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Phenolics profile and anti-proliferative activity of *Cyphomandra Betacea* fruit in breast and liver cancer cells

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Abstract

Background: *Cyphomandra betacea* (*C. betacea*) belongs to the Solanaceae family. This study was aimed to evaluate the anti-proliferative of *C. betacea* crude extract against selected cancer cell lines (breast and liver cancer) and to identify the polyphenolics profile (phenolic acids and flavonoids) of *C. betacea* fruits. Anti-proliferative effect of the extracts was examined using MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay, followed by cell morphology analysis using acridine orange and propidium iodide double staining.

Results: The phenolics profile was characterized using high-performance liquid chromatography (HPLC). *C. betacea* extract showed a high cytotoxic effect against liver and breast cancer cell lines with the IC₅₀ value of 30 and 80 µg/ml, respectively. Phenolics profiling showed that *C. betacea* extract has a wide variety of polyphenolic compounds which are the responsible substances underlying the cytotoxic activity. The microscopic examination analysed by acridine orange and propidium iodide double staining showed that viable cells of the liver and breast cells were well rounded, large and intact green nuclei compared with the treated cells that characterized by apoptotic features (shrunken nuclei with a less quantity of cytoplasm).

Conclusion: The present study demonstrated that the anti-proliferative properties of *C. betacea* fruits were partially attributed to the rich phenolics content. It supports the hypothesis that *C. betacea* fruits have potential as an effective agent in cancer therapy.

Keywords: Anti-proliferative activity, *Cyphomandra betacea*, Breast cancer cell, Liver cancer cell, Phenolics

Background

It is known that some of the medicinal plants possess anticancer activity. In addition, polyphenols from fruits or vegetables are the responsible molecules for the chemopreventive effects. Epidemiological studies have shown that high dietary intake of polyphenols from plant is associated with a decreased risk of the degenerative diseases, including cardiovascular diseases and certain cancers (Kumar and Pandey 2013). The major dietary polyphenols are flavonoids and the most anticancer compounds of which are catechins, curcumin, resveratrol and genistein (Dai and Mumper 2010). The process of carcinogenesis includes 3 important stages; initiation, promotion and progression (Wattenberg 1985). These phenolics substances have been shown to hinder cancer

progression by interfering with each of the stage of carcinogenesis. Several studies have proposed that the anticancer ability of some dietary polyphenols such as quercetin, genistein, apigenin was depending on the inhibition of the proliferation process of various cancers in vitro and in vivo (Fantini et al. 2015).

Phenolic acids are non-flavonoid polyphenolic compounds that are often in bound form which are abundant in fruits, vegetables, grains and seeds (Tsao 2010). They are commonly represented by vanillic acid, *p*-coumaric acid, caffeic acid, ferulic acid and gallic acid. Caffeic acid has been reported to suppress the activation of NF- κ B and AP1 which might contribute to their chemopreventive effects (Bharti and Aggarwal 2002). Flavonoids are further divided into several classes which include flavones, flavanones, isoflavones, anthocyanins, flavonols, and flavanols. Moreover, the Isoflavones such as genistein and daidzein that are known for their estrogenic activity have shown a putative role in the prevention of breast cancer (Barnes 2003).

The subtropical fruit *C. betacea* belongs to the Solanaceae family (Lim 2013). Commonly known as tamarillo, tree tomato or 'buah cinta' among the locals. *C. betacea* is considered as an undervalue fruit in Malaysia, which is grown in Cameron Highland (Peninsular Malaysia) and Kundasang (Sabah). Several of underutilized fruits are reported to be rich in bioactive compounds which make them good potential sources for the production of nutraceuticals, flavors and pharmaceuticals (Ali Hassan and Abu Bakar 2013). However, these types of fruits are often underutilized due to their unidentified features, and economical prospective. Hence, these underutilized fruits provide unlimited opportunities for using as a potential phytomedicine as they are known to possess an array of chemical diversity, which needs to be investigated. There is a limited knowledge about the phytochemical profiles, antioxidant and anticancer potential of *C. betacea*. Hence the present study has been aimed to understand the antioxidant activities and antiproliferative effects of *C. betacea*. It has been reported that *C. betacea* is rich in β -carotene and ascorbic acid which makes them good natural sources of provitamin A and vitamin C. The fruits also having rich contents of anthocyanin and carotenoids which are responsible for their colour due to its natural pigments and both demonstrate the important biological, therapeutic, and preventative properties (de Rosso and Mercadante 2007).

Previous study reported that *C. betacea* displayed higher antioxidant properties than *Lycopersicon esculentum* (cherry tomato) and *Solanum lycopersicum* (tomato). The total phenolic content of *C. betacea* was 7.63 mg gallic acid equivalent/g (Noor Atiqah et al. 2014). Various research groups have screened a variety of fruit for their antioxidant activity but none appear to have included *C. betacea*. Thus, in the present study we aimed to determine the antiproliferative activity of *C. betacea* fruits against breast and liver cancer lines and also to study the polyphenol distribution.

Methods

Fruit sampling

The fruits of *C. betacea* were collected from Cameron Highland, Pahang, Malaysia, during June 2013. The herbarium specimens were identified and deposited in BORNEENSIS, University Malaysia Sabah, Malaysia. The fruits were cleaned and weighed. The small cut pieces were freeze-dried, and ground into fine powder using a grinder. The

ground samples were sieved to get uniform size and then kept in an air-tight container and stored in a freezer ($-20\text{ }^{\circ}\text{C}$) until further analysis.

