JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemopreventive Activity of Polyphenolics from Black Jamapa Bean (*Phaseolus vulgaris* L.) on HeLa and HaCaT Cells

XOCHITL APARICIO-FERNÁNDEZ,[†] TERESA GARCÍA-GASCA,[‡] GAD G. YOUSEF,[§] MARY ANN LILA,[§] ELVIRA GONZÁLEZ DE MEJIA,[||] AND GUADALUPE LOARCA-PINA*,[†]

Programa de Posgrado en Alimentos del Centro de la Republica (PROPAC), Research and Graduate Studies in Food Science, School of Chemistry, Universidad Autónoma de Querétaro, Querétaro, Qro., 76010 México, Nutrition School, Natural Sciences Faculty, Universidad Autónoma de Querétaro, Querétaro, Qro., 76010 México, Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign, 1201 South Dorner Drive, Urbana, Illinois 61801, and Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, 228 ERML, 1201 W Gregory, Urbana, Illinois 61801

The antiproliferative effects of 100% methanol crude extract and of Toyopearl and silica gel fractions from the seed coats of black Jamapa beans (*Phaseolus vulgaris* L.) were evaluated using HeLa, human adenocarcinoma cells, and HaCaT, human premalignant keratinocytes. The 100% methanol crude extract [172.2 μM equiv of (+)-catechin] increased adhesion of HeLa cells; however, 3- and 5-fold higher concentrations decreased the number of cells attached as a function of the treatment time. The highest concentration tested diminished the cell adhesion until 40% (after 24 h) to almost 80% (after 72 h). The IC_{50} values showed that the 100% methanol crude extract was the most effective inhibitor of HeLa cell proliferation, even when it was dissolved in dimethylsulfoxide (DMSO) [34.5 μM equiv of (+)-catechin] or in medium [97.7 μM equiv of (+)-catechin]. The Toyopearl 5 (TP5) fraction and silica gel 2 (SG2) fraction inhibited 60% of the HeLa cell proliferation. The IC_{50} was 154 μM equiv of (+)-catechin of the 100% methanol crude extract on HaCaT cells. Toyopearl fractions TP4 and TP6 significantly inhibited HaCaT cell proliferation, but the silica gel fractions did not have a significant effect. The 100% methanol crude extract (35 μg of dry material/mL) decreased the number of HeLa cells in the G_0/G_1 phase from 68.9% (for control cells) to 51.4% (for treated cells) and increased apoptosis (2.9 and 21.2% for control and treated cells, respectively). The results indicated that black Jamapa beans could be a source of polyphenolic compounds, which have an inhibitory effect toward HeLa cancer cells but are less aggressive on HaCaT premalignant cells.

KEYWORDS: ***Phaseolus vulgaris*; polyphenolic compounds; cytotoxicity; HeLa cells; HaCaT cells**

INTRODUCTION

Over two-thirds of cancer-related death could be prevented through lifestyle modification, particularly through dietary means (*1*). Frequent consumption of diets rich in fruits and vegetables has been consistently shown to reduce the risk of multiple forms of human cancer (*2*). It has been proposed that the consumption of flavonoid-rich foods could bring diverse physiological benefits to the consumer, such as protection against human diseases associated with oxidative stress, like coronary heart disease and cancer (*3*−*5*).

Legumes contain a wide range of nutrients and non-nutrient bioactive constituents that may be protective against cancer (*6*). Beans are a staple food in Mexico and other Latin American countries (*7*). They are valued nutritionally because of their carbohydrate and protein quality. In addition, the presence of bioactive phytochemicals in beans has been recently described (*8*). The seed color of beans is determined by the presence and amount of flavonol glycosides, anthocyanins, and condensed tannins (proanthocyanidins) (*8*). In addition to their function as pigments, polyphenolics are bean constituents, which possess biological activity (*9*). The most widely distributed group of flavonoids in beans is proanthocyanidins (average reported: 9.4−37.8 mg of catechin equiv /1 g of bean), found mainly in the dried seed coat (*8*, *10*−*13*). The presence of anthocyanins has been reported only in black and blue−violet seed-colored beans (*8*, *14*−*15*).

Important biological activities, such as antioxidant (*8*, *10*, *16*) and antimutagenic (*11*, *13*, *17*) activities, have been discovered

* To whom correspondence should be addressed. Telephone: +52 (442) 192-1307. Fax: +52 (442) 192-1304. E-mail: loarca@uaq.mx.
[†] Research and Graduate Studies in Food Science, School of Chemistry, Universidad Autónoma de Querétaro.
[‡] Nutrition School, Natural Sciences Faculty, Universidad Autónoma de Querétaro.
[§] Department of Natural Resources and Environmental Sciences, University of Illinois at Urbana-Champaign.
[||] Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign.

10.1021/jf052974m CCC: $33.50
Published on Web 02/24/2006

in different flavonoid (mainly anthocyanins and proanthocyanidins) -rich extracts from beans. Flavonoids from plant sources have shown a variety of activities in *in vitro* studies, including the inhibitory activity on HeLa and other cancer cells (*18*−*23*) and different regulatory effects on normal and premalignant keratynocites HaCaT (*24*).

The objective of this research was to evaluate the antiproliferative bioactivity of polyphenolic compounds in the Jamapa black bean on HeLa (carcinoma) and HaCaT (precancerous) cells.

MATERIALS AND METHODS

Plant Material. Black Jamapa common beans (*Phaseolus vulgaris* L.) were grown in 2003 at "El Bajío" Experimental Station of the National Research Institute for Forestry Agriculture and Livestock (INIFAP), Mexico. The mature dry seed was stored at −20 °C until manual separation of the seed coats. The seed coats were lyophilized and stored in the dark at −20 °C until extraction and further analysis.

Extraction of Polyphenolics from Jamapa Bean Seed Coats. Independent samples of black bean seed coats were submitted to 100% methanol to obtain a crude extract rich in proanthocyanidins and other flavonoids.

Methanolic Extraction. The protocol for the extraction of phenolic compounds was previously described by Cardador-Martínez et al. (*11*). Lyophilized ground seed coats were placed in a flask and mixed with methanol [1:50 (w/v) ratio]. The flask was shaken for 24 h at 25 °C while wrapped in aluminum foil to protect the extract from light. The sample was then filtered using Whatman #4 paper, and methanol was removed under reduced pressure. The 100% methanol crude extract was lyophilized and stored in the dark at −20 °C until analysis.

