Screening of antiproliferative effect of aqueous extracts of plant foods consumed in México on the breast cancer cell line MCF-7

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Abstract
We evaluated the antiproliferative effect of aqueous extracts of 14 plant foods consumed in Mexico on the breast cancer cell line MCF-7. The plant foods used were avocado, black sapote, guava, mango, prickly pear cactus stems (called nopal in Mexico, cooked and raw), papaya, pineapple, four different cultivars of prickly pear fruit, grapes and tomato. β-Carotene, total phenolics and gallic acid contents and the antioxidant capacity, measured by the ferric reducing/antioxidant power and the 2,2-diphenyl-1,1-picrylhydrazyl radical scavenging assays, were analyzed in each aqueous extract. Only the papaya extract had a significant antiproliferative effect measured with the methylthiazolyldiphenyl-tetrazolium bromide assay. We did not notice a relationship between the total phenolic content and the antioxidant capacity with antiproliferative effect. It is suggested that each extract of plant food has a unique combination of the quantity and quality of phytochemicals that could determine its biological activity. Besides, papaya represents a very interesting fruit to explore its antineoplastic activities.

Keywords: Antioxidants, antiproliferative, breast cancer, fruit, vegetables

Introduction
It has been postulated that a high intake of fruits and vegetables could contribute to the prevention of cancer because of their high content of a variety of bioactive compounds called phytochemicals (Liu 2004). Phytochemicals are defined as bioactive non-nutrient plant compounds that have been linked to reduced risks of major chronic diseases (Liu 2004). Phytochemicals can be classified as carotenoids, phenolics (phenolic acids and flavonoids), alkaloids, and compounds containing nitrogen, sulfur or selenium (Liu 2004; Finley 2005). Because most of the phytochemicals present antioxidant capacity, it has been proposed that they could contribute to the prevention of the oxidative damage produced by an excess of reactive oxygen species (Liu 2004). Reactive oxygen species are involved in the initiation, promotion and progression steps of carcinogenesis (Brown and Bicknell...
Besides their antioxidant capacity, phytochemical compounds have diverse biological effects such as antimutagenic activity, inhibition of cell proliferation, induction of cell death and differentiation (Liu 2004). Cell culture systems have been a useful tool to study the effects and associated mechanisms of individual phytochemicals on cancer. Carotenoids, phenolics and organosulfur compounds are only some of the phytochemicals that have been studied with this approach (Prakash et al. 2001; Auborn et al. 2003; Brusselmans et al. 2005). Nevertheless, it was proposed that the health benefits of vegetables and fruits might result from multiple combined effects of their phytochemicals rather than from the action of a single active ingredient (Liu 2004; Percival et al. 2006). In this context, different combinations of phytochemicals have been tested in order to explore synergistic and additive effects on cancer cells proliferation (Meterns-Talcott et al. 2003; Campbell et al. 2006). It has been shown that several whole fruit and vegetable extracts have antiproliferative effects on the hepatocarcinoma cell line HepG2 (Chu et al. 2002; Sun et al. 2002). Percival et al. (2006) showed that aqueous extract of mango inhibited cell proliferation in leukemia cell line HL-60 as well as the neoplastic transformation of BALB/3T3 cells initiated by benzo(a)pyrene. All these studies strongly suggest that mixtures of phytochemicals and extracts of whole foods could be a good strategy to screen cancer chemopreventive activities of phytochemicals present in fruit and vegetables using cell culture models.

In this study we used 14 aqueous extracts from different plant foods consumed in Mexico to analyze their contents of total phenolic compounds, gallic acid, β-carotene and antioxidant capacity. Subsequently, we tested the antiproliferative effect of these extracts using the breast cancer cell line MCF-7. The interest in using a breast cancer cell line as an experimental system to test the potential anticancer properties of these extracts relies on data from in vitro and epidemiological studies that relate the phytochemical groups, including carotenoids and phenolics, with breast cancer chemoprevention (Prakash et al. 2001; Sato et al. 2002; Brusselmans et al. 2005; Fink et al. 2007).

**Materials and methods**

**Materials**

All chemicals used were of analytical grade and they were obtained from Sigma-Aldrich (St Louis, MO, USA) unless otherwise indicated. Acetone, n-hexane, ethanol, toluene, KOH and sodium carbonate were purchased from J.T. Baker (Mexico City, Mexico). Cell culture materials were obtained from Gibco Life Technologies (Grand Island, NY, USA).