Sample preparation

Fine powders (2.5 g) were extracted with 50 ml 80% ethanol. The dry residue (50 mg) was dissolved in DMSO (1 ml) to obtain working solution of 1 mg/ml. These final extracts were filtered through a $0.45\text{ }\mu\text{m}$ nylon membrane syringe filter before use.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

The human tumour cell lines HepG2 (liver), MDA-MB-231(breast) and 3T3 (normal mouse fibroblast) cell lines were obtained from the American Type Culture Collection (ATCC, VA, USA). Cells were routinely grown with RPMI-1640 media supplemented with 10% fetal calf serum and 1% penicillin/streptomycin and incubated at $37\text{ }^{\circ}\text{C}$ humidified atmosphere of 5% CO_2 in T75 (75 cm^2) flasks. The potential effects on cell viability were investigated using the MTT assay as an indicator of metabolically active cells. The cells were diluted in culture medium to a concentration of 1×10^5 cells/ml and were pipetted into 96-well plates and incubated at $37\text{ }^{\circ}\text{C}$ humidified, 5% CO_2 incubator atmosphere to attach for 24 h. Cells were then exposed with *C. betacea* extracts (6.25, 12.5, 25, 50, 100, 200 $\mu\text{g/ml}$) through twofold serial dilutions (Kaewpiboon et al. 2014). Cells were also treated with doxorubicin (0.20, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25 $\mu\text{g/ml}$) that served as a positive control and culture medium (1% DMSO) was used as a negative control. After 72 h of extracts and drug exposure, the culture medium was removed and 10 μl of MTT reagent was added. Following incubation for 4 h, the MTT/medium was removed and DMSO (100 μl) was added to dissolve the formazan crystals. Absorbance of the colored solution was measured on an ELISA plate reader. Percentage of cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{\text{OD sample}}{\text{OD negative control}} \times 100\%$$

All experiments were performed in triplicate. The concentration of substance required for 50% growth inhibition (IC_{50}) was estimated as that giving a 50% decrease in absorbance as compared to controls incubated simultaneously without substances.

Determination of apoptosis by acridine orange/propidium iodide (AO/PI) staining

HepG2 and MDA-MB-231 cancer cell lines were seeded into 6 well plate at a density of 1×10^5 cells/ml and incubated for 24 h at $37\text{ }^{\circ}\text{C}$ in 5% CO_2 incubator. After 24 h, cells were treated with *C. betacea* extract (IC_{50} values), doxorubicin (8 $\mu\text{g/ml}$) that served as positive control and culture medium (1% DMSO) that served as negative control for 24, 48 and 72 h. After exposure of grown cells in the extract, cells were washed with phosphate buffer saline (PBS) and harvested by centrifugation (2000 rpm for 5 min) and washed twice with PBS. The pellet (10 μl) were mixed with 5 μl (10 $\mu\text{g/ml}$) acridine orange and 5 μl (10 $\mu\text{g/ml}$) PI and observed under fluorescence microscope (Ekowati et al. 2010).

DNA ladder assay

Fragmentation of chromatin DNA is one of the hallmarks of apoptosis. The quality of DNA was obtained with DNA laddering kit following manufacturer's instructions Cayman Chemical (MI, USA). HepG2 and MDA-MB-231 (1×10^5 cells/ml) cancer cells were seeded into 6-well plate and incubated for 24 h. After 24 h, HepG2 and MDA-MB-231 cells were treated with 30 and 80 $\mu\text{g/ml}$ of *C. betacea* extracts, doxorubicin (8 $\mu\text{g/ml}$) and culture medium (1% DMSO) that served as negative control. After 72 h exposure of grown cells in the extract, cells were washed with phosphate buffer saline (PBS) and harvested by centrifugation at 2000 rpm for 5 min at room temperature (25 °C). The supernatant was discarded and pellet (1×10^5 cells) in 200 μl of samples volume was mixed with 100 μl of Lysis Buffer and vortexed for 10 s. Then, the mixture was centrifuged at 8000 rpm for 1 min at room temperature. The supernatant was collected and mixed with 20 μl of 10% SDS for each sample and vortexed again before added 20 μl of Enzyme A. Samples were vortexed and incubated at 56 °C for 1 h. Then, 20 μl of Enzyme B was added to each sample and incubated at 37 °C for another 1 h. Precipitating reagent (130 μl) and ice-cold ethanol (950 μl) were then added to each tube. Samples were vortexed and centrifuged at 12,000g for 5 min at 4 °C. After centrifugation, supernatant was discarded and pellet was washed once with ice-cold 80% ethanol. Pellets were then dried on the bench for 5–10 min. TE buffer (100 μl) was added in each sample by vigorous vortexing. Samples (15 μl) were mixed with 3 μl of Gel-Loading Buffer (6 \times) and electrophoresed on a 1% agarose gel pre-stained with ethidium bromide at 75 V for 60 min. The fragmented inter-nucleosomal DNA was visualized using FluorChem 5500 Chemiluminescent (Alpha Innotech, CA, USA).

Determination of polyphenol by HPLC

HPLC apparatus: The polyphenolic composition of the *C. betacea* fruits were determined by using HPLC–DAD (Agilent 1100, CA, USA). A reversed-phase C18 column (Nova-Pak, 150 mm \times 4 mm, 5 μm) from Waters (MA, USA) was used throughout this study. All reagents were obtained from Merck (Darmstadt, Germany) HPLC grade. The prepared mobile phase was degassed using ultrasonic agitation and filtered under vacuum through a 0.45 μm nylon membrane filter before analysis. The rate flow was set at 1 ml/min. The operating temperature was maintained at room temperature (25 °C).

Analysis of phenolic acids and flavonoids: The polyphenolic composition of the *C. betacea* fruits was determined according to method as described by Uddin et al. (2014) with slight modification. Samples (1 g) were mixed with 4 ml of 80% ethanol for 24 h. The mixture was later centrifuged (5000 rpm for 15 min) at room temperature (25 °C). The supernatant was filtered through 0.45 μm nylon membrane filter prior use. The mobile phase was composed of (A) 0.5% acetic acid, and (B) methanol, and the gradient elution was performed as follows: 0 min, 100:0; 20 min, 10:90; and 30 min, 100:0 and filtered under vacuum through 0.45 μm membrane filter before use. The detection and quantification of gallic acid and caffeic acid was done at 254 nm, chlorogenic acid, epigallocatechin gallate, vanillic acid, p-coumaric acid and naringin was done at 280 nm, trans-ferullic acid and ferullic acid at 329 nm while quercetin and kaempferol were detected at 370 nm.

