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Potentiating effect of β-caryophyllene on anticancer activity of α-humulene, isocaryophyllene and paclitaxel

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Abstract

β-caryophyllene is a sesquiterpene widely distributed in essential oils of various plants. Several biological activities are attributed to β-caryophyllene, such as anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities. In this work, the potentiating effect of β-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene and paclitaxel against MCF-7, DLD-1 and L-929 human tumour cell lines was evaluated. A non-cytotoxic concentration of β-caryophyllene significantly increased the anticancer activity of α-humulene and isocaryophyllene on MCF-7 cells: α-humulene or isocaryophyllene alone (32 μg mL^{-1}) inhibited cell growth by about 50% and 69%, respectively, compared with 75% and 90% when combined with 10 μg mL^{-1} β-caryophyllene. Moreover, β-caryophyllene potentiated the anticancer activity of paclitaxel on MCF-7, DLD-1 and L-929 cell lines. The highest potentiating effect was obtained in DLD-1 cells treated with paclitaxel combined with 10 μg mL^{-1} β-caryophyllene, which increased the paclitaxel activity about 10-fold. The intracellular accumulation of paclitaxel-oregon green was evaluated in combination with concentrations of β-caryophyllene ranging from 2.5 to 40 μg mL^{-1}. β-Caryophyllene (10 μg mL^{-1}) significantly increased the intracellular accumulation of paclitaxel-oregon green (about 64% over controls). Moreover, β-caryophyllene induced intracellular accumulation of calcein but not verapamil, an inhibitor of P-glycoprotein and multidrug resistance related protein transporters, suggesting that β-caryophyllene promotes drug accumulation by a different mechanism of action. These results suggest that β-caryophyllene facilitates the passage of paclitaxel through the membrane and thus potentiates its anticancer activity.

Introduction

The sesquiterpene β-caryophyllene is present in various essential oils such as *Eugenia caryophyllata, Myrica gale* and *Comptonia peregrina* (Zheng et al 1992; Sylvestre et al 2005; 2007). β-caryophyllene is known to possess anti-inflammatory (Tambe et al 1996; Cho et al 2007), anticarcinogenic (Kubo et al 1996), antibiotic (Alma et al 2003; Lourens et al 2004; Pichette et al 2006), antioxidant (Lourens et al 2004; Singh et al 2006) and local anaesthetic (Ghelardini et al 2001) activities. In essential oils this compound is frequently found mixed with γ-caryophyllene (isocaryophyllene) and/or α-caryophyllene (α-humulene) (Budavari 1996). In a previous study, we identified β-caryophyllene and α-humulene in *Abies balsamea* essential oil (Legault et al 2003) and showed that α-humulene was partly responsible for the cytotoxicity of this oil. We also showed that α-humulene induces intracellular depletion of glutathione and increases production of reactive oxygen species, which may be responsible for its cytotoxicity. Moreover, isocaryophyllene was found to be cytotoxic against all tumour cell lines tested. In contrast, β-caryophyllene did not inhibit tumour cell growth. The concentration of α-humulene in *A. balsamea* essential oil was not sufficient to explain the activity of the oil, and all other compounds identified were found to be inactive. We therefore suggested that β-caryophyllene could increase the anticancer activity of α-humulene. Indeed, β-caryophyllene has been shown to promote the absorption of 5-fluorouracil across human skin, suggesting that this compound could increase intracellular accumulation of anticancer agents, thereby potentiating their cytotoxicity (Cornwell & Barry 1994). Moreover, several sesquiterpenes, such as nerolidol and bisabolol, have been shown to sensitize bacteria to various antibiotic agents, thus increasing their antimicrobial activity (Brehm-Stecher & Johnson 2003).

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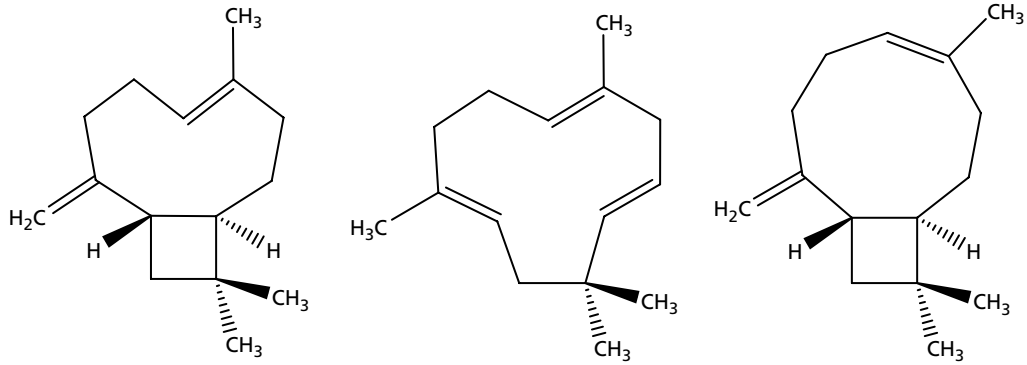

Figure 1 Molecular structures of β-caryophyllene (left), α-humulene (middle) and isocaryophyllene (right).

