

# Effect of the Water Extracts of Avocado Fruit and Cherimoya Leaf on Four Human Cancer Cell Lines and *Vicia faba* Root Tip Cells

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## Abstract

The main objective of this study is to determine the effect of the water extract of *Persea americana* Mill. (avocado fruit) and *Annona cherimolia* Mill (cherimoya leaf) on living cells. The antiproliferative properties of avocado fruit water extract (AFWE) and cherimoya leaf water extract (CLWE) were determined using four human cancer cell lines: lung (A549), liver (HepG-2), colon (HT-29) and breast (MCF-7). Cancer cells were incubated with 100 µg/ml of AFWE or CLWE for 48 hours, then the cell viability was measured using colorimetric tetrazolium cleavage test (MTT). Both extracts resulted in more than 90% mortality in treated cells. *Vicia faba* root tip assay was used to determine the effect of AFWE and CLWE on mitotic index (MI), Micronuclei (MN) formation rate and chromosomal abnormalities. *Vicia faba* roots were soaked in 100, 1250, 2500, 5000, 10000, 20000 µg/ml of AFWE or CLWE for 4 and 24 h. AFWE and CLWE were mitodepressive and resulted in a significant decrease of MI in a dose dependant manner. CLWE treatment resulted in a decrease in prophase cell percentage and an increase in MN & chromosomal abnormalities. On contrary, the prophase cell percentage was linearly increasing with the applied concentration without micronuclei formation in avocado treatment. Our results strongly indicate that avocado and cherimoya extracts were highly cytotoxic and mitodepressive on cancer and plant cells, respectively.

**Keywords:** *Annona cherimolia*, *Persea americana*, micronuclei, mitotic index, *Vicia faba* root assay

## 1. Introduction

The diverse plant kingdom is gaining global recognition as unique and renewable resource for the discovery of phytochemicals that may represent pharmacological active compounds (Am, Al-Malki, Refai, Kumosani, & Moselhy, 2013). Phytochemicals are used in many aspects such as food supplements (Abrahim, Kanthimathi, & Abdul-Aziz, 2012; Adebajo et al., 2012), immunity enhancers (Bayrami & Boskabady, 2013; Benmebarek, Zerizer, Laggoune, & Kabouche, 2013; Buapool et al., 2013), wound healers (Chandra, Yadav, Mani, Ghosh, & Sachan, 2013), cancer preventive and therapeutic agents (Bhagat, Sharma, & Saxena, 2012). Cancer prevention through the diet can be considered as an effective tool to improve public health and reduce the cost for healthcare (Haniadka, Popouri, Palatty, Arora, & Baliga, 2013). According to Newman et al. (2007), 40% of all anticancer drugs are developed from natural products while 20% are synthetic derived from natural ones. However, the detection of an active compound with a biological activity of interest in about 250000-500000 unexplored plant species is similar to find a needle in a haystack (Rates, 2001).

The study of natural bioactive compounds in plants has required the development of bioassay techniques. It is not always possible to test against cancer in animal models due to the system complexity. In vitro methods allow the screening of large numbers for cytotoxicity against many types of cancer cell lines and usually require less test material, time and money (El-Menshawi et al., 2010).

Plant bioassays that measure mitotic cell cycle, micronuclei induction rate and the frequency of chromosomal aberrations are both time and cost effective. These tests are helpful in screening the bioactivity of plant extracts at large scale, particularly in areas with limited funds. They will also eliminate the hazards of using cultured human cells and experimental animals. Plant bioassays are mainly used in most ongoing research to determine the genotoxic effect of chemical compounds rather than testing their pharmaceutical properties.

Root tip meristems of *Vicia faba* (broad beans) have been used as a pioneer cytogenetic material for the detection of genotoxicity in many studies (Dong & Zhang, 2010; Ma, Cabrera, & Owens, 2005). This bioassay was validated by the International Programme on Chemical Safety (IPCS, WHO) and the United Nations Environment Program (UNEP). As far as the authors are aware, *Vicia faba* root tip assay had not been used for screening natural plant extracts for their anticancer potential.