Standards: All standards were dissolved in 80% ethanol and prepared between the range of 20 and 100 µg/ml. All standards used were of the highest purity (HPLC grade) were obtained from Sigma (Darmstadt, St. Louis), were filtered through 0.45 µm nylon membrane syringe filter before injections.

Statistical analysis

Data are presented as mean ± S.E.M and all experiments were carried out with three replicates. The data was statistically analysed using SPSS version 21.0. One-way analysis of variance (ANOVA) with Tukey's test was used to test for differences between multiple groups. Level of significance was set at $p \leq 0.05$. Independent-Samples *T* Test was used to test for significant differences in phenolic compounds of both extracts which was considered at the level of $p \leq 0.05$.

Results and discussions

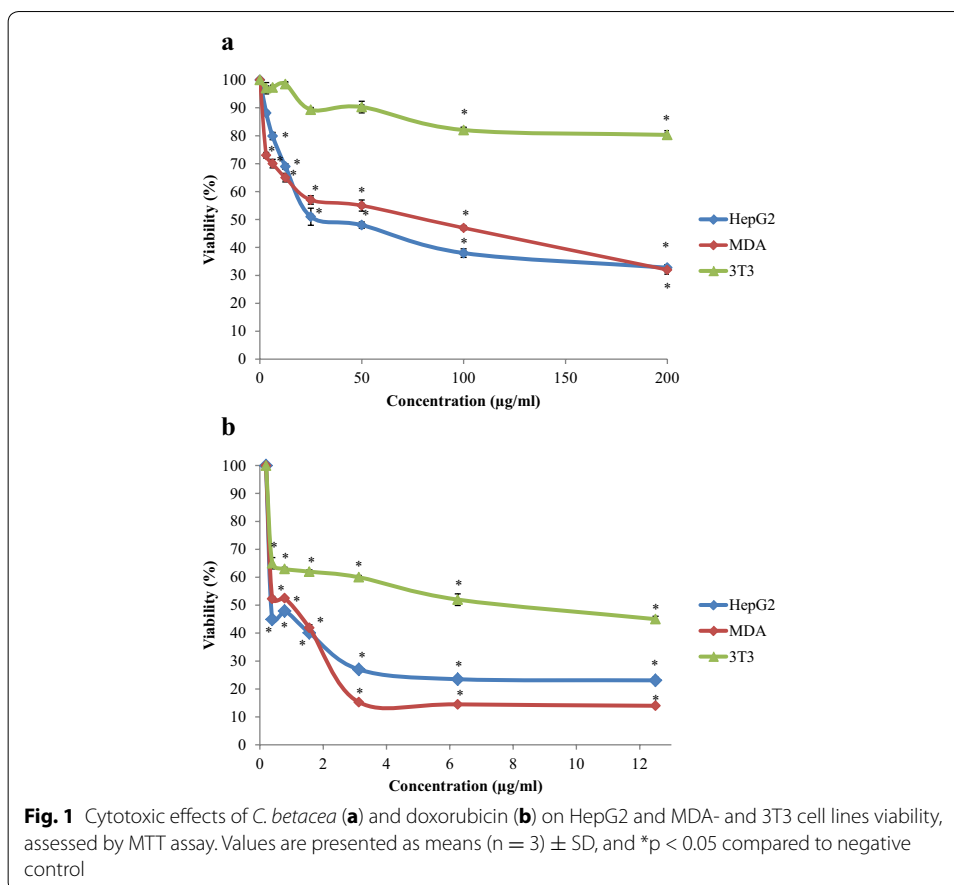
Cytotoxicity activity

The cytotoxic behaviour of *C. betacea* fruits were tested in two different human cancer cell lines: liver (HepG2) and mammary gland (MDA-MB-231) adenocarcinoma. The experiments were also carried out in normal mouse fibroblast (3T3) to investigate its toxicity towards normal cells. The endpoint criteria for antiproliferative activity was selected according to the cytotoxic activity of IC_{50} values that should be below 100 µg/ml for crude extracts and less than 25 µM for pure compounds (Cos et al. 2006). Figure 1a shows that *C. betacea* extract strongly inhibited the proliferation of HepG2 and MDA-MB-231 cell lines in a dose-dependent manner with IC_{50} values of 30 and 80 µg/ml, respectively. It was verified that the increased in concentration of *C. betacea* fruits leads to a higher cytotoxic activities. However all the sample extracts did not exert any significant cytotoxic effect against 3T3 cell ($IC_{50} > 200.00$ µg/ml). Doxorubicin (chemotherapy drug) was capable to induce cytotoxicity in HepG2 and MDA-MB-231 with IC_{50} value of 0.35 and 0.78 µg/ml respectively, as shown in Fig. 1b. Nevertheless, results also proved that doxorubicin showed superior cytotoxic activity against 3T3 normal cell with IC_{50} value of 8 µg/ml (Fig. 1b).

Previous study reported that the cancer preventing action of fruits and vegetables is most probably due to the several bioactive compounds that act simultaneously to prevent cancer rather than being one or two potent anticarcinogens (Tomás-Barberán and Gil 2008). As chemopreventive agents, phytochemicals can interfere in different step of carcinogenesis (promotion, initiation and progression) by two major actions; cancer blocking agents and cancer suppressing agent (Surh 2003). Some chemopreventive phytochemicals inhibit the metabolic action of the pre-carcinogens, consequently block the tumor initiation.