Together, the results reported in the literature suggest that β-caryophyllene could increase the membrane permeation and consequently the biological activity of drugs. In this study, we have investigated the potentiating activity of β-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene and paclitaxel (Figure 1) on MCF-7, DLD-1 and L-929 tumour cell lines. The effect of β-caryophyllene on the intracellular accumulation of paclitaxel was also tested.

Materials and Methods

Cell culture

Human breast cancer adenocarcinoma MCF-7, colon adenocarcinoma DLD-1 (ATCC #CCL-221) and murine fibroblast L-929 (ATCC #CCL-1) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The MCF-7, DLD-1 and L-929 cell lines were grown in Dulbecco's minimum essential medium (DMEM) with Earle's salts (Mediatech Cellgro, Herndon, USA). The culture medium was supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, USA), a solution of vitamins, sodium pyruvate and non-essential amino acids (all at 1:100 v/v dilution of supplied solutions), penicillin (100 IU) and streptomycin (100 μg mL^{-1}) (Mediatech Cellgro). Cells were cultured in a humidified atmosphere at 37°C in 5% CO_2.

Cytotoxicity assay

Exponentially growing cells were plated at a density of 5×10^3 cells per well in 96-well microplates (Costar, Corning Inc., Lowell, MA, USA) in 100 μL culture medium and were allowed to adhere for 16 h before treatment. Cells were then treated with α-humulene (16–64 μg mL^{-1}), isocaryophyllene (16–64 μg mL^{-1}) or paclitaxel (0.008–1 μg mL^{-1}), alone or in combination with β-caryophyllene (2.5 or 10 μg mL^{-1}). Ethanol 100% (Sigma-Aldrich, Oakville, Ontario, Canada) was used as a solvent. The final concentration of ethanol in the culture medium was 0.5% (volume/volume) to avoid solvent toxicity. After 48 h, the cytotoxicity was assessed using the resazurin reduction test (O'Brien et al 2000). Fluorescence was measured on an automated 96-well Fluoroskan Ascent Fl plate reader (Labsystems, Vienna, VA, USA) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxicity was expressed as the concentration that inhibited cell growth by 50% (IC50).

Evaluation of the intracellular accumulation of paclitaxel-oregon green

Exponentially growing cells were plated at a density of 15×10^3 cells per well in 96-well microplates (Costar, Corning Inc.) in 100 μL culture medium. The plates were incubated at 37°C and 5% CO_2 for 24 h before treatment. Cells were washed with 100 μL DMEM without FBS. A 100 μl volume of DMEM containing 2.5, 5, 10, 20 or 40 μg mL^{-1} β-caryophyllene with 0.5 μM (0.6 μg mL^{-1}) paclitaxel-oregon green was added to each well. The final concentration of solvent in the culture medium was 0.5% (volume/volume) to avoid solvent toxicity. The cells were incubated for 60 min at 37°C and 5% CO_2. Cells were then washed three times with phosphate-buffered saline. Fluorescence was measured on an automated 96-well Varioskan plate reader (Thermo, Waltham, MA, USA) using excitation and emission wavelengths of 495 nm and 525 nm, respectively.

Statistical analysis

Values are expressed as mean ± s.d. of three determinations. The results were analysed by the Kruskal–Wallis test followed by a Student–Newman–Keuls' test or Dunn's post-hoc test. P values of 0.05 or less were considered significant.

Results and Discussion

Previous studies have shown that α-humulene and isocaryophyllene inhibit growth of tumour cells in-vitro. In contrast, β-caryophyllene was not found to be cytotoxic (Legault et al 2003). In this study, we have evaluated the potentiating effect of β-caryophyllene on the anticancer activity of α-humulene, isocaryophyllene and paclitaxel against tumour cell lines.