*Persea americana* Mill. (avocado) is an economically important tropical tree belonging to family Lauracea. From phytochemical perspective, extracts of the plant yield functionalized alkanols known as aliphatic acetogenins (Kawagishi et al., 2001). Avocado is used in treating tumor in ethnomedicine and exhibits a chemoprotective effect on human cells (Paul, Kulkarni, & Ganesh, 2011).

*Annona cherimolia* Mill. (Cherimoya) is a subtropical tree that belongs to family Annonaceae. Cherimoya showed a significant cytotoxic potential in pancreatic, mammary, prostatic, and kidney cancer cells (Alali, Liu, & McLaughlin, 1999). Members of this family gained more interest due to the presence of the secondary metabolite acetogenins. Acetogenins can be used as antiparasitic, antimicrobial, antimicotic, immunosuppressive, cytotoxic and antitumorous agents (Garcia-Aguirre, Zepeda- Vallejo, Gallegos, Alvarez-Gonzalez, & Madrigal-Bujaidar, 2008).

Avocado and cherimoya are known to possess stronger cytotoxic effect in tumorous than in normal cells. Some studies showed that plant and animal cells exhibited similar responses towards treatments with bioactive compounds (Konuk, Liman, & CİĞERCİ, 2007). To determine if this is the case, we tested the cytotoxic effect of avocado fruit water extract (AFWE) and cherimoya leaf water extract (CLWE) on four human cancer cells and meristematic plant cells. The bioactivity of the water extracts of avocado and cherimoya had never been tested on human cancer cell lines or plant cells elsewhere. Therefore, this study will be very helpful in highlighting the activities of both extracts and also to compare between the efficiency of the two bioassays in determining their bioactivity. For this aim, the anticancer properties of ALWE and CLWE were determined using four human cancer cell lines: A549 (human lung carcinoma), HePG2 (human liver carcinoma), HT29 (human colon carcinoma) and MCF-7 (human breast carcinoma). The genotoxic effects of the two extracts were determined by measuring the mitotic index (MI), micronuclei (MN) formation rate and chromosomal abnormalities in *Vicia faba* root tip cells.

## 2. Materials and Methods

### 2.1 Plant Materials

Fresh samples of *Persea americana* fruit and *Annona cherimoli* leaves were obtained from El Orman Botanical Garden (Giza, Egypt). The plants were identified by the herbarium officer of El Orman Garden: Mrs Teriz Labib. Voucher specimens were kept in the herbarium, Botany Department, Faculty of Science, Ain Shams University, under the name of Noha-Dina (2009). Leaves were collected at approximately similar age (the third fully expanded leaves from the top of each branch) and fully ripened dry fruits of *P. americana* were used. Seeds of *Vicia faba* (cultivar: Windsor white) were kindly provided by the Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt and was used as a reference model in the root tip bioassay.

### 2.2 Preparation of Extracts

Water extracts were prepared by dissolving 2 grams of fresh material in 100 ml of 90°C distilled water, incubated at the same temperature for 15 min, cooled, filtered and used as a stock (2000000 µg/ml). Water extracts were prepared and used the same day of collection to avoid any change in its activity.

### 2.3 Cell Culture

A549 (human lung carcinoma), HePG2 (human liver carcinoma), HT29 (human colon carcinoma) and MCF-7 (human breast carcinoma) were obtained from Karolinska Institutet, Stockholm, Sweden. All cells were maintained in RPMI 1640 (Lonza Biowahittkar) medium except for MCF-7 cells which were maintained in DMEM medium (Lonza Biowahittkar). All the media were supplemented with 1% antibiotic-antimycotic mixture (10.000 U/ml Potassium Penicillin, 10.000 µg/ml Streptomycin Sulfate and 25 µg/ml Amphotericin B and 1% L-glutamine. All antibiotics and L- glutamine were purchased from Biowest, France.