AO/PI double staining and fluorescent microscopy

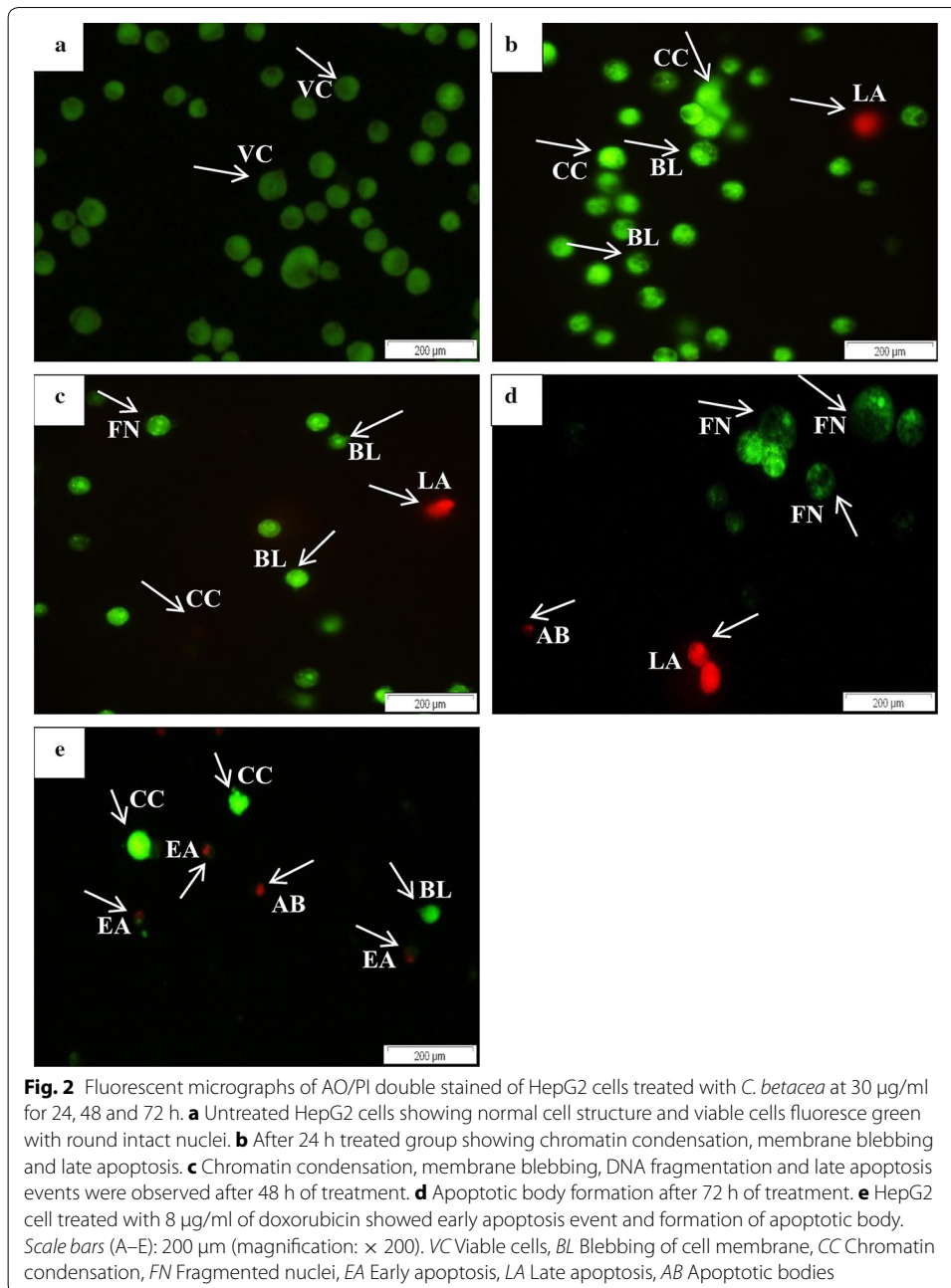
Staining cells with fluorescent dyes is useful in evaluating the nuclei morphology of apoptotic cells and mode of cell death. Apoptotic and viable cells of HepG2 and MDA-MB-231 were evaluated using fluorescent microscope. Healthy viable cells of HepG2 and MDA-MB-231 were visualized well rounded, large and intact green nuclear structure (Figs. 2a, 3a) while observations of cells treated with *C. betacea* extract based on their



IC₅₀ values were identified shrinking with condensed fragmented nuclei that confirmed apoptosis in a time dependent manner (Figs. 2b–d, 3b–d). Early apoptosis features (Figs. 2e, 3e) were seen for HepG2 and MDA-MB-231 treated with 0.35 and 0.78 µg/ml of Doxorubicin, respectively. AO penetrated within the fragmented DNA (FN) and displayed nuclear condensation that indicates moderate apoptosis. Late stage of apoptosis (LA) showed the presence of apoptotic bodies (AB) observed as orange-red fluorescence represents the hallmark of late apoptosis (Figs. 2b–d, 3c, d). In addition, cells were noticeable with asymmetrically localized, membrane blebbing (BL) and nuclear margination (CC) as can be seen in Figs. 2b–e and 3b–e for HepG2 and MDA-MB-231, respectively.

DNA laddering

Detection of DNA fragmentation become a widely accepted biochemical hallmark of apoptotic cytotoxicity and drug induced apoptosis in cancer cells (Kroemer et al. 1995). Figure 4 shows the DNA fragmentation which was detected in HepG2 (lane b and c) and MDA-MB-231 (Lane D and E) cancer cell lines treated with *C. betacea* extract based on their IC₅₀ and IC₇₅ values after 72 h incubation. However, there is no DNA



fragmentation was observed in control (untreated) cells as shown in Lane D and Lane G for HepG2 and MDA-MB-231 cells, respectively. Conversely, the standard doxorubicin induced the fragmentation of the DNA at very low concentration (8 µg/ml) as shown in Lane H and Lane I for HepG2 and MDA-MB-231 cells, respectively. These results indicate that cytotoxic activity is present in *C. betacea* and exerted substantial DNA damage leading to apoptotic cell death in HepG2 and MDA-MB-231 cancer cell lines.