Condensed tannins and total anthocyanins were quantified according to Deshpande and Cheryan (*25, 26*) and Abdel-Aal and Hucl (*27*), respectively.

Fractionation of 100% Methanol Crude Extract. Two fractionation methods were independently applied to the 100% methanol crude extract, using vacuum liquid chromatography and two different column supports including: (a) fractionation on Toyopearl (TP) and (b) fractionation on silica gel (SG). Toyopearl fractionation yielded six fractions coded TP1, TP2, TP3, TP4, TP5, and TP6. Silica gel fractionation yielded 22 fractions, and similar fractions based on the TLC profile were recombined to create six major fractions as follows: SG 1 (SG 1−3), SG 2 (SG 4−8), SG 3 (SG 9−12), SG 4 (SG 13−17), SG 5 (SG 18−20), and SG 6 (SG 21−22). All fractions were evaporated under reduced pressure, lyophilized, and stored at −20 °C until bioassay. The fractionation and fraction composition analysis were performed according to the method described by Aparicio-Fernandez et al. (*28*).

Cells and Cell Culture. HeLa, human cervix adenocarcinoma cells (ATCC, CCL-2), and HaCaT, human premalignant keratinocytic cells (kindly donated by Dr. Mark Lingen, University of Chicago), were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco). Cells were cultured at 37 °C in 5% CO_2 in air.

Treatment of Cells with Polyphenolics from Black Bean: Adhesion Assay. HeLa cells were subcultured into 24-well plates with 500 μL of 10% FBS DMEM (1×10^4 cells/well) and incubated. After 24 h of incubation, the medium was changed by adding DMEM containing 1% bovine serum albumin (BSA) and different concentrations of 100% methanol crude extract [50, 150, and 250 μg of dry extract/mL corresponding to 172.2, 516.6, and 861 μM equiv of (+)-catechin]. Cells were treated for 8−72 h in duplicate in at least two independent experiments. Attached cell number was estimated using a DNA carmine-based colorimetric method (*29*). Briefly, cells were fixed with 100% ethanol, dried, and stained with alcoholic/HCl carmine. Colorant was extracted with 0.01 N NaOH, and absorbance was determined at 531 nm. The cell number was estimated using a titration curve of cell density ($y = 1 \times 10^{-6}x + 0.0099$; $R^2 = 0.8385$), and results were given as a percentage of the cell number with respect to control cells. For the titration curve, cells were plated at densities ranging from 1×10^4 to 7×10^5 cells/well in 24-well plates using serial dilutions of concentrated cell suspensions. After adhesion, some wells of each density were harvested with trypsin and cells were counted in a hemacytometer; meanwhile, parallel cultures were fixed and stained as described above (*29*). A relationship between the cell number and resultant absorbance after the colorant extraction, for each cell density, was accomplished for cell-density titration-curve construction, which measured cell adhesion.

Treatment of Cells with Polyphenolics from Black Bean: Proliferation Assay. Cell proliferation inhibition was assessed using a cell counting kit (CCK-8) assay (Dojindo Molecular Technologies, Inc., Gaithersburg, MD). Cells were subcultured into 96-well plates in 100 μL of complete DMEM medium, 5×10^3 cells/well for HeLa and 1×10^4 cells/well for HaCaT, and incubated for 24 h. Then, the medium was replaced by 100 μL of fresh medium without FBS and containing the test sample (flavonoid standards, 100% methanol crude extract, TP fractions, silica gel fractions, or camptothecin) at the desired concentration (0.12−100 μg/mL for quercetin; 0.5−500 μg of dry material/mL for 100% methanol crude extract; all TP and SG fractions were tested at 35 μg of freeze-dry extract/mL; 0.62−5 μg/mL camptothecin). Camptothecin (an anticancer agent), quercetin, and (+)-catechin (important polyphenols present in fruits and vegetables) were used as controls. Each test was carried out in triplicate, and each experiment was repeated twice. The plates were incubated in a 37 °C humidified incubator under an atmosphere of 5% CO_2 for 24 h. After incubation, the medium containing the tested extracts or compounds was discarded and the plates were washed with phosphate-buffered saline (PBS). A total of 100 μL of serum-free medium containing 10 μL of CCK-8 solution were added to each well of the plate. In all experiments, a control without any treatment was carried out. The plate was incubated for 2 h, and the absorbance was measured at 450 nm using a microplate reader (microQuant, Bio-Tek Instruments, Inc., Winooski, VT). At the end, IC_{50} (concentration that caused 50% of growth inhibition) was calculated for the extracts and standards according to the protocols described by Monks et al. (*30*). The lower the IC_{50} value, the highest the potency against cell proliferation.

Assesment of Cell-Cycle Distribution and Apoptotic Cells by Flow Cytometry. The cell cycle of HeLa cells was analyzed by flow cytometry (Beckman-Coulter Cell Lab Quanta, Miami, FL). Briefly, 2×10^5 HeLa cells/mL were subcultured into 6-well plates in 2.5 mL of complete DMEM and incubated for 24 h. Then, the medium was replaced by 2.5 mL of fresh medium without FBS and 35 μg/mL 100% methanol crude extract and compared to the response of untreated cells (control) in triplicate. Cells were collected and rinsed with PBS; the pellets were suspended in 1 mL of 4,6-diamidino-2-phenylindole (DAPI) solution (Beckman Coulter), and the florescence was measured by flow cytometry to determine the distribution of cells in the various phases of the cell cycle, including apoptotic cells.

Data Analysis. ANOVA (*31*) was used to analyze the cell counts measured by CCK-8, the proportion of the specific cycle phase of cells (G_0/G_1), measured by flow cytometry, and the proportion of apoptotic cells. A pairwise comparison was performed using Tukey's procedure when the overall effect was significant.

RESULTS AND DISCUSSION

The concentration of condensed tannins in 100% methanol extract was 586 ± 93.7 mg of (+)-catechin equiv/1 g of lyophilized extract and anthocyanins were present in 110.8 ± 6.73 mg of cyanidin 3-glucoside equiv/1 g of lyophilized extract, as reported in our previous work (*13*).