### 2.4 MTT Viability Assay

The viability of cancer cell lines was determined after the exposure to water extracts for 48 h using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described in Mosmann (1983).

### 2.5 Root Tip Preparations and Treatment

*Vicia faba* dry seeds were rinsed and then soaked in distilled water for 24 h. Germinating seedlings were kept at 25°C on moist gauze until their primary roots were 1-2 cm in length (Cotelle, Masfaraud, & Férard, 1999). Root

tips were soaked for 4 or 24 h in different concentrations of water extracts (100, 1250, 2500, 5000, 10000, 20000 µg/ml). Distilled water was used as negative control. All treatments were done in triplicates at temperature of  $25 \pm 2^{\circ}\text{C}$ .

### 2.6 Cytological Study and Slide Preparation

Root-tips were cut directly into Carnoy's solution [absolute alcohol: glacial acetic acid (3:1)] and incubated at room temperature for 24 h. Roots are then hydrolyzed in 1N analar HCl at 58-60°C for 10 min (Qian, 2004). Root tips- 3 mm long- were squashed and stained according to Darlington and La-Cour (1976) Leuco-basic Fuchsin technique. Light green dye (0.3%) was used for background staining of the cytoplasm. Root tips were squashed in 45% acetic acid. Dehydration was done using ascending series of ethyl alcohols; 30%, 50%, 70%, 96%, absolute alcohol; absolute alcohol: xylene (1:1) and xylene, keeping root tips for 5 min in each concentration. Preparations were mounted in Canada balsam and placed at 45°C in the oven (until completely dry). Root tips were examined for micronuclei frequencies and other abnormalities at 4000x magnification using Leica light microscope. The photomicrographs were taken from the prepared slides using digital camera (Sony, 8Mp).

### 2.7 Mitotic Indices (MI) were Calculated Using the Following Formula:

$$\text{Mitotic index (MI)} = \frac{\text{Number of dividing cells} \times 100}{\text{Total cells examined}}$$

### 2.8 Statistical Analysis

Data shown are the means and standard errors of three or more independent experiments. Statistical comparisons between groups were made by Student's t-test using Microsoft excel program, and a P-value<0.05 considered to be statistically significant.

## 3. Results

### 3.1 Cytotoxic Effect of Plant Extracts on Cancer Cell Lines

Avocado fruit water extract (AFWE) exhibited strong cytotoxic effect against all cancer cell lines used in this study (Table 1). The extract resulted in lethal percentages of 93.3%, 98.3%, 97.8% and 91.7% in A-549 HepG-2 HT-29 and MCF-7 human cancer cell lines, respectively. The cytotoxic effect of avocado extract was more pronounced as compared to that of cherimoya leaf water extract (CLWE) in HepG-2 and HT-29 cancer cell lines. The cytotoxic concentrations of the two extracts were also tested over a range of dilutions on the four cancer cell lines to determine their LC<sub>50</sub> (Table 2 and Figure 1). Both *P. americana* and *A. cherimolia* water extracts showed LC<sub>50</sub> value less than 30 µg/ml in HepG-2 cell lines with LC<sub>50</sub> =13.3 & 10 µg/ml and HT-29 cell line with LC<sub>50</sub>= 13.3 & 16 µg/ml, respectively.

Table 1. In vitro cytotoxic activity of crude methanolic extracts tested against human carcinoma cell lines (A549, HepG-2, HT-29 & MCF-7) after 24 hours

Plant name	Mean A-549	Mean HepG-2	Mean HT-29	Mean MCF-7
Persea americana fruit	93.3	98.3	97.8	91.7
+ve control ( <i>Annona Cherimolia</i> )	95	94	93	94
DMSO	0	0	0	0
-Ve control	0	0	0	0
Blank	-	-	-	-

Table 2. LC<sub>50</sub> values related to MTT assay of the water extract of avocado fruit and cherimoya leaf on human cancer cell lines. Values are in µg/ml