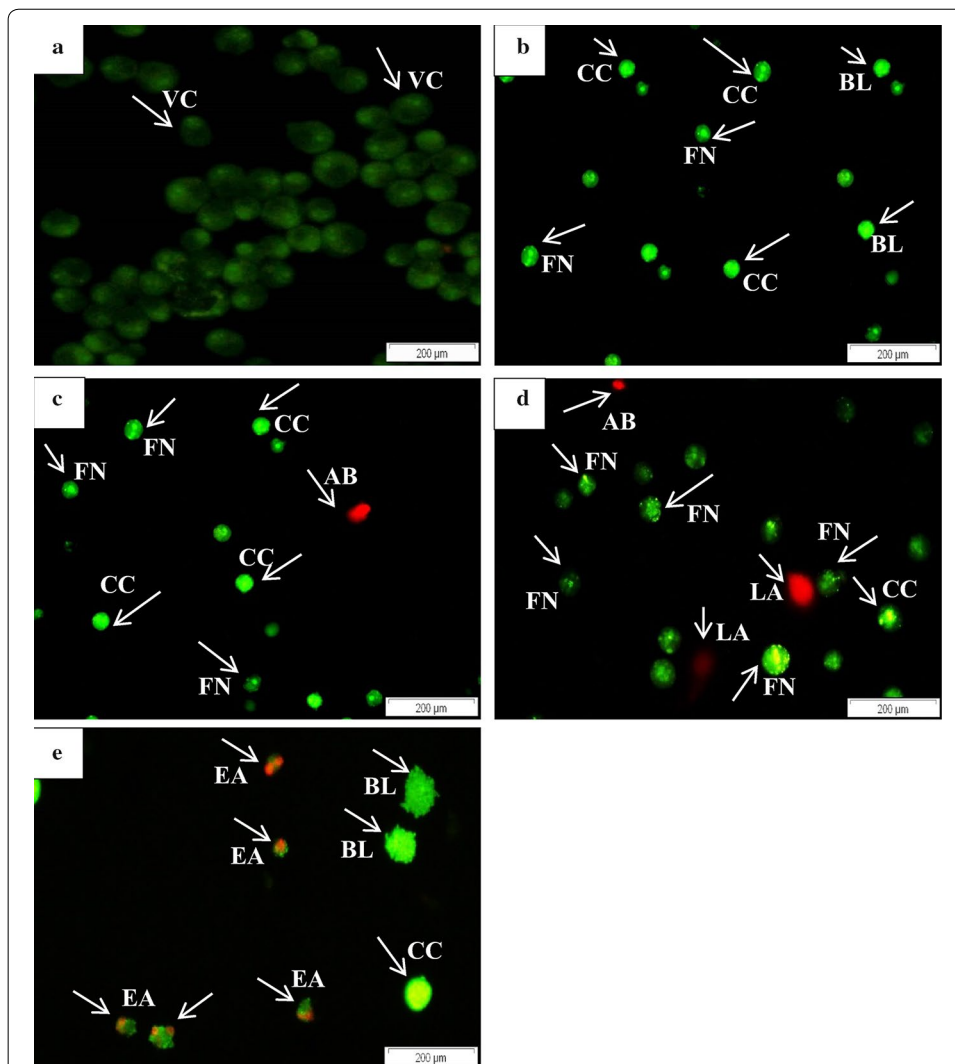
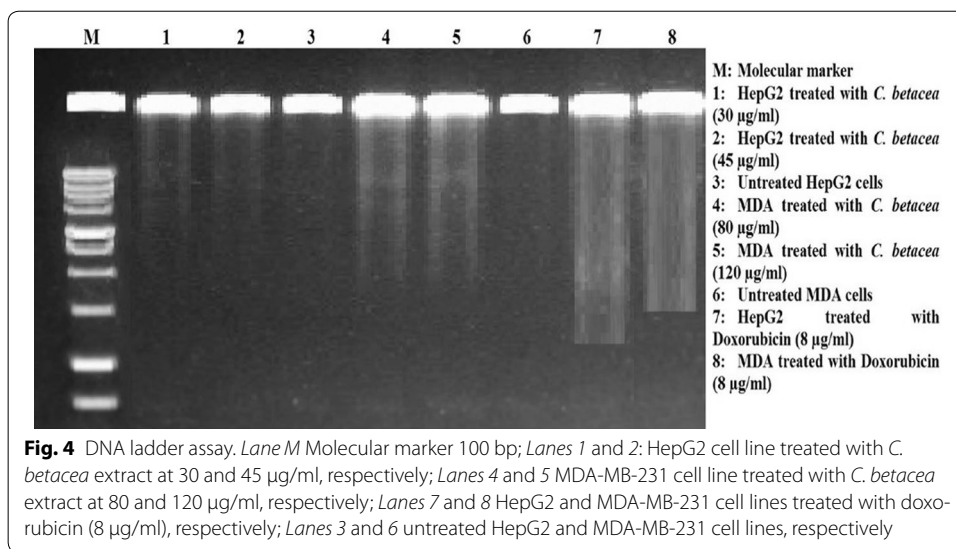


Fig. 3 Fluorescent micrographs of AO/PI double stained of MDA-MB-231 cells treated with *C. betacea* at 80 µg/ml for 24, 48 and 72 h. **a** Untreated MDA-MB-231 cells showing the normal viable cells with circular nucleus uniformly distributed in the centre of the cell. **b** Blebbing or signs of membrane protrusions and fragmented of nuclei were evident after 24 h of treatment. Nucleus showed green fluorescence and concentrated into a granular that indicates chromatin condensation. **c** 48 h treated cells detected as rounding off, reduction in cell size, damaged membrane and appearance of apoptotic body. **d** Necrotic cells: 72 h treated cells showed late apoptosis events with formation of apoptotic body, unsymmetrical shape and size, uneven and disintegrated membrane surface. **e** MDA-MB-231 cell treated with 8 µg/ml of doxorubicin showed early apoptosis events with appearance of membrane blebbing and chromatin condensation. Scale bars (**a–e**): 200 µm (magnification: × 200). VC Viable cells, BL Blebbing of cell membrane, CC Chromatin condensation, FN Fragmented nuclei, EA Early apoptosis, LA Late apoptosis, AB Apoptotic bodies