Effect of 100% Methanol Bean Extract on HeLa Cell Adhesion. HeLa cells were treated with different concentrations of 100% methanol crude extract (0, 172.2, 516.6, and 861 μM (+)-catechin equiv) at different time intervals. Cells exposed to all concentrations of Jamapa bean 100% methanol crude extract remained firmly attached to the surface of the culture dish. All treated cells were incapable of resuspension using trypsin and presented a different appearance compared to control cells [0 μM (+)-catechin equiv]. **Table 1** shows that the lowest 100% methanol crude extract concentration tested (172.2 μM (+)-catechin equiv or 50 μg of (+)-catechin equiv/mL) main-

Table 1. Percentage of HeLa Cell Adhesion by Different Concentrations of 100% Methanol Crude Extract[a]

μg/mL (μM)[b]	8 h	12 h	24 h	72 h
0	100.0 ± 2.4 a	100.0 ± 8.7 a	100.0 ± 3.2 a	100.0 ± 9.6 a
50 (172.2)	102.9 ± 7.1 a	90.5 ± 4.9 a,b	84.2 ± 7.8 a	102.3 ± 14.8 a
150 (516.6)	91.0 ± 14.1 a,b	81.8 ± 8.4 a,b	52.0 ± 4.2 b	44.3 ± 19.1 b
250 (861)	60.3 ± 3.8 b	65.9 ± 12.7 a,b	36.3 ± 0.7 b	22.1 ± 2.8 b

[a] Results are the average of two independent experiments. Duplicate wells were tested per dose per experiment. All samples were directly dissolved in the growth media. Different letters in the same column mean a statistically significant difference (Tukey $p \leq 0.05$). [b] Micrograms of 100% methanol crude extract per milliliter or micromolar equivalent to (+)-catechin.

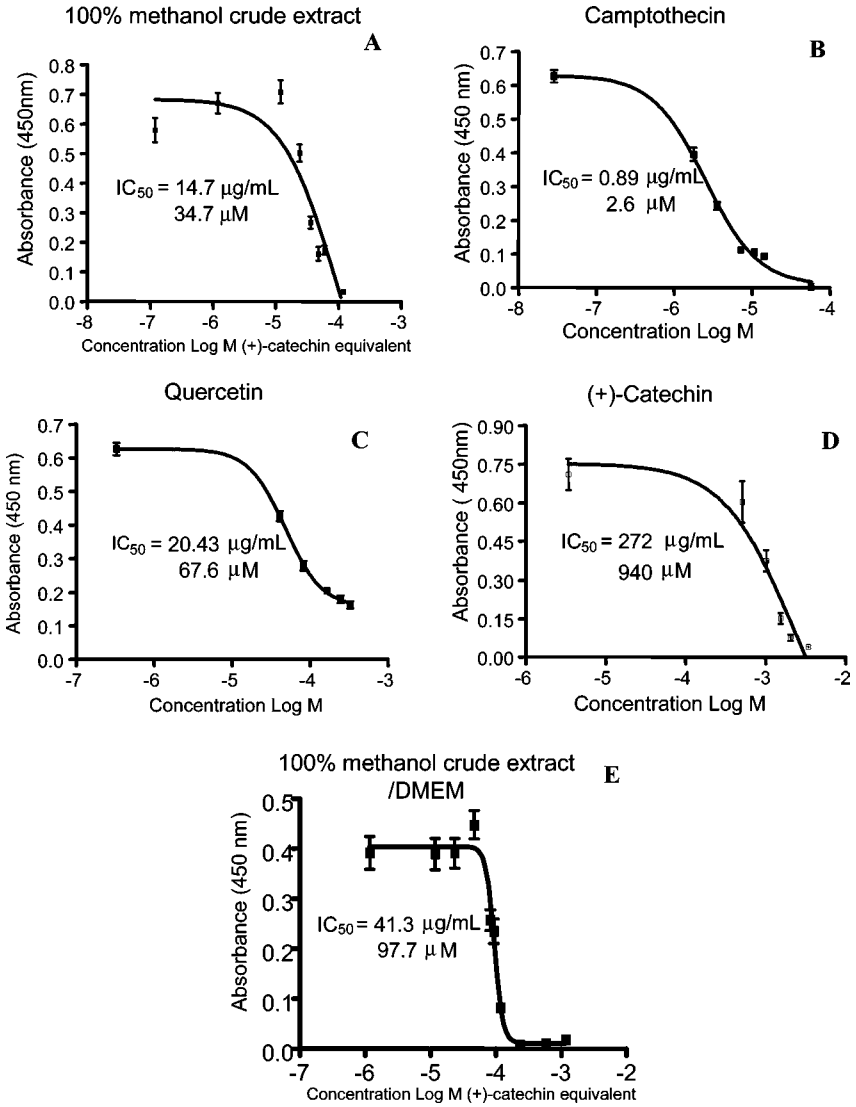

Figure 1. Dose–response curve of (A) 100% methanol crude extract, (B) camptothecin, (C) quercetin, and (D) (+)-catechin in DMSO and (E) 100% methanol crude extract in growth medium (DMEM) on HeLa cells. IC_{50} was calculated from the anti-log of the *x*-axis value at the inflection point of the sigmoid-curve fit generated for the treatment (GraphPad software Prism version 4). The curve represents the average of three independent experiments.

tained 100% of cells attached to the dish through 72 h. When the concentration was increased 3-fold, cells remained attached to the cell-culture dish, but in this case, a cytotoxic effect was observed until almost 60% at 72 h of treatment. At the highest concentration tested (861 μM (+)-catechin equiv), cell adhesion was inhibited until 78% after 72 h of treatment, suggesting a cytotoxic effect. The increment of cell adhesion of HeLa cells could be related to the ability of proanthocyanidins to enhance the collagen structure (*32*). Goo et al. (*33*) developed a collagen-resistant matrix using epigallocatechin-3-gallate that provided a favorable support for cell function and cell adhesion in human dermal fibroblasts. However, cell adhesion inhibition has been observed by many authors when cells are exposed to flavonoids; for example, intercellular adhesion molecule type 1 (ICAM-1) expression is inhibited by taxifolin (*24*). We observed that, at low concentrations, cells remained strongly attached to the culture dish, probably because of the enhancement of stability on collagen fibers. Using higher concentrations (3−5-fold) or increasing treatment duration, the number of attached cells was lower as a result of the effect of the extract on cell survival and adhesion. Cell counting with a hemacytometer was not possible because of changes in cell adhesion; therefore, the effect of the extract on cell proliferation was evaluated using an enzymatic test.