Potentiating effect of β-caryophyllene on α-humulene and isocaryophyllene

The potentiating effect of β-caryophyllene has been evaluated in combination with α-humulene on the human breast adenocarcinoma cell line, MCF-7. Cells were treated with increasing concentrations (16, 32 and 64 $\mu g\,mL^{-1}$) of β-caryophyllene, α-humulene or a combination of the two sesquiterpenes. β-Caryophyllene did not inhibit cell growth at all doses tested but α-humulene was cytotoxic at 32 and 64 $\mu g\,mL^{-1}$ (Figure 2A). Interestingly, the two sesquiterpenes together were significantly more cytotoxic than α-humulene alone: α-humulene (32 $\mu g\,mL^{-1}$) inhibited cell growth by 50 ± 6% alone, but by 75 ± 6% when combined with 10 $\mu g\,mL^{-1}$ β-caryophyllene. These results indicate that β-caryophyllene potentiates the cytotoxicity of α-humulene.

The potentiating effect of β-caryophyllene was also evaluated with isocaryophyllene. As shown in Figure 2B, non-toxic concentrations of β-caryophyllene significantly improved the activity of isocaryophyllene. Thus, isocaryophyllene (16 $\mu g\,mL^{-1}$) alone did not inhibit cell growth whereas inhibition was 52 ± 4% when combined with 10 $\mu g\,mL^{-1}$ β-caryophyllene. β-Caryophyllene increased the cytotoxicity of isocaryophyllene 32 $\mu g\,mL^{-1}$ from 69% to 90%. Altogether, the results indicate that β-caryophyllene potentiates the activity of α-humulene and isocaryophyllene in-vitro.

Potentiating effect of β-caryophyllene with paclitaxel

The potentiating activity of β-caryophyllene was also evaluated in combination with paclitaxel, a diterpene originally isolated from *Taxus brevifolia* (Wani et al 1971) and then from *T. canadensis* (Zamir et al 1992). This antitumour agent is used clinically to treat breast, ovarian and lung cancers.

MCF-7, DLD-1 and L-929 cell lines were treated with paclitaxel alone or in combination with β-caryophyllene (2.5 and 10 $\mu g\,mL^{-1}$). β-Caryophyllene did not inhibit the growth of any of the cell lines tested (data not shown) whereas 0.025 $\mu g\,mL^{-1}$ paclitaxel significantly inhibited the growth of MCF-7 (28 ± 2%), DLD-1 (17.3 ± 0.2%) and L-929 (18.4 ± 0.2%), compared with untreated cells (Figure 3). β-Caryophyllene significantly increased the activity of paclitaxel (0.025 $\mu g\,mL^{-1}$) in all tumour cell lines tested. In MCF-7 cells, growth inhibition by paclitaxel was increased by about 50% and 68% with 2.5 and 10 $\mu g\,mL^{-1}$ β-caryophyllene, respectively, and by about 36% and 123%, respectively in L-929 cells. The greatest potentiating effect of β-caryophyllene was obtained in DLD-1 cell lines. At concentrations of 2.5 and 10 $\mu g\,mL^{-1}$, β-caryophyllene increased growth inhibition induced by paclitaxel by about 91% and 189%, respectively. Moreover, the IC50 calculated from the survival curve shows that β-caryophyllene (10 $\mu g\,mL^{-1}$) increased the cytotoxicity induced by paclitaxel by about 10-fold (Table 1). Similar results were obtained with L-929 cells. These results suggest that β-caryophyllene promotes the intracellular accumulation of paclitaxel.