Phytochemical compositions

Phenolic acids composition

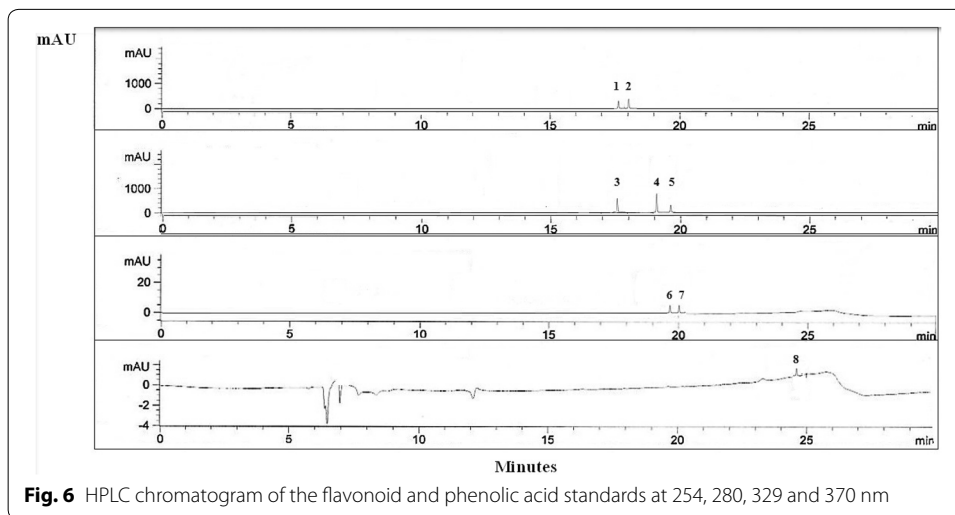
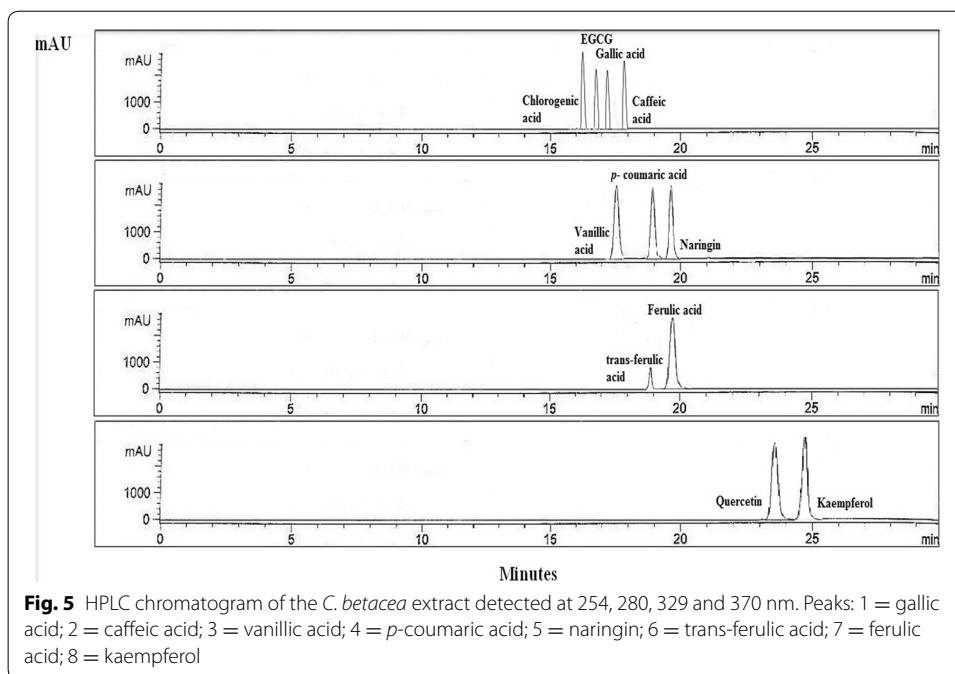
The major secondary metabolites in plants include terpenoids, alkaloids, sulphur-containing compounds and phenolic compounds (Dillard and Bruce 2000). The main phenolic subclass in fruits are phenolic acids (hydroxybenzoic and hydroxycinnamic acid), flavonoids, and coumarin (Hounsoume et al. 2008). Phenolic acids in general are phenols



with one carboxylic acid group. Hydroxybenzoic acids (vanillic and gallic acid) have the carboxylic acid group directly attached to the ring, while hydroxycinnamic acids (*p*-coumaric acid, caffeic acid, ferulic acid, *trans*-ferulic acid and chlorogenic acid) have a three-carbon side chain. Phenolic compounds have been reported to possess antioxidants, antibacterial, anticancer and anti-inflammatory properties (Ghasemzadeh et al. 2010).

Figure 5 illustrates the separation of flavonoids and phenolic acids in *C. betacea* extract, while Fig. 6 shows the mixture of 11 external standards for flavonoids and phenolic acids. A good separation was achieved in a short separation time of 26 min. Phenolic acid contents achieved from *C. betacea* extract were presented in Table 1. Out of seven phenolic acids tested, gallic, caffeic and vanillic acids were found to be most abundant in the samples tested. While ferulic, *p*-coumaric and *trans*-ferulic acids were found in minor quantity in the samples tested ranged from 0.05 to 0.49 µg/g DW. Caffeic and gallic acids display a wide variety of biological functions including their primary antioxidant activity, which are mainly related to the modulation of carcinogenesis. In addition, gallic acid has been proven to protect against oxidative damage induced by reactive oxygen species (ROS) and act as chemopreventive agents that cause apoptosis in the proliferation of several tumour cell lines. For an instance, Lim (2013) has reported that *C. betacea* prevented oxidative stress-induced cell death in HepG2 cells in a dose-dependent manner.