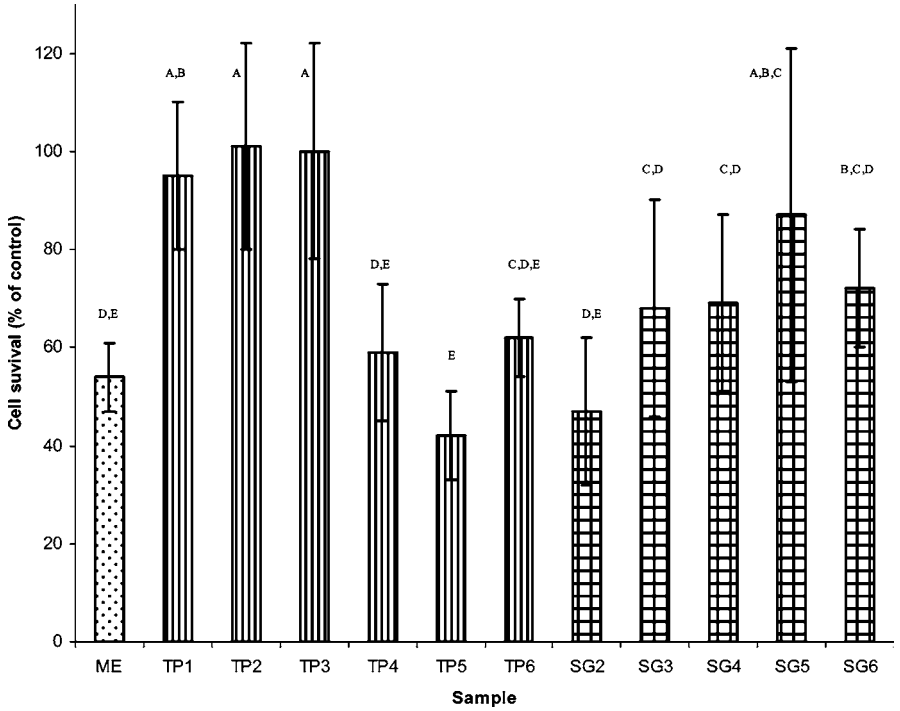

Figure 2. HeLa cell growth inhibition by 35 μg of dry material/mL of 100% methanol crude extract and fractions. (ME) 100% methanol crude extract, (TP1) Toyopearl fraction 1, (TP2) Toyopearl fraction 2, (TP3) Toyopearl fraction 3, (TP4) Toyopearl fraction 4, (TP5) Toyopearl fraction 5, (TP6) Toyopearl fraction 6, (SG2) silica gel fractions 4–8, (SG3) silica gel fractions 9–12, (SG4) silica gel fractions 13–17, (SG5) silica gel fractions 18–20, and (SG6) silica gel fractions 21–22. All of the samples were directly dissolved in the growth media. Different letters on the bars mean a statistically significant difference (Tukey $p \leq 0.05$).

Effect of 100% Methanol Crude Extract and Standards on HeLa Cell Proliferation. Cell proliferation was analyzed 24 h after HeLa cells had been cultured with increasing concentrations of 100% methanol crude extract equiv to 1.18−1181 μM (+)-catechin in the media. Parts **A**−**D** of **Figure 1** show the dose−response curves and IC_{50} values of standards and 100% methanol crude extract dissolved in dimethylsulfoxide (DMSO). A parallel experiment demonstrated that the final concentration of DMSO in the medium (0.5%) did not produce any effect on HeLa cell proliferation (data not shown).

All of the 100% methanol crude extract concentrations tested inhibited HeLa cell proliferation in a dose-dependent manner. The inhibitory 50% concentration (IC_{50}) was 34.7 μM (+)-catechin equiv, as shown in **Figure 1A**. As previously reported, (+)-catechin is a component present in the 100% methanol crude extract and in some fractions. In our previous study, (+)-catechin was present in its monomeric form as well as forming oligomers up to hexamers (*28*). Different flavonols in beans, including quercetin have also been reported by others (*8*, *15*, *28*). These flavonoids (mainly quercetin) were found to inhibit the proliferation of different cancer cell lines, including HeLa (*34*−*37*), and we included them as phenolic standards. Camptothecin (**Figure 1B**), an anticancer drug, presented the lowest IC_{50} value (2.6 μM); therefore, camptothecin was the most potent compound inhibiting HeLa cell growth. This result is similar to a previously reported observation by Hara et al. (*38*), who reported that 5 μM camptothecin induced S-phase arrest and apoptosis in HeLa cells. The inhibitory activity of quercetin on different cancer cell lines had been reported; for example, Mori et al. (*37*) found an IC_{50} value for quercetin on HeLa-S3 cells of 16.9 μg/mL. This value is slightly lower than the value found in the present investigation of 20.43 μg/mL (67.6 μM) (**Figure 1C**). This difference could be due to differences between HeLa, used in the present study, and the HeLa-S3 cell line used by Mori et al. (*37*). (+)-Catechin (**Figure 1D**) presented the lowest inhibitory potency (IC_{50} = 272 μg/mL, 940 μM) in agreement to similar findings reported by Mori et al. (*37*), who determined an IC_{50} value greater than 100 μg/mL. It is well-known that cell adhesion is closely related to cell proliferation because the interaction between integrins and the extracellular matrix results in survival transduction signals (*39*), and also, intercellular adhesion mediated by cellular adhesion mollecules (CAMs) is related to cell proliferation (*40*). Some authors have shown that integrin expresion is enhanced by flavonoids (*41*), and also, collagen stability is enhanced (*32*, *33*). On the other hand, intercellular adhesion mediated by CAMs is negatively affected by flavonoids (*40*); therefore, the relationship between cell adhesion and cell proliferation observed in our results is probably due to blocking CAM expression, leading to cell death. Because the inhibitory potency of 100% methanol crude extract on HeLa cells (14.7 μg/mL, 34.7 μM) was higher (the lowest IC_{50} value, the highest potency) than the inhibitory potency for quercetin (20.43 μg/mL, 67.6 μM) and (+)-catechin (272 μg/mL, 940 μM), it is hypothesized that the inhibitory activity is not due to a single polyphenolic compound but to the sum of the effects of all of the different flavonoids present in the complex bean extract. It has been described that potentiating interactions (synergies and additive effects) may play a role in the bioactivity of fruit and vegetable natural extracts (*23*, *42*). The 100% methanol crude extract was tested again and, this time, dissolved directly in the medium. Similar to previous results, 100% methanol crude extract inhibited the proliferation of HeLa cells in a dose-dependent manner. **Figure 1E** shows the dose−response curve of the 100% methanol crude extract dissolved directly in the growth medium. The IC_{50} value [41.3 μg/mL, 97.7 μM (+)-catechin equiv] was higher (meaning a lower potency) than the IC_{50} when the extract was previously dissolved in DMSO (14.7 μg/mL, 34.7 μM, **Figure 1A**). These results imply that DMSO is probably acting as a carrier to take the extract into the cells, and that is why the IC_{50} value is lower