**Plant foods**

Lycopersicon esculentum Mill., Cv. Clermon). Plant foods were obtained from commercial sources, except for nopal and PP that were harvested from Campo Experimental Norte de Guanajuato, Instituto Nacional de Investigaciones Forestales y Agrícolas y Pecuarias, Gto., México. PP cultivars used in this study have different color characteristics: 'Reina' is a clear green/white, 'Rojo pelota' is purple red, whereas 'Naranjona' and '2-651' are yellow/orange. The colors presented in PP are mainly due to a combination of two antioxidant betalain pigments, the purple red betanin and the yellow/orange indicaxantin (Butera et al. 2002).

**Plant food characterization and preparation**

Plant foods used did not show any physical defects, signs of pathogen or degradation process, or other characteristics unsuitable for marketing and consumption. All of the plant foods were processed immediately after arrival to the laboratory, where they were cleaned and dried before processing. Because nopal is a vegetable commonly consumed after cooking, both cooked and raw products were included. Cooking of nopal was as follows: an edible portion of fresh nopal was cut into small rectangles (0.5 mm × 30 mm) and plunged into one volume of boiling water for 12 min. After boiling, the water was strained and nopal was cooled at room temperature for 80 min. For objective characterization of the plant foods, the external (peel) and internal (pulp) color characteristics were defined, on the basis of the CIELAB color system ($L^*$, $a^*$, $b^*$) with a Minolta CM-2002 spectrophotometer (Minolta Co., Osaka, Japan) and the program Spectra Match 3.3.7. Within the uniform space of CIELAB, two color coordinates ($a^*$ and $b^*$) as well as an index of lightness ($L^*$) are defined. The coordinate $a^*$ indicates positive values for reddish colors and negative values for greenish ones, whereas $b^*$ indicates positive values for yellowish colors and negative values for bluish ones. $L^*$ is an approximate measurement of luminosity, which is the property according to which each color can be considered equivalent to a member of the gray scale, between blank and white within the range 0–100. In general, external and internal color was longitudinally determined at least on three points of the plant products. On the other hand, the edible portions of the fruit were cut and pooled, and the total soluble solids expressed as degree Brix (°B) were determined in three aliquots of the plant products using a hand refractometer with automatic temperature compensation (Atago Co. Ltd., Osaka, Japan). After this, aliquots of the plant foods were flash frozen in liquid nitrogen, and kept at $-70^\circ$C until analysis. Table I presents the color characteristics and °B of plant foods.