The genus of *Solanum* (Solanaceae) can be an important source of natural antioxidants, particularly the phenolic acid compositions. Some of these phytochemicals include 2-methyl[1,3,4]oxadiazole, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one and thiazole that may have the role in anti-inflammatory effects while n-hexadecanoic acid may be responsible in the antioxidant activities. The other compound identified was 1,3,4-Oxadiazole (OXD) products that are well-known for their anti-inflammatory, (Dr. Duke's phytochemical and ethnobotanical databases), antibacterial, antifungal activity (El-Emam et al. 2004) and HIV replication inhibition (Sahin et al. 2002). Furthermore,



thiazole was also reported previously to have anticancer properties which suggest the potential of the medicinal activities of tamarillo fruits. It was reported that the hydroxycinnamic acid derivatives is dominant in the peel and pulp of *C. betacea* (Vasco et al. 2009). Noor Atiqah et al. (2014) found that *C. betacea* contain considerable high mean of total phenolic content (7.63 ± 0.37 mg GAE/g). The same study also reported there was also a positive correlation existed between antioxidant activity and total phenolic content of *C. betacea* which suggest that the predominant source of antioxidant potential of *C. betacea* derives from the polyphenolic compounds present in the fruits. Overall, the

Table 1 Phenolic acid compositions ($\mu\text{g/g DW}^a$) of *C. betacea*

Samples	$\mu\text{g/g DW}^a$	RT (min)	Area (mAU)
Ferulic	0.05	19.630	31.44
<i>p</i> -coumaric	0.41 \pm 0.47	19.578	38.86
Caffeic	1.65 \pm 0.72	17.677	28.25
Vanillic	1.11 \pm 1.82	17.682	15.99
Trans-ferulic acid	0.49 \pm 0.51	19.622	32.35
Gallic	3.02 \pm 1.36	17.676	26.99

^a Dry weight; data are presented in mean \pm S.E.M ($n = 3$); N.D. - not detected

data suggested that *C. betacea* may be a good source of antioxidant compounds in nutraceutical or functional food products.

Flavonoids composition

Flavonoids are the most diverse group of secondary metabolite that involve in plant growth, reproduction, seed germination and protection against pathogen (Agati et al. 2012). The structural subclass of flavonoids depend on the modifications of the C-ring and they can be divided into; flavonol (quercetin, rutin and kaempferol), flavone (apigenin, luteolin), flavan-3-ol (epicatechin, catechin), anthocyanin (delphinidin and cyaniding), isoflavone (genistein and diazein) and flavanone (hesperetin and naringenin). Flavonoids are the most studied phenolic compounds, due to their biological effects including antioxidant, antimutagenic, anticarcinogenic, and antibacterial properties (Kumar and Pandey 2013). The amount of selected polyphenols (flavonols and flavanones) in *C. betacea* were reported in Table 2.

Flavonols

Flavonols compound such as quercetin and kaempferol in *C. betacea* were examined and shown in Table 2. We detected kaempferol at lower levels with the values of $0.5 \pm 0.80 \mu\text{g/g DW}$ in *C. betacea* however, quercetin was not detectable. Tokuşoğlu et al. (2003) reported that kaempferol levels in *S. esculentum* (tomato) which share the same genus with *C. betacea* were identified in the range between $0.2\text{--}0.6 \mu\text{g/g FW}$, which were in accordance to our findings. Food derived flavonoids particularly flavonols (kaempferol, quercetin, and myricetin) are reported to exhibit multiple biological functions such as antioxidant, anti-allergenic, anti-inflammatory, cardio-protective and vasodilatory effects. In addition to all these activities, flavonols also has strong anticancer

Table 2 Flavanoids composition ($\mu\text{g/g DW}^a$) of the fruits

Samples	$\mu\text{g/g DW}^a$	RT (min)	Area (mAU)
Flavonols			
Quercetin	N.D.	N.D.	N.D.
Kaempferol	0.5 \pm 0.80	24.693	13.17
Flavanones			
Naringin	3.32 \pm 1.50	19.582	37.95

^a Dry weight; data are presented in mean \pm S.E.M ($n = 3$); N.D. - not detected

activity on several cancer cells such as leukaemia, breast, ovarian, gastric and liver (Joshi et al. 2011).

Flavanones

Table 2 shows the amount of flavanones (naringin) present in *C. betacea* fruits with the values of 3.32 ± 1.50 $\mu\text{g/g}$ DW. Flavanones accounted for 90-98% of flavonoids in citrus fruits such as sweet oranges, lemons, limes and grapefruit (Fowler and Koffas 2009). Flavanones have been demonstrated to induce cytotoxic activity toward various human cancer cell lines with little effect on normal cells. Patil et al. (2009) found that lime juice rich in hesperidin inhibits human pancreatic cancer cell, while Harmon and Patel (2004) reported that the regular intake of flavanones inhibits proliferation of breast cancer cell. This suggests that *C. betacea* fruit with moderate amount of flavanones content making them ideal candidates in developing the potential flavonoid-based chemotherapeutics for anticancer treatment.

Conclusions

In conclusion, the present study demonstrated that the crude extract of *C. betacea* possessed antiproliferative activity against HepG2 and MDA-MB-231 cancer cell lines, and more importantly did not exerted an inhibitory effect on the proliferation of the normal cells (3T3). The profiling of phenolic acids and flavonoids by HPLC-DAD-MS has identified some of the major polyphenols that are likely to contribute to the chemopreventive activity. Previous study has found that *C. betacea* exhibited high antioxidant activity largely derived from polyphenolic, flavonols and anthocyanins compounds. It can be concluded from this study that *C. betacea* has a surprisingly wide range of beneficial properties including antioxidant and anticancer properties and has good potential to be further developed in nutraceutical or functional-food products.

Abbreviations

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); HPLC: high performance liquid chromatography; IC_{50} : concentration to inhibit cellular proliferation by 50%; DMSO: dimethyl sulfoxide; HepG2: liver hepatocellular carcinoma; MDA: homo sapiens mammary gland; 3T3: normal mouse fibroblast; CO_2 : carbon dioxide; ELISA: enzyme-linked immunosorbent assay; OD: optical density; PBS: phosphate buffered saline; PI: propidium iodide; HPLC-DAD-MS: high-performance liquid chromatography-diode array detection-mass spectrometry; SPSS: statistical package for the social sciences; ANOVA: analysis of variance; AO/PI: acridine orange/propidium iodide; AO: acridine orange; DNA: deoxyribo nucleic acid; GAE: gallic acid equivalent; ROS: reactive oxygen species; FW: fresh weight; DW: dry weight; EGCG: epigallocatechin 3-gallate; SEM: standard error of the mean.