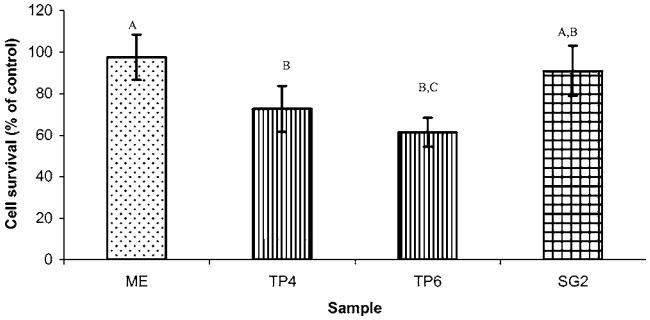

Figure 3. HaCaT cell growth inhibition by 35 μg of dry material/mL of 100% methanol crude extract and fractions. (ME) 100% methanol crude extract, (TP4) Toyopearl fraction 4, (TP6) Toyopearl fraction 6, and (SG2) silica gel fractions 4–8. All of the samples were directly dissolved in the growth media. Different letters on the bars mean a statistically significant difference (Tukey $p \leq 0.05$).

when DMSO is present. Bawadi et al. (*43*) reported that water-soluble condensed tannins isolated from black beans, at 0.24−24 μM, did not affect the growth of normal cells (human fibroblast lung cells) but induced cancer cell death (Caco-2 colon, MCF-7 and Hs578T breast and DU 145 human prostatic cells) in a dose-dependent manner by apoptosis.

Comparative HeLa Cell Growth Inhibition by 100% Methanol Crude Extract and TP and SG Fractions. Most of the fractions could not be dissolved easily in DMSO. Therefore, the cytotoxicity of 100% methanol crude extract and fractions was estimated when samples were dissolved directly in the culture media. **Figure 2** shows the effect of 100% methanol crude extract, Toyopearl fractions 1−6 (TP1−TP6), and silica gel major fractions (SG2−SG6) on HeLa cell growth at 35 μg/mL. Fractions TP1, TP2, TP3, and SG5 did not result in significant inhibitory effects on HeLa cells. The remaining fractions and the 100% methanol crude extract significantly inhibited the growth of HeLa cells between 30 and 60%. TP4, TP5, TP6, and SG2 fractions showed the highest activity against HeLa cell proliferation; the composition analysis showed that these fractions presented the highest concentration of proanthocyanidins (*28*). Meanwhile, conversely to some papers about inhibitory activity of anthocyanins from different vegetables on cancer cell proliferation (*44*, *45*), TP1, TP2, and TP3 fractions, rich in black bean anthocyanins (*28*), did not have any biological activity against HeLa cancer cells. The results could suggest that the antiproliferative activity of Jamapa common beans could be attributable to proanthocyanidins, as shown for other cells and products (*43*, *46*).

Effect of 100% Methanol Crude Extract and Some TP and SG Fractions on HaCaT Cells. HaCaT, a human cell line of spontaneously immortalized keratinocytes, was used as noncancerous control. A dose−response curve of 100% metha-