**Aqueous extract preparation**

Aqueous extracts were prepared as described by Percival et al. (2006) with some modifications. Briefly, the frozen edible portions of plant foods were thawed, mixed 1 + 1 (w/v) with high-performance liquid chromatography (HPLC)-grade water, homogenized and sonicated for 5 min. After centrifugation at 9,000 × g for 20 min at 4°C, the supernatant was set aside. The pellet was resuspended with HPLC-grade water, shaken and centrifuged, and the procedure was repeated a third time before supernatants were pooled and one aliquot of each extract was filtered twice through a sterile 0.45 μm pore filter (Chromaphil® PET-45/25; Macherey-Nagel; Düren, Germany). Both filtered and non-filtered supernatant samples were stored at $-70^\circ$C until utilization in cell culture or chemical analysis. Table II presents the
<table>
<thead>
<tr>
<th>Plant food</th>
<th>n</th>
<th>(L^*)</th>
<th>(a^*)</th>
<th>(b^*)</th>
<th>(nL)</th>
<th>(a^*)</th>
<th>(b^*)</th>
<th>(B) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avocado</td>
<td>12</td>
<td>37.9 ± 7.3</td>
<td>7.3 ± 0.2</td>
<td>0.3 ± 1.2</td>
<td></td>
<td></td>
<td></td>
<td>7.5 ± 0.02</td>
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<tr>
<td>Black sapote</td>
<td>60</td>
<td>41.2 ± 1.4</td>
<td>4.5 ± 1.4</td>
<td>6.2 ± 1.8</td>
<td>18</td>
<td>22.5 ± 4.8</td>
<td>16.5 ± 3.6</td>
<td>3.0 ± 1.9</td>
</tr>
<tr>
<td>Guava</td>
<td>42</td>
<td>76.2 ± 1.3</td>
<td>2.1 ± 1.2</td>
<td>47.4 ± 4.0</td>
<td>27</td>
<td>82.2 ± 2.3</td>
<td>2.6 ± 1.3</td>
<td>23.2 ± 2.9</td>
</tr>
<tr>
<td>Mango</td>
<td>48</td>
<td>66.5 ± 1.8</td>
<td>20.1 ± 1.9</td>
<td>38.2 ± 2.3</td>
<td>48</td>
<td>61.4 ± 4.1</td>
<td>23.7 ± 3.2</td>
<td>49.7 ± 7.0</td>
</tr>
<tr>
<td>Nopal (cooked)</td>
<td>18</td>
<td>50.8 ± 2.0</td>
<td>4.3 ± 0.6</td>
<td>16.5 ± 3.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Nopal (raw)</td>
<td>30</td>
<td>50.4 ± 2.6</td>
<td>3.7 ± 1.3</td>
<td>14.8 ± 3.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Papaya</td>
<td>9</td>
<td>55.9 ± 4.5</td>
<td>17.4 ± 3.4</td>
<td>28.3 ± 5.1</td>
<td>6</td>
<td>46.6 ± 6.5</td>
<td>31.5 ± 3.8</td>
<td>33.9 ± 4.6</td>
</tr>
<tr>
<td>Pineapple</td>
<td>6</td>
<td>58.1 ± 2.1</td>
<td>15.8 ± 4.5</td>
<td>35.5 ± 7.1</td>
<td>6</td>
<td>75.6 ± 2.6</td>
<td>5.1 ± 0.6</td>
<td>34.2 ± 2.3</td>
</tr>
<tr>
<td>PP ‘Naranjona’</td>
<td>60</td>
<td>52.3 ± 1.3</td>
<td>7.8 ± 2.6</td>
<td>13.0 ± 1.5</td>
<td>48</td>
<td>41.3 ± 5.1</td>
<td>22.2 ± 3.7</td>
<td>23.7 ± 5.6</td>
</tr>
<tr>
<td>PP ‘Reina’</td>
<td>69</td>
<td>55.1 ± 5.7</td>
<td>-7.3 ± 1.2</td>
<td>26.7 ± 4.7</td>
<td>27</td>
<td>58.0 ± 4.0</td>
<td>0.2 ± 1.2</td>
<td>14.7 ± 2.2</td>
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<tr>
<td>PP ‘Rojo Pelota’</td>
<td>45</td>
<td>43.5 ± 2.2</td>
<td>7.5 ± 2.0</td>
<td>3.1 ± 2.7</td>
<td>48</td>
<td>30.2 ± 4.3</td>
<td>29.4 ± 4.1</td>
<td>2.1 ± 1.2</td>
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<tr>
<td>PP ‘2-651’</td>
<td>78</td>
<td>56.8 ± 2.4</td>
<td>1.5 ± 3.1</td>
<td>16.2 ± 3.3</td>
<td>78</td>
<td>43.4 ± 7.3</td>
<td>19.7 ± 5.5</td>
<td>14.7 ± 4.2</td>
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<tr>
<td>Red Grape</td>
<td>45</td>
<td>34.0 ± 3.2</td>
<td>12.6 ± 2.6</td>
<td>4.1 ± 1.8</td>
<td>15</td>
<td>31.7 ± 1.5</td>
<td>11.2 ± 1.0</td>
<td>7.7 ± 1.7</td>
</tr>
<tr>
<td>Tomato</td>
<td>42</td>
<td>55.4 ± 8.7</td>
<td>25.3 ± 2.5</td>
<td>24.0 ± 4.4</td>
<td>42</td>
<td>47.5 ± 2.6</td>
<td>26.4 ± 3.0</td>
<td>18.5 ± 3.4</td>
</tr>
</tbody>
</table>

Color characteristics were determined on the basis of the CIE-LAB color system (\(L^*\), \(a^*\) and \(b^*\)). \(n\), number of observations; ND, not determined.
proportion between the grams of plant foods and the total water used to prepare the aqueous extracts as well as the $\beta$ values. Aqueous extracts are more convenient for use in tests for in vitro assays with cultured cells since they do not contain toxic compounds usually employed in other types of chemical extractions.