Authors' contributions

AR, FO and RR made substantial contributions to conception, design, and acquisition of data. MAM contributed for the analysis, interpretation of data and revised the manuscript. FA was reviewed and edited the manuscript. All authors participated in the initial draft of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

- Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. *Plant Sci* 196:67–76
- Ali Hassan SH, Abu Bakar MF (2013) Antioxidative and anticholinesterase activity of *Cyphomandra betacea* fruit. *Sci World J* 2013:1–7
- Barnes S (2003) Phyto-oestrogens and osteoporosis: What is a safe dose? *Br J Nutr* 89:S101–S108
- Bharti AC, Aggarwal BB (2002) Nuclear factor- κ B and cancer: its role in prevention and therapy. *Biochem Pharmacol* 64:883–888
- Cos P, Arnold JV, Dirk VB, Louis M (2006) Anti-infective potential of natural products: How to develop a stronger in vitro 'proof of concept'. *J Ethnopharmacol* 106:290–302
- Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15:7313–7352
- de Rosso VV, Mercadante AZ (2007) HPLC-PDA-MS/MS of anthocyanins and carotenoids from dovalis and tamarillo fruits. *J Agric Food Chem* 55:9135–9141
- Dillard CJ, Bruce GJ (2000) Phytochemicals: nutraceuticals and human health. *J Sci Food Agric* 80:1744–1756
- Dr. Duke's Phytochemical and Ethnobotanical Database (2014) <http://www.ars-gov/duke/>. Accessed 19 Jan 2015
- Ekowati H, Astuti I, Mustofa M (2010) Anticancer activity of Calanone on HeLa cell line. *Indonesian J Chem* 10:240–244
- El-Ermam AA, Al-Deeb OA, Al-Omar M, Lehmann J (2004) Synthesis, antimicrobial, and Anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones. *Bioorg Med Chem* 12:5107–5113
- Fantini M, Benvenuto M, Masuelli L, Frajese GV, Tresoldi I, Modesti A, Bei R (2015) *In vitro* and in vivo antitumoral effects of combinations of polyphenols, or polyphenols and anticancer drugs: perspectives on cancer treatment. *Int J Mol Sci* 16:9236–9282
- Fowler ZL, Koffas MAG (2009) Biosynthesis and biotechnological production of flavanones: current state and perspectives. *Appl Microbiol Biot* 83:799–808
- Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules* 15:4324–4333
- Harmon AW, Patel YM (2004) Naringenin inhibits glucose uptake in MCF-7 breast cancer cells: a mechanism for impaired cellular proliferation. *Breast Cancer Res Treat* 85:103–110
- Hounscome N, Hounscome B, Tomos D, Edwards-Jones G (2008) Plant metabolites and nutritional quality of vegetables. *J Food Sci* 73:R48–R65
- Joshi UJ, Gadge AS, D'Mello P, Sinha R, Srivastava V, Govil G (2011) Anti-inflammatory, antioxidant and anticancer activity of quercetin and its analogues. *IJRPBS* 2:1757–1766
- Kaewpiboon C, Winayanuwattikun P, Yongvanich T, Phuwapraisrisan P, Assavalapsakul W (2014) Effect of three fatty acids from the leaf extract of *Tiliacora triandra* on P-glycoprotein function in multidrug-resistant A549RT-eto cell line. *Pharmacogn Mag* 10:S549–S556
- Kroemer G, Petit P, Zamzami N, Vayssi re JL, Mignotte B (1995) The biochemistry of programmed cell death. *FASEB J* 9:1277–1287
- Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013:1–16
- Lim TK (2013) Fruits: edible medicinal and non-medicinal plants, vol 6. Springer, New York, pp 326–332
- Noor Atiqah AAK, Maisarah AM, Asmah R (2014) Comparison of antioxidant properties of tamarillo (*Cyphomandra betacea*), cherry tomato (*Solanum lycopersicum* var. cerasiform) and tomato (*Lycopersicon esulentum*). *Int Food Res J* 21:2355–2362
- Patil JR, Chidambara Murthy KN, Jayaprakasha GK, Chetti MB, Patil BS (2009) Bioactive compounds from Mexican lime (*Citrus aurantifolia*) juice induce apoptosis in human pancreatic cells. *J Agric Food Chem* 57:10933–10942
- Sahin K, Kucun O, Sahin N, Sari M (2002) Effects of vitamin C and vitamin E on lipid peroxidation status, some serum hormone, metabolite and mineral concentrations of Japanese quail, reared under heat stress (34 °C). *Int J Vitam Nutr Res* 72:91–100
- Surh YJ (2003) Cancer chemoprevention with dietary phytochemicals. *Nat Rev Cancer* 3:768–780
- Tokuşođlu O, Unal MK, Yildirim Z (2003) HPLC-UV and GC-MS characterization of the flavonol aglycons quercetin, kaempferol, and myricetin in tomato pastes and other tomato-based products. *Acta Chromatogr* 13:196–207
- Tom s-Barber n FA, Gil MI (eds) (2008) Improving the health-promoting properties of fruit and vegetable products. Woodhead, London
- Tsao R (2010) Chemistry and biochemistry of dietary polyphenols. *Nutrients* 2:1231–1246
- Uddin R, Saha MR, Subhan N, Hossain H, Jahan IA, Akter R, Alam A (2014) HPLC-analysis of polyphenolic compounds in *Gardenia jasminoides* and determination of antioxidant activity by using free radical scavenging assays. *Adv Pharm Bull* 4:273–281
- Vasco C, Avila J, Ruales J, Svanberg U, Kamal-eldin A (2009) Physical and chemical characteristics of golden-yellow and purple-red varieties of tamarillo fruit (*Solanum betaceum* Cav.). *Int J of Food Sci Nutr* 60:278–288
- Wattenberg LW (1985) Chemoprevention of cancer. *Cancer Res* 45:1–8