Cell line	Lethal concentration (LC <sub>50</sub> )	
	Avocado fruit water extract (AFWE)	Cherimoya Leaf Water extract (ALWE)
A-549	35.4	9.8
HepG-2	13.3	10
HT-29	22	9.7
MCF-7	54.5	6.5

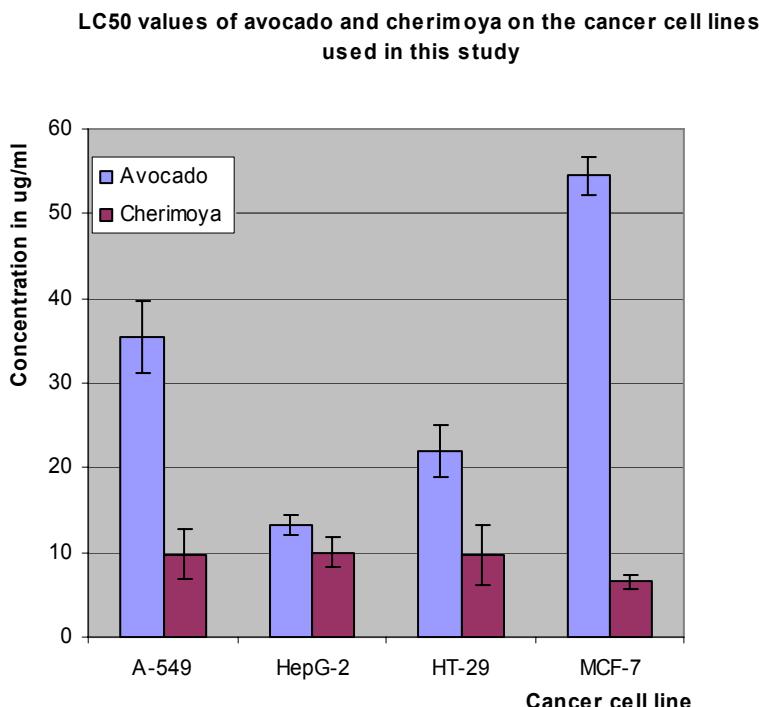


Figure 1. LC<sub>50</sub> of the water extract of *Persea americana* fruit and *Annona cherimolia* leaves in relation to the four cancer cell lines

### 3.2 The Effect of Plant Extracts on Mitotic Indices

As shown in Table (3), we observed a decrease in the mitotic indices of root tip cells of *Vicia faba* in a dose-dependant manner in all plant extracts used. Differences in mitotic indices between the treated plants and the untreated control were significant at the highest concentrations of 10000 and 20000 ppm, applied for 4 h, for all treatments applied. In 24 h treatments, the mitotic indices were significantly reduced as compared with control at concentrations of 5000, 10000 and 20000 ppm.

Table 3. The effect of plant extracts on the mitotic index of *Vicia faba* L.

	Plant extract	Persea americana		Annona cherimolia	
		Conc. µg/ml	Number of cells observed	MI(X ± SE)	Number of cells observed
4 hours	Control				
	100	3360		2.98 ± 0.57	3078
	1250	4200		2.86 ± 0.39	3850
	2500	4117		2.77 ± 0.49	4077
	5000	3640		1.92 ± 0.68	4428
	10000	4200		1.50 ± 0.61*	3630
24 hours	20000	4944		1.03 ± 0.14**	3376
	Control				
	100	3516		8.08 ± 0.68	3033
	1250	3450		7.68 ± 0.90	4015
	2500	4322		6.94 ± 0.77	4115
	5000	4200		4.29 ± 0.33 **	3321
	10000	4023		3.11 ± 0.38**	3130
	20000	3697		2.11 ± 0.24 **	3900

Note: \* P<0.05, \*\*P<0.001 as compared to the untreated control plants.

### 3.3 Micronuclei Formation Rate of *Vicia faba* Root Tip Cells

The water extract of *A. cherimolia* was able to induce micronuclei formation (Table 4). In plants treated with higher concentrations (10000, 20000 ppm) of *A. cherimolia* water extract, we observed more than one micronucleus per cell (Figure 2a) with the formation of nuclear bud (Figure 2b). Interestingly, no micronuclei were detected in the root cells of *Vicia faba* plants treated with all the used concentrations of *P. americana* either for 4 or 24 h (Table 4).