**Extraction and quantification of all-trans-$\beta$-carotene in aqueous extracts**

All-trans-$\beta$-carotene was extracted from aqueous extracts according to Lee and Castle (2001). An aliquot of each aqueous extract (10 ml) was mixed with 20 ml extracting solvent (hexane:acetone:ethanol, 50:25:25, v/v/v), agitated, and centrifuged for 5 min at 12,000 $\times g$ at 4°C. The top layer of hexane containing the carotenoids was recovered and dried using a rotary evaporator under vacuum at 40°C, and the residue was dissolved using 1 ml methanol. The procedure was conducted at 4°C under dimmed light and all samples were wrapped in foil. Samples were automatically injected into an HP 1100 series HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany) equipped with an online degasser and a diode array detector (DAD). The HPLC system was equipped with a 150 mm $\times$ 4.6 mm i.d., 3 $\mu$m C30 reversed-phase column (YMC Inc., Milford, MA, USA), which was kept at 15°C. After pilot studies, the most appropriate mobile phase was found to be composed of methanol (solvent A) and tert-butyl methyl ether (solvent B) with the following gradient program: 0–100% tert-butyl methyl ether, in 20 min, at a flow rate of 1 ml/min. The injection volume for samples was 50–100 μl and the detector was set at 450 nm. All-trans-$\beta$-carotene from analytical samples was identified and quantified by comparing its retention time and UV–vis absorption spectra with those of the standards in the library that was built using the inline DAD and by calibration curves constructed with pure compound.

**Determination of phenolic content in aqueous extracts**

The total phenolic content in aqueous extracts was analyzed by the Folin–Ciocalteu colorimetric method described previously (Singleton et al. 1999), with some modifications. Briefly, 30 μl appropriate dilutions of aqueous extracts were oxidized...
with 150 μl Folin–Ciocalteu reagent, and after 5 min the reaction was neutralized with 120 μl of 7.5% (w/v) sodium carbonate. The absorbance of the resulting blue color was measured at 620 nm after 2 h in a microplate reader (Dynex Technologies, Inc., Chantilly, VA, USA). Gallic acid was used as standard, and results were expressed as micrograms of gallic acid equivalents (GAE) per milliliter.

**Extraction and determination of gallic acid content in aqueous extracts**

Because phenolic compounds commonly occur in plants as simple glycosides esters, we used acid to hydrolyze the conjugated forms to aglycons according to the method described by Hämäkinen et al. (1998), with some modifications. Briefly, an aliquot of each aqueous extract (2 ml) was mixed with 2 ml of 2.6 M HCl and incubated at 80°C for 6 h. After hydrolysis the extracts were cooled in ice and filtered through a nylon 0.45 μm pore filter. Samples were automatically injected into an HP 1100 series HPLC system (Hewlett-Packard GmbH) equipped with an online degasser, a DAD. Separation of phenolics was carried out using a Waters X-Terra™ RP18 column (250 mm × 4.6 mm i.d.; particle size, 5 μm). The binary mobile phase consisted of water 1% formic acid (solvent A) and acetonitrile (solvent B). The flow rate was kept constant at 0.5 ml/min for a total run time of 90 min. The system was run with a gradient program: 2–100% acetonitrile in 60 min. The injection volume for samples was 20 μl and the detector was set at 280, 320 and 360 nm for simultaneous monitoring of different groups of phenolics. Identification of compounds was achieved by comparing their retention times and UV–vis spectra with those of the standards in the library that was built using the inline DAD.

**Ferric reducing/antioxidant power assay**

The ferric reducing/antioxidant power assay (FRAP) was measured using the method reported by Benzie and Strain (1999) with some modifications. Three reagents were used: sodium acetate and acetic acid buffer (300 mM, pH 3.6); 10 mM solution of 2,4,6-tripyridyl-s-triazine in a 40 mM solution of hydrochloric acid; or 20 mM solution of ferric chloride hexahydrate prepared with HPLC-grade water. A FRAP reagent was prepared fresh prior to each analysis by combining 300 mM sodium acetate buffer, 10 mM 2,4,6-tripyridyl-s-triazine and 20 mM ferric chloride in the proportions of 10:1:1. An ascorbic acid standard series was prepared fresh prior to analysis. The FRAP assay was carried out in a microplate reader (Dynex Technologies, Inc.) and absorbance readings were taken at 620 nm. The plates were manually loaded with sodium acetate buffer (60 μl), ascorbic acid standards (60 μl) or samples (60 μl) into the respective wells, after which 240 μl freshly prepared FRAP reagent was added into each well, and readings taken 4 min thereafter. Antioxidant capacity was expressed as micromoles of ascorbic acid equivalents (AAE) per liter.