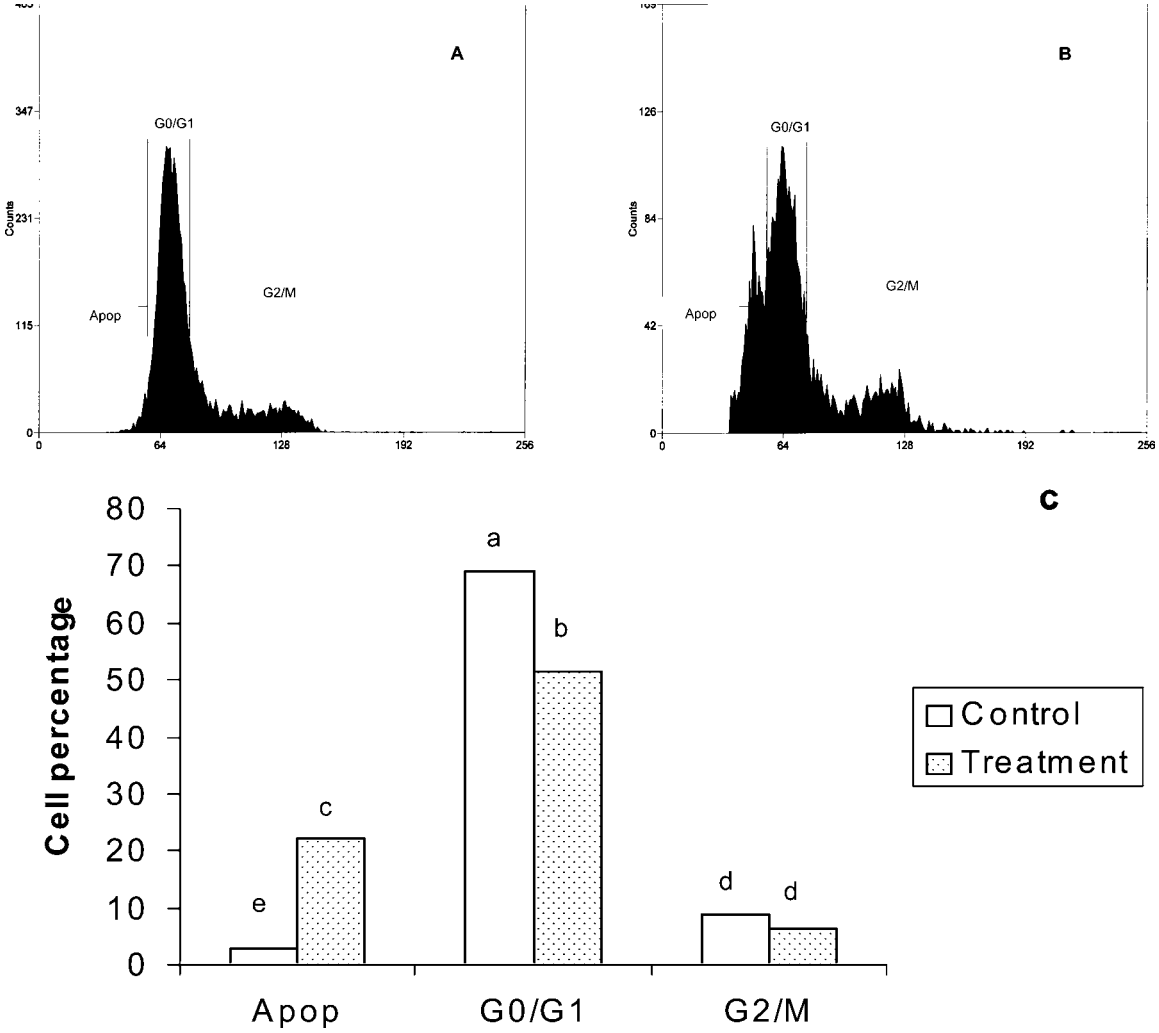

Figure 4. Effect of 100% methanol crude extract of the black Jamapa bean on HeLa cell apoptosis. (A) Flow cytometric determination of apoptosis of control cells and (B) after 24 h of treatment with 35 μg of dry 100% methanol crude extract/mL. (C) Percentage of apoptotic control and treated cells. Different letters on the bars mean a statistically significant difference (Tukey $p \leq 0.05$).

nol crude extract (dissolved directly in the medium) on HaCaT cells was tested. The IC_{50} value [65.2 μg/mL, 154 μM (+)-catechin equiv] was about 1.6 times higher than the IC_{50} for HeLa cells treated under the same conditions. The inhibitory activity of 35 μg/mL 100% methanol crude extract and some of the fractions with the highest inhibitory activity on HeLa cells (TP4, TP6, and SG2) were tested on HaCaT cells. **Figure 3** shows that neither the 100% methanol crude extract nor the fraction SG2 significantly affected the growth of premalignant HaCaT cells; meanwhile, the two TP fractions tested significantly inhibited the growth on HaCaT cells. Proanthocyanidin dimers in the form of catechin/epicatechin (*m*/*z* 579) and anthocyanins in the form of delphinidin 3-glycoside (*m*/*z* 465), petunidin 3-glycoside (*m*/*z* 479), and malvidin 3-glycoside (*m*/*z* 493) were identified in fraction TP3. The TP4 fraction contained higher concentrations of flavan-3-ol oligomers. TP4 appeared rich in heterogeneous dimers mainly composed of (epi)-afzelechin, (epi)catechin, and (epi)gallocatechin in addition to the above anthocyanins. The SG2 fraction contained a mixture of monomer−hexamer catechin-based proanthocyanidins (*28*). The results show a lower inhibitory effect of bean polyphenolics on premalignant cells compared to the effect observed toward cancer cells that may be of clinical interest. Flavonoids from different food sources have been demonstrated to inhibit selectively cancerous cells (*47*−*49*). Nair et al. (*47*) demonstrated that the antitumor effects of quercetin directly correlate with the aggressive potential of prostate cancer cells and that the mechanism(s) of quercetin-mediated antitumor effects may involve the upregulation of tumor-suppressor genes and the reciprocal downregulation of oncogenes and cell-cycle genes. More research is needed to prove the potential inhibitory effect and mechanism of action of polyphenolics from beans on different cancer cells and their possible clinical use.

Effect of 100% Methanol Crude Extract on HeLa Cell-Cycle Distribution and Apoptosis. The cell-cycle distribution of HeLa cells was analyzed after exposure to 35 μg/mL 100% methanol crude extract. A decrease in the number of cells in the G_0/G_1 phase was observed from 68.9% in control cells to 51.4% in treated cells. The percentage of cells in the G_2/M phase was not affected by the treatment (8.4 and 6.4% for control and treated cells, respectively). The treatment of HeLa cells with 35 μg/mL 100% methanol crude extract caused an increment in the DNA molecule breakage in cells (from 2.9% in control cells to 22.1% in treated cells), which could mean that the tested amount of 100% methanol crude extract caused apoptosis to HeLa cells (**Figure 4**). Different investigations have reported the effect polyphenolics have on the cycle of cancer cells; many of them attribute an apoptotic effect to polyphenolics from diverse natural sources (*20*, *21*, *44*−*46*).

In summary, polyphenolic compounds obtained from the black Jamapa bean had an inhibitory effect toward HeLa cells; HaCaT cells were affected to a lesser extent. The increment in cell adhesion, at the highest concentrations of 100% methanol crude extract tested, could be an interesting aspect to study a possible antiangiogenic effect of polyphenolics from beans. The cytotoxic effect of 100% methanol crude extract on HeLa cells may not be due to a single polyphenolic compound in the complex extract but to the sum of effects from different flavonoids in beans, which was better observed when the 100% methanol crude extract was fractionated. It could be concluded that fractionating extracts on silica gel into 22 fractions and recombining similar fractions resulted in the loss of some type of interactions between flavonoids and their effects compared to Toyopearl fractionation with a smaller number of fractions. It is preferred to use Toyopearl because it is relatively gentle on complex proanthocyanidins and does not tend to degrade phytochemical molecules during separation, as some other matrices are prone to do. When these results are taken together, they indicate that the black Jamapa bean could be an important source of polyphenolic compounds with potential anticancer activity.

LITERATURE CITED

(1) Barnard, R. J. Prevention of cancer through lifestyle changes. *Evidence-Based Complement Alternat. Med*. **2004**, *1*, 233−239.

(2) Chen, Ch.; Kong, A. T. Dietary cancer-chemopreventive compounds: From signaling and gene expression to pharmacological effects. *Trends Pharmacol. Sci*. **2005**, *26*, 318−326.