Table 4. Effect of plant extracts on micronucleus formation rate in *Vicia faba* root tip cells

Time	Treatment	Micronuclei formation rate (%)	
		<i>Persea americana</i>	<i>Annona cherimolia</i>
4 hours	Control	---	---
	100	---	1.68
	1250	---	1.86
	2500	---	2.12
	5000	---	2.83
	10000	---	3.23
24 hours	20000	---	3.40
	Control	---	---
	100	---	1.79
	1250	---	1.93
	2500	---	2.03
	5000	---	2.45
	10000	---	3.37
	20000	---	3.80

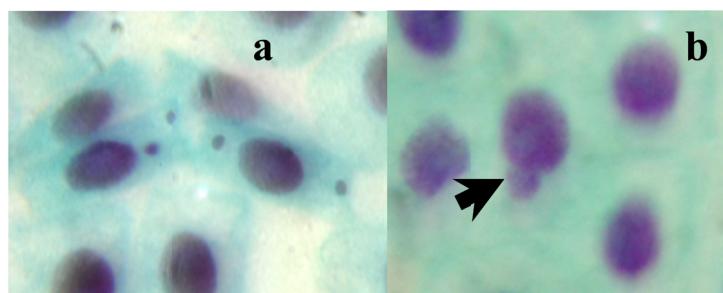


Figure 2. Effect of *A. cherimolia* water extract on micronuclei formation rate. Micronuclei were linearly increasing in a dose dependant manner (a) Two micronuclei per cells and (c) nuclear bud were observed at higher concentrations

### 3.4 Chromosome Aberration Rate of *Vicia faba* Root Tip Cells

Root tip cells treated with the plant extract of *P. americana* exhibited the least chromosomal abnormalities as compared with the corresponding control. This result applies for both treatment duration used (4 or 24h). The most common abnormalities were stickiness (Figure 3a) in plants treated with the water extract of *P. americana* fruit at relatively high concentrations. On the other hand, Chromosome laggards (Figure 3b) and disturbed metaphase chromosomes (Figure 3c), in addition to stickiness, are the most common abnormalities detected in plants treated with the leaf water extract of *A. cherimolia*.

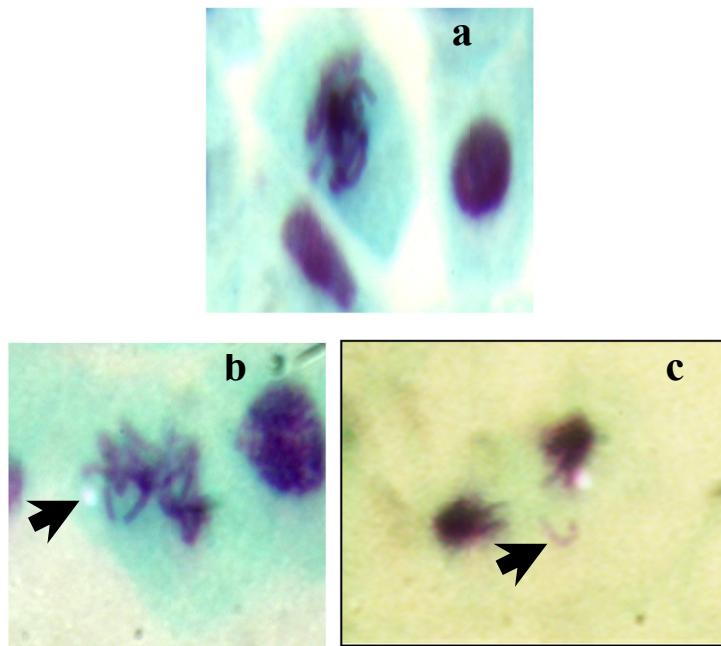


Figure 3. Chromosomal abnormalities induced upon treatments with the water extracts under study. Representative micrograph pictures showing the most common abnormalities. (a) Chromosomal stickiness is commonly observed in plants treated with the highest concentrations of *P. americana* and most of the concentrations of *A. cherimolia* (b) Disturbed metaphase and (c) chromosomal laggards were only observed in plants treated with the water extract of *A. cherimolia* leaves