**2,2-Diphenyl-1-1-picrylhydrazyl radical scavenging assay**

The 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) assay is based on the measurement of the scavenging capacity of antioxidants towards the stable radical DPPH. The free radical DPPH is reduced to the corresponding hydrazine when it reacts with hydrogen donors (Sanchez-Moreno 2002). This capacity is measured using a decoloration assay, which evaluate the decrease of absorbance produced by addition of the
antioxidant to a DPPH solution in methanol. Here, DPPH radical scavenging assay was measured according to Brand-Williams et al. (1995) with some modifications. The aqueous extracts (10 μl) reacted with 290 μl methanolic DPPH solution (60 μM). After 30 min of incubation, the absorbance at 490 nm was determined in a microplate reader (Dynex Technologies, Inc.). The absorbance of samples with DPPH at steady state was subtracted from absorbance of control DPPH. The change in absorbance was compared with a standard curve of ascorbic acid, and the scavenging of the stable radical DPPH antioxidant capacity was expressed as micromoles AAE per liter.

Cell culture

The breast cancer cell line MCF-7 (ATCC HTB-22) was kindly supplied by Dr C. Aceves (Instituto de Neurobiología, UNAM, Mexico). MCF-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin (basal medium). Cultures were incubated at 37°C in a humidified 5% CO2, 95% air atmosphere.

Antiproliferative assay

The antiproliferative activity of plant food extracts was measured by the methythiazolyldiphenyltetrazolium bromide (MTT) assay described previously by Arroyo-Helguera et al. (2006) with some minor modifications. The MTT assay is based on the conversion by viable cells of the yellow tetrazolium dye MTT to purple formazan crystals, and it provides an indirect but quantitative determination of metabolically active cells. The cells were seeded in a 96-well plate at a density of 5 x 10^3 cells/well in a final volume of 100 μl and incubated for 24 h. After which, 100 μl fresh medium per well was added together with the different doses of aqueous extracts. The final concentrations of aqueous extracts were 0%, 0.01%, 0.1%, 0.5%, 1% (v/v). Also 2% and 4% (v/v) of aqueous extracts from guava, mango, nopal (raw), papaya and black sapote were assayed. We included in each assay a treatment with 250 nM thapsigargin, an endoplasmic reticulum Ca^{2+}-ATPase inhibitor and a strong inducer of apoptosis in the MCF-7 cell line, as a positive control of cell proliferation inhibitor (Jackisch et al. 2000). Thapsgargin was dissolved in dimethyl sulfoxide (DMSO) and an appropriate control was included. The DMSO used was 0.1% (v/v) of the total culture medium. One hour ahead of 24, 48 and 72 h of incubation, 20 μl MTT (5 mg/ml in phosphate-buffered saline) were added to cell culture. After 1 h the medium was removed and formazan crystals were dissolved with 100 μl DMSO. The absorbance was measured at 550 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell proliferation (percentage) was determined at different times of treatment from the MTT absorbance reading for each concentration compared with the control. At least three replicates for each sample were used to determine cell proliferation (percentage values). All experiments were performed at least in duplicate.

Statistical analysis

Results from antiproliferative assays were analyzed by one-way analysis of variance using a Tukey test as the post-test, and P < 0.05 was considered statistically significant. Presented data represent the mean ± standard deviation. Correlations between the phenolic content and antioxidant capacity by the FRAP and DPPH
assays were performed with the two-tailed Pearson correlation test, and $P < 0.05$ was considered statistically significant.