(3) Duthie, G. G.; Duthie, S. J.; Kyle, J. A. M. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. *Nutr. Res. Rev*. **2000**, *13*, 79−106.

(4) Olthof, M. R.; Hollman, P. C. H.; Buijsman, M. N. C. P.; van Amelsvoort, J. M. M.; Katan, M. B. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. *J. Nutr*. **2003**, *133*, 1806−1814.

(5) Mukhtar, H.; Ahmad, N. Tea polyphenols: Prevention of cancer and optimizing health. *Am. J. Clin. Nutr*. **2000**, *71*, 1698S−1702S.

(6) Mathers, J. C. Pulses and carcinogenesis: Potential for prevention of colon, breast and other cancers. *Br. J. Nutr*. **2002**, *88*, S273−S279.

(7) Shellie-Dessert, K. C.; Bliss, F. A. Genetic improvement in food quality factors. In *Common Beans: Research for Crop Improvement*; Schoonoven, A., van-Voysest, O., Eds.; CAB International; Wallinford, U.K., 1991; pp 649−673.

(8) Beninger, C. W.; Hosfield, G. L. Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *J. Agric. Food Chem*. **2003**, *51*, 7879−7883.

(9) Guzmán-Maldonado, S. H.; Paredes-López, O. Functional products of plants indigenous to Latin America: Amaranth, quinoa, common beans and botanicals. In *Functional Foods. Biochemical and Processing Aspects*; Mazza, G., Ed.; Technomic: Lancaster, PA, 1998; pp 293−328.

(10) Cardador-Martínez, A.; Loarca-Piña, G.; Oomah, B. D. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem*. **2002**, *50*, 6975−6980.

(11) Cardador-Martínez, A.; Castaño-Tostado, E.; Loarca-Piña, G. Antimutagenic activity of natural phenolic compounds in the common bean (*Phaseolus vulgaris*) against aflatoxin B_1. *Food Addit. Contam*. **2002**, *19*, 62−69.

(12) de Mejia, E.; Guzman-Maldonado, S. H.; Acosta-Gallegos, J. A.; Reynoso-Camacho, R.; Ramirez-Rodriguez, E.; Pons-Hernandez, J. L.; Gonzalez-Chavira, M. M.; Castellanos, J. Z.; Nelly, J. D. Effect of cultivar and growing location on the trypsin inhibitors, tannins and lectins of common beans (*Phaseolus vulgaris* L.) grown in semiarid highlands of Mexico. *J. Agric. Food Chem*. **2003**, *51*, 5962−5966.

(13) Aparicio-Fernandez, X.; Manzo-Bonilla, L.; Loarca-Piña, G. Comparison of antimutagenic activity of phenolic compounds in newly harvested and stored common beans *Phaseolus vulgaris* against aflatoxin B_1. *J. Food Sci*. **2005**, *70*, S73−S78.

(14) Takeoka, G. R.; Dao, L. T.; Full, G. H.; Wong, R. Y.; Harden, R. A.; Edwards, R. H.; Berrios, J. D. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. *J. Agric. Food Chem*. **1997**, *45*, 3395−3400.

(15) Romani, A.; Vignolini, P.; Galardi, C.; Mulinacci, N.; Benedettelli, S.; Heimler, D. Germplasm characterization of zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. *J. Agric. Food Chem*. **2004**, *52*, 3838−3842.

(16) Wu, X. L.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Gebhardt, S. E.; Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *J. Agric. Food Chem*. **2004**, *52*, 4026−4037.