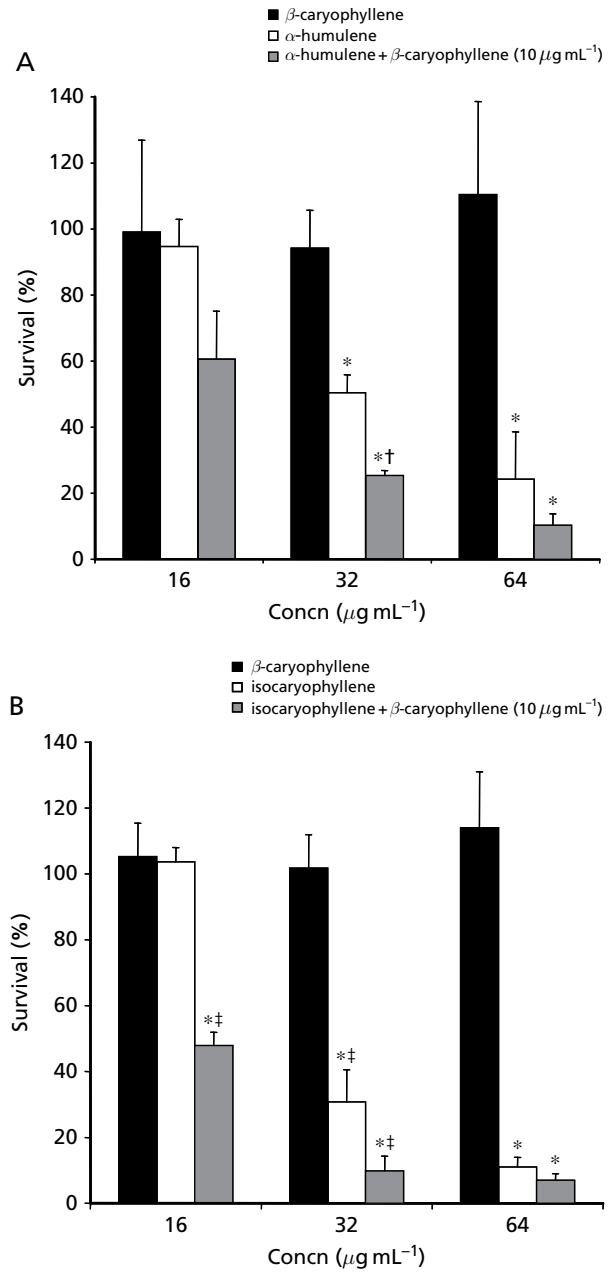

Figure 2 Potentiating activity of β-caryophyllene combined with α-humulene (A) or isocaryophyllene (B) on MCF-7 tumour cell lines. β-Caryophyllene (10 $\mu g\,mL^{-1}$) did not inhibit cell growth (data not shown). Data represent the mean ± s.d. of three determinations. *$P \leq 0.05$ vs β-caryophyllene alone; †$P \leq 0.05$ vs α-humulene alone; ‡$P \leq 0.05$ vs isocaryophyllene alone (Kruskal–Wallis test and post-hoc Student–Newman–Keuls' test).

Intracellular accumulation of paclitaxel and calcein induced by β-caryophyllene

The effect of β-caryophyllene on the accumulation of paclitaxel was evaluated in DLD-1 cells (colorectal cancer cells). The cells were incubated with paclitaxel-oregon green, alone

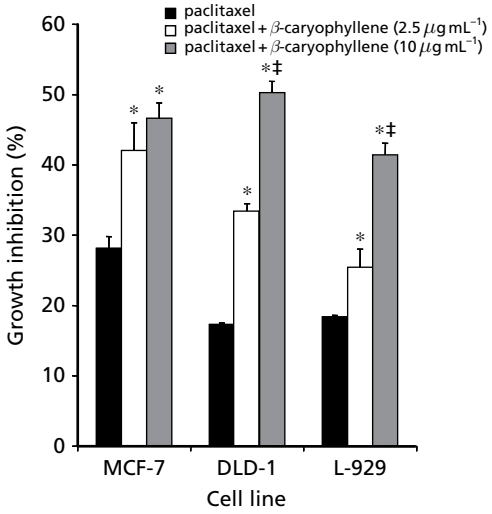

Figure 3 Potentiating activity of β-caryophyllene combined with paclitaxel (0.025 μg mL^{-1}) on MCF-7, DLD-1 and L-929 tumour cell lines. β-Caryophyllene (2.5 and 10 μg mL^{-1}) did not inhibit cell growth (data not shown). Data represent the mean ± s.d. of three determinations. *$P \leq 0.05$ vs paclitaxel alone; $^{\ddagger}P \leq 0.05$ vs β-caryophyllene (2.5 μg mL^{-1}) combined with paclitaxel (Kruskal–Wallis test and post-hoc Student–Newman–Keuls' test).