**Results and discussion**

In the present work we studied the effect of 14 aqueous extracts obtained from plant foods consumed in Mexico on cell proliferation of breast cancer cell line MCF-7. The aim of our study was to test complete and not fractioned extracts. Initially, the total content of $\beta$-carotene and phenolics, including gallic acid, in the aqueous extracts were analyzed. Among all the plant foods analyzed we only detected $\beta$-carotene in papaya extract ($21.3 \pm 2.3 \, \mu g/ml$), and were unable to detect $\beta$-carotene in other aqueous extracts from fruit and vegetables rich in this compound, such as mango (Ornelas et al., 2007), presumably because of the very low dilutions used. This could also be explained because the papaya matrix is softer than other plant foods used in this study. In papaya and other yellow fruits, carotenoids are dissolved in oil droplets in chromoplasts and can be readily extracted whereas in other foods, such as nopal, carotenoids could be entrapped and form a complex with proteins in diverse cellular structures (Castenmiller and West 1998). This result is very important because carotenoids are a family of nearly 600 fat-soluble plant pigments that have received substantial attention because some act as provitamin A, and many have antioxidant activity (Liu 2004). Figure 1a shows the values of phenolic contents, expressed as micrograms of GAE per milliliter, of the 14 fruits and vegetable aqueous extracts tested in this study. Among all the aqueous extracts analyzed, guava had the highest phenolic content, followed by black sapote, nopal (cooked and raw), mango, pineapple, papaya, PP ‘Rojo pelota’, red grapes, PP ‘2-651’, PP ‘Reina’, and avocado. Tomato extract had the lowest phenolic content. It is convenient to indicate that Folin–Ciocalteu is not specific for analysis of individual compounds; however, it gives a global quantity of phenolics present in each sample (Singleton et al. 1999). Among all the aqueous extract analyzed, grape had the highest gallic acid content, followed by mango, pineapple, papaya, PP ‘Reina’, PP ‘Naranjona’, guava, black sapote, PP ‘2-651’, PP ‘Rojo pelota’, and tomato (Figure 1b). Finally, in avocado and nopal (cooked and raw) extracts, we are unable to detect gallic acid (Figure 1b). Phenolic compounds are important constituents in fruits and vegetables, and their presence relates to the antioxidant capacity, food quality, and potential health benefits (Mahattanatawee et al. 2006).

The antioxidant capacity by FRAP and DPPH assays in aqueous extracts of plant foods (Figure 2) showed a very similar pattern. The five aqueous extracts with highest antioxidant capacity were guava, mango, black sapote, papaya and pineapple. It is interesting to note that the aqueous extract of black sapote, a fruit poorly characterized, had high phenolic content and high antioxidant capacity. Further research is warranted to explore the correlation between the phenolic content, antioxidant capacity and potential health benefits of this fruit. Extracts with lower antioxidant capacity were avocado, tomato and nopal (cooked and raw). Both nopal extracts, cooked and raw, had high phenolic content (Figure 1a) but very low antioxidant capacity (Figure 2).

Figure 3 shows the Pearson correlations between the total phenolic content and the antioxidant capacity in the 14 aqueous extracts used in this study. Figure 3a shows a significant difference in correlation between total phenolic contents and antioxidant
capacity measured with the FRAP and DPPH assays ($R^2 = 0.42$ and $R^2 = 0.29$, respectively) when all values of aqueous extracts were included. However, the correlation was increased when data from nopal (cooked and raw) was excluded from the analysis (with FRAP assay, $R^2 = 0.92$; with DPPH assay, $R^2 = 0.66$; Figure 3b). Previous studies demonstrated a high correlation between total phenolic content and antioxidant capacity (Sun et al. 2002; Mahattanatawee et al. 2006), but there are exceptions (Kähkönen et al. 1999; Wu et al. 2004). It is known that different phenolic compounds are detected differently when they are quantified by the Folin–Ciocâlteu method (Kähkönen et al. 1999; Singleton et al. 1999). Similarly, the molecular antioxidant response of phenolic compounds during the FRAP and DPPH assays could vary depending on their chemical structure, as in the situation with the oxygen radical absorbance capacity and oxidation methyl linolate assays (Kähkönen et al. 1999; Wu et al. 2004). The antioxidant capacity of an extract therefore cannot be predicted on the basis of its total phenolic content.

