carcinoma epidermoid cells (Guilet et al 2001), human renal cell lines (Kawase et al 2005), and cells from the uterine cervix (HeLa), larynx (Hep2), prostate gland (PC3), lymphoma (K562) and neural tissue (U251) (Reyes-Chilpa et al 2004).

One aspect of neoplastic cells is their proclivity to replicate. Depending on their mechanism of action, antineoplastic agents may exert their effects specifically during one of the phases or they may act during any phase of the cell cycle to cause cell death (Dictor et al 2004). We analysed the cell cycle in cells treated with coumarins. Interestingly, in BMK cells we observed arrest in the S-phase of the cell cycle. Damage to DNA may prevent replication by altering DNA, RNA or their function. Agents that work by this mechanism inhibit the proliferation of neoplastic cells (they cannot divide). Other drugs may cause damage to DNA, which can result in cell death. After treatment, inhibition of the mitotic division of BMK cells occurred. The inhibition depended on the concentration of the coumarins. This inhibition was accompanied by the appearance of large numbers of apoptotic cells. Therefore, it can be speculated that the cells enter apoptosis at the border of the G1 phase and cells that have alterations in their DNA are arrested so to repair the damage.

The effects of coumarins on the cell cycle have been studied previously. Coumarins and 7-hydroxycoumarin have antitumour actions in-vitro and in-vivo (Marshall et al 1999; Lacy & O'Kennedy 2004; Elinos-Baez et al 2005). Coumarins and 7-hydroxycoumarin inhibit cell growth by inducing cell cycle arrest in the G1 phase in lung carcinoma cell lines (López-González et al 2004). Jimenez-Orozco et al (2001) observed that 7-hydroxycoumarin 1 mM inhibited the G1/S transition of the cell cycle in the human lung adenocarcinoma cell line A-427. They also found that 7-hydroxycoumarin significantly reduced cyclin D1 expression, which appeared to indicate an action of 7-hydroxycoumarin in early events of phase G1. Finally, studies using nitro-derivatives of coumarins showed that they caused dose-dependent inhibition of the S-phase regulatory protein, cyclin A, in an irreversible manner. Therefore, these and other nitro-derivatives of 7-hydroxycoumarin possess selective and irreversible cytotoxicity (Finn et al 2004). Recent studies using *C. brasiliense* collected in Brazil have shown that it inhibited the growth of leukaemic cells and induced caspase-mediated and p53-independent apoptosis

(Kimura et al 2005). Our results agreed with those previous studies; we found that coumarins isolated from *C. brasiliense* collected in Mexico displayed potent cytotoxic and antiproliferative activity in-vitro.

Tumorigenesis is an extremely complex multistep process that leads to the pathological expansion of a tissue. Tumour cells gain an unlimited replicative potential because of an imbalance between finely tuned proliferate, growth-inhibitory and apoptotic signals. Animal models that accurately represent the cellular and molecular changes associated with the initiation and progression of cancer have significant potential to facilitate the development of better methods for the early detection and treatment of this illness. Chemotherapeutic drugs can be divided into several categories, based on the way they act. Antineoplastic agents act primarily on rapidly dividing and growing cells.

To date, there are no reports regarding the assay of the effects of coumarins in animal models. In this study, we have evaluated the antitumoral effects of coumarins in tumours induced by subcutaneous injection of BMK cells into mice. Our in-vitro results demonstrated that coumarins isolated from *C. brasiliense* had cytotoxic and antiproliferative effects. Additionally, we demonstrated that coumarins were better than vincristine in inhibiting tumour growth.

## Conclusion

Coumarins isolated from *C. brasiliense* had cytotoxic effects and were able to alter the G1/S transition of the cell cycle in-vitro. In addition, they had antitumour effects. Therefore, coumarins have the potential to be used alone or in combination with other antineoplastic drugs, and they might increase the effectiveness of other treatments against cancer.

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