### 3.5 Percentage of Different Mitotic Phases

As shown in Figure 4, the percentage of prophase cells were decreasing in a dose dependant manner in plants treated with *P. americana* or *A. cherimolia* water extracts for 4 h. The same is true for *A. cherimolia* treated plants for 24 h. On the other hand, the percentage of prophase cells was increasing linearly with the concentration applied in plants treated with *P. americana* for 24 h (Figure 4b). The metaphase and ana-telophase percentages were increasing as well in plants treated with CLWE for 24 h, while they decreased in plants treated with AFWE for 24 h.

Table 5. The percentage of normal and abnormal mitosis in plant cells treated with the water extracts of *P. americana* and *A. cherimolia* at different concentrations

Time	Plant extract	<i>Persea americana</i>			<i>Annona Cherimolia</i>		
		Conc. µg/ml	Normal Metaphase (%)	Anaphase (%)	Abn. Mitosis (%)	Normal Metaphase (%)	Anaphase (%)
4 hours	Control	26.40	23.58	1.88 ± 0.61	26.40	23.58	1.88 ± 0.61
	100	25.00	25.00	3.00 ± 0.92	29.25	20.75	3.92 ± 0.60
	1250	24.17	23.33	4.17 ± 0.69	27.05	25.41	5.74 ± 1.85
	2500	26.32	26.32	4.39 ± 1.18	29.06	28.20	6.84 ± 2.43
	5000	28.57	25.71	5.71 ± 1.95	36.84	25.26	8.40 ± 2.47*
	10000	30.16	23.80	7.94 ± 2.60*	39.00	23.73	16.95 ± 1.32**
	20000	33.33	23.53	13.73 ± 1.35**	40.00	26.00	26.19 ± 1.4**
hours 24	Control	24.30	27.50	1.60 ± 0.57	24.30	27.50	1.60 ± 0.18
	100	24.65	26.76	2.46 ± 0.46	22.45	27.76	1.63 ± 0.143
	1250	24.15	26.42	3.77 ± 1.00	28.67	26.88	7.17 ± 3.03
	2500	25.67	22.67	5.00 ± 1.91	33.85	24.62	10.40 ± 3.38*
	5000	20.56	23.89	7.78 ± 2.66	45.16	17.42	23.23 ± 0.83**
	10000	20.00	21.60	16.00 ± 1.41**	41.18	18.49	36.13 ± 1.2**
	20000	20.51	21.79	24.36 ± 0.55**	47.50	19.17	45.83 ± 1.45**