According to the mentioned results, complex mixtures of phytochemicals from each tested plant food present a unique quantitative and qualitative combination of phytochemicals that could determine its overall biological activity. The potential antiproliferative effect of the aqueous extracts was proved using the widely accepted method of MTT assay (Arroyo-Helguera et al. 2006). We used a treatment with 250 nM thapsigargin, which has been reported as a potent inhibitor of cell proliferation.
and inducer of apoptosis in MCF-7 cells, as an antiproliferative positive control (Jackisch et al. 2000). The strong inhibitory effect of thapsigargin after 24, 48 and 72 h on cell proliferation of MCF-7 cells is evident in Figure 4. These data indicate that the technique to test the potential antiproliferative action of the aqueous extracts is adequate.

On the other hand, after testing the 14 aqueous extracts at 0.01%, 0.1%, 0.5% and 1% for 24, 48 and 72 h, we found that only papaya extract had a significant but modest inhibitory effect on proliferation of MCF-7 cells after 72 h of treatment (Figure 5c). Therefore, we increased the doses to 2% and 4% for black sapote, guava, mango, papaya and raw nopal extracts, which were those with the highest phenolic content and antioxidant capacity in the case of the four first extracts, and with high phenolic content but poor antioxidant capacity in the case of nopal (Figure 1a and 2). With the higher doses, we have noticed that only mango and papaya extracts had a significant inhibitory effect on cell proliferation (Figure 5). The effect of the mango extract was significant only at 4% and after 72 h of treatment (Figure 5c). However, papaya extract was more effective, showing an inhibitory effect on cell proliferation at 4% after 24 h (Figure 5a). At longer treatment duration (48 and 72 h), the papaya’s antiproliferative action was more evident (Figure 5b and c). The maximum effect of papaya extract was shown after 72 h of treatment at 2% and 4%, causing 30% and 53% inhibition of MCF-7 cells proliferation, respectively (Figure 5c).

Figure 2. Total antioxidant capacity measured by the (a) FRAP or (b) DPPH assays for aqueous extracts of plant foods. Vertical bars, standard deviation of the mean, n = 3; dashed lines, mean of all values of each graph.
One of the major hypotheses related with cancer chemoprevention and phytochemicals indicates that high antioxidant capacity is correlated with a high concentration of phytochemicals and therefore with more anticancer activity (Percival et al. 2006). However, some reports have indicated that antioxidant capacity is more relevant in the initiation step of carcinogenesis when reactive oxygen species and other pro-oxidant carcinogens oxidize DNA to generate mutations (Liu 2004; Finley 2005). According to this assumption, some authors have suggested that in promotion and progression steps of carcinogenesis the antioxidant capacity of phytochemicals appeared to play a minor role in anticancer activity (Finley 2005). Our results and those of others (Chu et al. 2002; Sun et al. 2002; Percival et al. 2006), using different whole foods extracts, have shown that an antiproliferative effect in cancer cells does not correlate with phenolic content nor with antioxidant capacity. Besides the role played by phytochemicals as antioxidants, they also participate in the regulation of cell cycle, induction of apoptosis, regulation of detoxification enzymatic system, inhibition of angiogenesis, modulation of immune system, and so forth (Finley 2005). Studies using isolated phenolics revealed different mechanisms of anticancer activity in breast cancer cells, such as induction of tumor suppressor genes p53 and p21, increment in connexin43, inhibition of fatty acid synthase, and reduction in the expression cyclins D1 and D3 (Brusselsmans et al. 2005; Mertens-Talcott et al. 2005; Calcabrini et al. 2006; Murray et al. 2006; Conklin et al. 2007). On the other hand, lycopene, a potent

![Figure 3. Pearson correlations between total phenolic compounds and antioxidant capacity measured by the FRAP (squares) or DPPH (triangles) assays for aqueous extracts of plant foods. Analyses were done (a) with and (b) without nopal (cooked and raw).](image-url)
antioxidant carotenoid, inhibits cell proliferation of breast and endometrial cancer cells through the inhibition of phosphorylation of retinoblastoma oncoprotein, reduction in cyclin D levels and retention of p27 in the cyclin E–cyclin-dependent kinase complexes (Nahum et al. 2001). Mertens-Talcott et al. (2003) showed that quercetin and ellagic acid interact synergistically in the induction of apoptosis and reduction of proliferation on MOLT-4 leukemia cells. Ellagic acid potentiates the effect of quercetin on the increase of p53 and p21 in MOLT-4 cells (Mertens-Talcott et al. 2005). Besides, Campbell et al. (2006) showed in the mouse heptocarcinoma cell line (Hpa-1c1c7) that the combinations of flavonoid aglycones quercetin, kaempferol and naringenin have a clear synergistic effect, but in human prostate cancer cells (LNCaP) the effect is not clear. Moreover, the combinations at a 25 μM dose of kaemferol–naringenin and quercetin–naringenin seem to increase cell proliferation in LNCaP cells (Campbell et al. 2006).