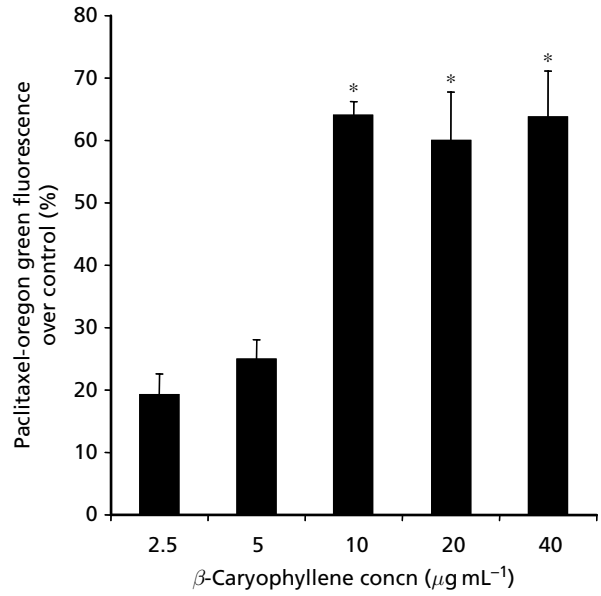

Figure 4 Intracellular accumulation of paclitaxel-oregon green induced by β-caryophyllene on DLD-1 colorectal cancer cells. Data represent the mean ± s.d. of three determinations. *$P \leq 0.05$ vs control without β-caryophyllene (Kruskal–Wallis test and Dunn's post-hoc test).

Table 1 Cytotoxic activity of paclitaxel combined with β-caryophyllene against DLD-1 and L-929 cell lines. Values are the concentration that inhibits 50% of cell growth (IC50; μg mL^{-1})

	DLD-1	L-929
β-caryophyllene	> 40	> 40
Paclitaxel	0.43 ± 0.09	0.7 ± 0.3
β-caryophyllene + paclitaxel (2.5 μg mL^{-1})	0.16 ± 0.03*	0.4 ± 0.2
β-caryophyllene + paclitaxel (10 μg mL^{-1})	0.042 ± 0.008*,†	0.06 ± 0.01*,†

*$P \leq 0.05$ vs paclitaxel alone; $^{\dagger}P \leq 0.05$ vs β-caryophyllene + paclitaxel 2.5 μg mL^{-1} (Kruskal–Wallis test and Student–Newman–Keuls' method).

or in combination with concentrations of β-caryophyllene ranging from 2.5 to 40 μg mL^{-1}. β-Caryophyllene induced an increase in the accumulation of paclitaxel-oregon green compared with untreated cells (Figure 4). The percentage of paclitaxel-oregon green accumulation induced by 2.5, 5 and 10 μg mL^{-1} β-caryophyllene was 19 ± 3%, 25 ± 3% and 64 ± 2% higher than controls, respectively ($P \leq 0.05$). The quantity of intracellular paclitaxel-oregon green remained stable at higher doses of 20 μg mL^{-1} (+60 ± 3%; $P \leq 0.05$) and 40 μg mL^{-1} (+64 ± 7%; $P \leq 0.05$) β-caryophyllene.

Drug transporters such as P-glycoprotein (or multidrug resistance 1 (MDR1)) and multidrug resistance related protein (MRP1) can decrease drug accumulation; thus, inhibition of these transporters can increase intracellular accumulation of anticancer agents such as paclitaxel. P-gp and MRP1 are found in DLD-1 cells, but their levels depend on the culture conditions of the cells (Nakumura et al 2003). Therefore, β-caryophyllene may inhibit drug transporters and increase the accumulation of intracellular paclitaxel, potentiating its activity. To test this hypothesis, the effect of β-caryophyllene and verapamil, an inhibitor of P-gp and MRP1, was evaluated in DLD-1 using a calcein accumulation assay (Hollo et al 1994). Verapamil did not induce a significant accumulation of intracellular calcein (data not shown), suggesting that P-gp and MRP1 were weakly active in the culture conditions used. In contrast, β-caryophyllene (10 μg mL^{-1}) significantly increased the accumulation of intracellular calcein by 25 ± 1% ($P = 0.004$, one-way analysis of variance; data not shown). These results suggest that β-caryophyllene promotes drug accumulation by another mechanism. Several cyclic hydrocarbons, including terpenes, have been shown to accumulate in the biological membrane bilayer, causing it to swell and resulting in increased membrane fluidity (Sikkema et al 1994). Therefore, β-caryophyllene may accumulate in the membranes of cancer cells and increase membrane permeability. This membrane alteration could facilitate the passage of bioactive compounds through the cytoplasmic membrane of cancer cells. Consequently, β-caryophyllene could increase intracellular accumulation of antitumour drugs such as paclitaxel and potentiate their anticancer activity.

In conclusion, this study shows that non-cytotoxic concentrations of β-caryophyllene increase the growth inhibition induced by α-humulene, isocaryophyllene and paclitaxel on tumour cell lines. The potentiating effect of β-caryophyllene could be due in part to alteration of membrane permeability.