(17) González de Mejía, E.; Castaño-Tostado, E.; Loarca-Piña, G. Antimutagenic effects of natural phenolic compounds in beans. *Mutat. Res. Genetic Toxicol. Environ. Mut.* **1999**, *44*, 1−9.
(18) de Bruyne, T.; Pieters, L.; Deelstra, H.; Vlietinck, A. Condensed vegetable tannins: Biodiversity in structure and biological activities. *Biochem. Syst. Ecol.* **1999**, *27*, 445−459.
(19) Rafi, M. M.; Vastano, B. C.; Zhu, N.; Ho, C.; Ghai, G.; Rosen, R. T.; Gallo, M. A.; DiPaola, R. S. Novel polyphenol molecule isolated from licorice root (*Glycrrhiza glabra*) induces apoptosis, G2/M cell cycle arrest, and Bcl-2 phosphorylation in tumor cell lines. *J. Agric. Food Chem.* **2002**, *50*, 677−684.
(20) Katsube, N.; Iwashita, K.; Tsushida, T.; Yamaki, K.; Kobori, M. Induction of apoptosis in cancer cells by billberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* **2003**, *51*, 68−75.
(21) Lazzè, M. C.; Savio, M.; Pizzala, R.; Cazzalini, O.; Perucca, P.; Scovassi, A. I.; Stivala, L. A.; Bianchi, L. Anthocyanins induce cell cycle perturbations and apoptosis in different human cell lines. *Carcinogenesis* **2004**, *25*, 1427−1433.
(22) Schmidt, B. M.; Howell, A. B.; McEniry, B.; Knight, C. T.; Seigler, D. S.; Erdman, J. W.; Lila, M. A. Effective separation of potent antiproliferation and antiadhesion components from wild blueberry (*Vaccinium angustifolium* Ait.) fruits. *J. Agric. Food Chem.* **2004**, *52*, 6433−6442.
(23) Seeram, N. P.; Adams, L. S.; Hardy, M. L.; Heber, D. Total cranberry extract versus its phytochemical constituents: Antiproliferative and synergistic effects against human tumor cell lines. *J. Agric. Food Chem.* **2004**, *52*, 2512−2517.
(24) Bito, T.; Roy, S.; Sen, C. K.; Shirakawa, T.; Gotoh, A.; Ueda, M.; Ichihashi, M.; Packer, L. Flavonoids differentially regulate IFN γ-induced ICAM-1 expression in human keratinocytes: Molecular mechanisms of action. *FEBS Lett.* **2002**, *520*, 145−152.
(25) Deshpande, S. S.; Cheryan, M. Evaluation of vanillin assay for tannin analysis of dry beans. *J. Food Sci.* **1985**, *50*, 905−910.
(26) Deshpande, S. S.; Cheryan, M. Determination of phenolic compounds of dry beans using vanillin, redox and precipitation assays. *J. Food Sci.* **1987**, *52*, 332−334.
(27) Abdel-Aal, E. S.; Hucl, P. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chem.* **1999**, *76*, 350−354.
(28) Aparicio-Fernandez, X.; Yousef, G. G.; Loarca-Pina, G.; de Mejia, E.; Lila, M. A. Characterization of polyphenolics in the seed coat of black Jamapa bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **2005**, *53*, 4615−4622.
(29) García-Gasca, T.; Paz-González, V.; Moncada-Álvarez, M. C.; Blanco-Labra, A.; Salazar-Olivo, L. A. Colorimetric quantitation of *in vitro* cell density using carmine, a chromosome-specific stain. *Toxicol. in Vitro* **2002**, *16*, 573−579.
(30) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. Feasibility of high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(31) SAS Institute. *SAS User's Guide: Statistics*, 8th ed.; SAS Institute: Cary, NC, 1999.
(32) Han, B.; Jaurequi, J.; Tang, B. W.; Nimni, M. E. Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices. *J. Biomed. Mater. Res.* **2003**, *65A*, 118−124.
(33) Goo, H. C.; Hwang, Y. S.; Choi, Y. R.; Cho, H. N.; Suh, H. Development of collagenase-resistant collagen and its interaction with adult human dermal fibroblasts. *Biomaterials* **2003**, *24*, 5099−5113.
(34) Deschner, E. E.; Ruperto, J.; Wong, G.; Newark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **1991**, *12*, 1193−1196.
(35) de Vincenzo, R.; Ferlini, C.; Distefano, M.; Gaggini, C.; Riva, A.; Bombardelli, E.; Morazzoni, P.; Valenti, P.; Belluti, F.; Ranelletti, F. D.; Mancuso, S.; Scambia, G. *In vitro* evaluation of newly developed chalcone analogues in human cancer cells. *Cancer Chemother. Pharmacol.* **2000**, *46*, 305−312.
(36) Jakubowicz-Gil, J.; Rzymowska, J.; Gawron, A. Quercetin, apoptosis, heat shock. *Biochem. Pharmacol.* **2002**, *64*, 1591−1595.
(37) Mori, A.; Nishino, C.; Enoki, N.; Tawata, S. Cytotoxicity of plant flavonoids against HeLa cells. *Phytochemistry* **1988**, *27*, 1017−1020.
(38) Hara, T.; Ishii, T.; Fujishiro, M.; Masuda, M.; Ito, T.; Nakajima, J.; Inoue, T.; Matsuse, T. Glutathione *S*-transferase P1 has protective effects on cell viability against camptothecin. *Cancer Lett.* **2004**, *203*, 199−207.
(39) Hood, J. D.; Cheresh, D. A. Role of integrins in cell invasion and migration. *Nature Rev.* **2002**, *2*, 91−101.
(40) Kim, Y. G.; Kim, M. J.; Lim, J. S.; Lee, M. S.; Kim, J. S.; Yoo, Y. D. ICAM-3-induced cancer cell proliferation through the PI3K/Akt pathway. *Cancer Lett.* **2005**, Epub ahead of print.
(41) Pilorget, A.; Berthet, V.; Luis, J.; Moghrabi, A.; Annabi, B.; Beliveau, R. Medulloblastoma cell invasion is inhibited by green tea (−)epigallocatechin-3-gallate. *J. Cell. Biochem.* **2003**, *90*, 745−755.
(42) Shafiee, M.; Carbonneau, M.; Huart, J.; Descomps, B.; Leger, C. Synergistic antioxidative properties of phenolics from natural origin toward low-density lipoproteins depend on the oxidation system. *J. Med. Food* **2002**, *5*, 69−78.
(43) Bawadi, H. A.; Bansode, R. R.; Trappey I. I. A.; Truax R. E.; Losso, J. N. Inhibition of Caco-2 colon, MCF-7 and Hs578T breast, and DU 145 prostatic cancer cell proliferation by water-soluble black bean condensed tannins. *Cancer Lett.* **2005**, *218*, 153−162.
(44) Martin, S.; Favot, L. Matz, R.; Lugnier, C.; Andriantsitohaina, R. Delphinidin inhibits endothelial cell proliferation and cell cycle progression through a transient activation of ERK-1/-2. *Biochem. Pharmacol.* **2003**, *65*, 669−675.
(45) Hyun, J. W.; Chung, H. S. Cyanidin and malvidin from *Oryza sativa* cv. Heugjinjubyeo mediate cytotoxicity against human monocytic leukemia cells by arrest of G_2/M phase and induction of apoptosis. *J. Agric. Food Chem.* **2004**, *52*, 2213−2217.
(46) Yang, L.-L.; Chang, C.-C.; Chen, L.-G.; Wang, C.-C. Antitumor principle constituents of *Myrica rubra* var. *acuminata*. *J. Agric. Food. Chem.* **2003**, *51*, 2974−2979.
(47) Nair, H. K.; Rao, K. V. K.; Aalinkeel, R.; Mahajan, S.; Chawda, R.; Schwartz, S. A. Inhibition of prostate cancer cell colony formation by the flavonoid quercetin correlates with modulation of specific regulatory genes. *Clin. Diagn. Lab. Immunol.* **2004**, *11*, 63−69.
(48) Kawaii. S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. Antiproliferative activity of flavonoids on several cancer cell lines. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 896−899.
(49) Hakimuddin, F.; Paliyath, G.; Meckling, K. Selective cytotoxicity of a red grape wine flavonoid fraction against MCF-7 cells. *Breast Cancer Res. Treat.* **2004**, *85*, 65−79.

Received for review November 28, 2005. Revised manuscript received January 31, 2006. Accepted February 2, 2006. The authors wish to thank the United States Agency for International Development through a grant from the Association Liaison Office for University Cooperation in Development (TIES-Enlaces/US-AID), Consejo Nacional de Ciencia y Tecnología (CONACYT) Grant 31623-B, and USDA/IFAFS Grant 00-52101-9695 and the "El Bajío" Experimental Station of the National Research Institute for Forestry Agriculture and Livestock (INIFAP) for Black Jamapa common beans (*Phaseolus vulgaris* L.) donated.

JF052974M