On the other hand, there are a few studies of synergic effects between isolated carotenoids or other compounds in cancer cells. Lu et al. (2005) showed that lutein alone at 1–8 μM did not have the same potent inhibitory effect on cell proliferation in human prostate cancer cells (LNCaP and PC-3) as avocado extract with less than 0.015 μM lutein. Besides lutein, the avocado extract contained several other carotenoids (lutein, β-carotene, α-carotene, β-criptoxanthin and zeaxanthin) and tocopherols, suggesting that these compounds and others might have contributed to the observed inhibition of prostate cancer cells. Papaya extract constitutes a very complex mixture of phytochemicals. In general, papaya is considered a good source of β-carotene and β-criptoxanthin (Breithaupt and Bamedi 2001; Perez-Carrillo and Yahia 2004), and ‘Maradol’ papaya is also rich in lycopene (Perez-Carrillo and Yahia 2004). Moreover, it has been reported that papaya contains chlorogenic acid, catechin and catechin conjugates but has few quantities of flavonols, negligible flavones or hydroxyicinnamates (Franke et al. 2004; Mahattanatawee et al. 2006). On the other hand, in our aqueous extracts the main phenolic found was gallic acid.
One of the most common criticisms of this kind of study is the large dose of phytochemicals used in cell cultures to test anticancer activity. The usual doses to test biological actions of isolated phenolic compounds in cell culture are between 6 and 50 μM, but the plasma concentration attained after a phenolic-rich meal is only 0.1–10 μM (Kroon et al. 2004). It is difficult to compare in vitro studies that use a single phenolic or a whole food extract. For example, 0.1, 10 and 50 μM quercetin, the most ubiquitous phenolic in plant foods, are equal to 0.3, 3 and 15 μg/ml. In our study, the highest dose of phenolic compounds used was 24 μg GAE/ml guava extract (4%) whereas the highest doses of black sapote, mango and papaya extracts used were 16.6, 8.5 and 7 μg GAE/ml, respectively, indicating that the 2% and 4% papaya extracts are in a supraphysiologic range. In contrast, doses between 0.01% and 1% of this extract that promoted a significant antiproliferative effect are within the physiological range (0.017–1.75 μg GAE/ml). The papaya extract used was rich in β-carotene (the highest dose used had 0.85 μg/ml), whereas circulating values of total carotenoids in women are usually in a range of 0.135–2.56 μg/ml, and concentrations of carotenoids used in in vitro studies were 0.054–10.7 μg/ml (1–10 μM) (Prakash et al. 2001; Sato et al. 2002). This suggests that papaya extract treatments provided physiological doses of carotenoids. These data agree with the hypothesis that the additive and synergistic effects of mixtures of phytochemicals in whole fruits and vegetables are responsible for their potent antioxidant and anticancer activities. Hence, it is very likely that the health benefits of fruits and vegetables are due to the complex mixtures of phytochemicals present in whole foods (Liu 2004; Lu et al. 2005; Percival et al. 2006).

Figure 5. Effect of several doses of mango and papaya aqueous extracts on cell proliferation in MCF-7 cells after (a) 24 h, (b) 48 h and (c) 72 h of treatment. Control = 0. Vertical bars, standard deviation of the mean, n = 6. ∗Significant difference (P < 0.05%) with respect to the control.
Conclusions

From 14 extracts of 13 different fruits and vegetables common in the Mexican diet, the extract derived from papaya was the one with the highest antioxidant and antiproliferative activities. It is recommended that future research investigates the active compound(s) present in the papaya extract to explore the cellular and molecular mechanisms associated with antiproliferative action, as well as to extend the observations of our work on MCF-7 cells to other neoplastic cell lines.

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