References

Alma, M. H., Mavi, A., Yildirim, A., Digrak, M., Hirata, T. (2003) Screening chemical composition and in vitro antioxidant and antibacterial activities of the essential oils from Origanum syriacum L. growing in Turkey. *Biol. Pharm. Bull*. **26**: 1725–1729

Brehm-Stecher, B. F., Johnson, E. A. (2003) Sensitization of Staphylococcus aureus and Escherichia coli to antibiotics by the sesquiterpernoids nerolidol, farnesol, bisabolol, and apritone. *Antimicrob. Agents Chemother*. **47**: 3357–3360

Budavari, S. (ed) (1996) *The Merck Index: An encyclopedia of chemicals, drugs and biologicals*. 12th edn. Merck & Co Inc., New Jersey, pp 308

Cho, J. A., Chang, H. J., Lee, S.-K., Kim, H.-J., Hwang, J.-K., Chun, H. S. (2007) Amelioration of dextran sulphate sodium-induced colitis in mice by oral administration of β-caryophyllene, a sesquiterpene. *Life Sci*. **80**: 932–939

Cornwell, P. A., Barry, B. W. (1994) Sesquiterpene components of volatile oil as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. *J. Pharm. Pharmacol*. **46**: 261–269

Ghelardini, C., Galeotti, N., Di Cesare, M. -L., Mazzanti, G., Bartolini, A. (2001) Local anaesthetic activity of β-caryophyllene. *Farmaco* **56**: 387–389

Hollo, Z., Homolya, L., Davis, C. W., Sarkadi, B. (1994) Calcein accumulation as a fluorometric functional assay of the multidrug transporter. *Biochim. Biophys. Acta* **1191**: 384–388

Kubo, I., Chaudhuri, S. K., Kubo, Y., Sanchez, Y., Ogura, T., Saito, T. Ishikawa, H. (1996) Cytotoxic and antioxidative sesquiterpenoids from Heterotheca inuloides. *Planta Med*. **62**: 427–430

Legault, J., Dahl, W., Debiton, E., Pichette, A., Madelmont, J. C. (2003) Antitumor activity of balsam fir oil: production of reactive oxygen species induced by alpha-humulene as possible mechanism of action. *Planta Med*. **69**: 402–407

Lourens, A. C., Reddy, D., Baser, K. H., Viljoen, A. M., Van Vuuren, S. F. (2004) In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. *J. Ethnopharmacol*. **95**: 253–258

Nakumura, T., Sakaeda, T., Ohmoto, N., Moriya, Y., Komoto, C., Shirakawa, T., Gotoh, A., Matsuo, M., Okmura, K. (2003) Gene expression profiles of ABC transporters and cytochrome P450 3A in Caco-2 and human colorectal cancer cell lines. *Pharm. Res*. **20**: 324–327

O'Brien, J., Wilson, I., Orton, T., Pognan, F. (2000) Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem*. **267**: 5421–5426

Pichette, A., Larouche, P.-L. Lebrun, M., Legault, J. (2006) Composition and antibacterial activity of *Abies balsamea* essential oil. *Phytother. Res*. **20**: 371–373

Sikkema, J, de Bont, J. A., Poolman, B. (1994) Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem*. **269**: 8022–8028

Singh, G., Marimuthu, P., de Heluani, C. S., Catalan, C. A. (2006) Antioxidant and biocidal activities of Carum nigrum (seed) essential oil, oleoresin, and their selected components. *J Agric. Food Chem*. **54**: 174–181

Sylvestre, M., Legault, J., Dufour, D., Pichette, A. (2005) Anticancer activity of leaf essential oil of *Myrica gale* L. *Phytomedicine* **12**: 299–304

Sylvestre, M., Pichette, A., Longtin, A., Legault, J. (2007) Composition and cytotoxic activity of the leaf essential oil of Comptonia peregrina (L.) Coulter. *Phytother. Res*. **21**: 536–540

Tambe, Y., Tsujiuchi, H., Honda, G., Ikeshiro, Y., Tanaka, S. (1996) Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, β-caryophyllene. *Planta Med*. **62**: 469–470

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P., McPhail, A. T. (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia. J. Am. Chem. Soc*. **93**: 2325–2327

Zamir, L. O., Nedea, M. E., Garneau, F.-X. (1992) Biosynthetic building blocks of *Taxus canadensis* taxanes. *Tetrahedron Lett*. **33**: 5235–5236

Zheng, G. Q., Kenney, P. M., Lam, L. K. (1992) Sesquiterpenes from clove (Eugenia caryophyllata) as potential anticarcinogenic agents. *J. Nat. Prod*. **55**: 999–1003