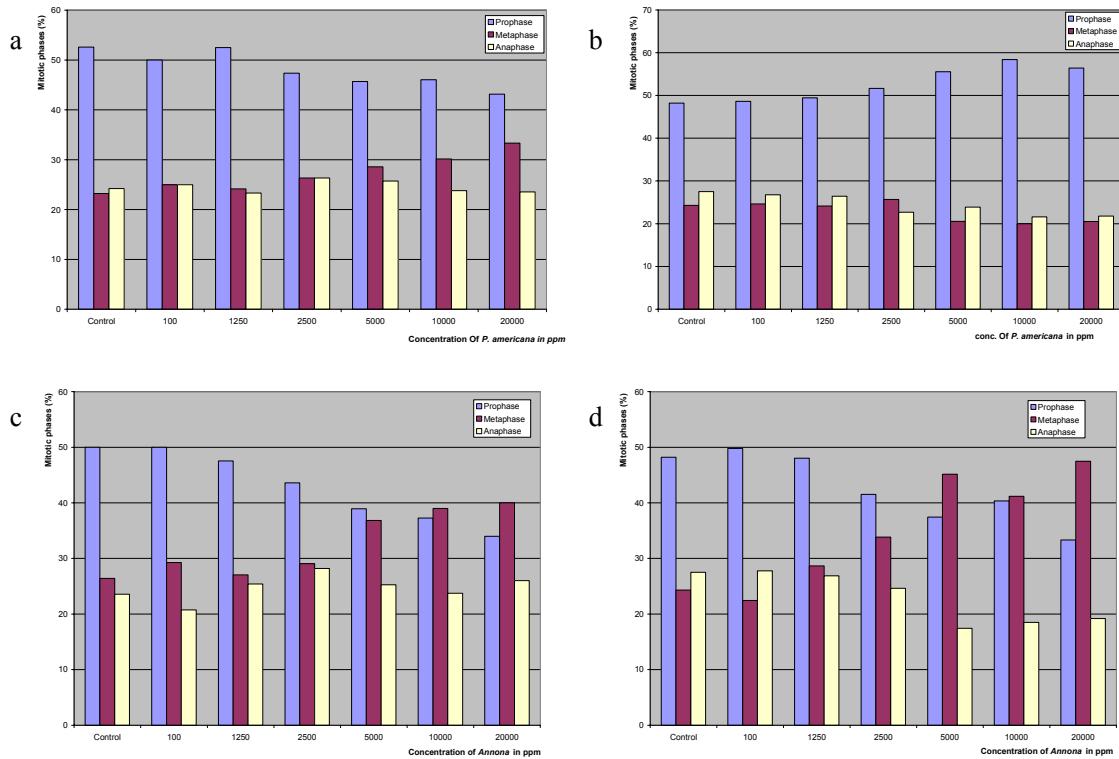


Figure 4. Mean values of different mitotic phases of treated and untreated *V. faba* root tip cells. Effect of water extract of *P. americana* (a): for 4 and (b): 24 h. Effect of the water extract of *A. cherimolia* leaves (c): for 4 and (d): 24 h

#### 4. Discussion

Many studies, including our own, showed that phytochemicals extracted from *Persea americana* (avocado) and *Annona cherimolia* (cherimoya) can selectively induce cell cycle arrest, inhibit growth and enhance apoptosis in some cancer cell lines other than those used here (Ambrosio, Chunhua, Li, Kinghorn, & Ding, 2011; Ding, Chin, Kinghorn, & D'Ambrosio, 2007; Garcia-Aguirre et al., 2008). For instance, in vitro studies had shown that the acetone extract of avocado fruit inhibited the growth of prostate cancer cells (Lu et al., 2005) while its chloroform extract inhibited the growth of oral cancer cells (Ding et al., 2009). Fractions from the ethanol extract of cherimoya inhibited the growth of human colon cancer and increased the micronuclei formation in mice erythrocytes (Garcia-Aguirre et al., 2008). However, the effect of avocado fruit water extract (AFWE) and cherimoya leaf water extract (ALWE) have not been reported before either on cancer or plant cells, as far as the authors are aware of. This prompted us to test the properties of AFWE and ALWE in order to mimic the natural way of their consumption by humans. This was carried out using two bio-screeing approaches: 1- cancer cell MTT and 2- *Vicia faba* root tip - bioassays.

In order to measure their cytotoxic properties, Four cancer cell lines [lung (A549), liver (HepG-2), colon (HT-29) and breast (MCF-7)] were incubated with 100 µg/ml of AFWE or CLWE for 48 h. The Viability of cancer cells was measured using MTT assay. Both extracts resulted in more than 90% growth inhibition in all cancer cell lines as shown in Table 1. This indicates that AFWE and CLWE posses the ability to arrest the growth of cancer cells used in this study. To determine the concentration at which AFWE and ALWE can inhibit 50% of cancer cell growth, we treated all cell lines with series of diluted concentrations and measured cell viability using MTT assay. LC<sub>50</sub> values are shown in Table 2 for both extracts. Avocado and cherimoya water extract exhibited low LC<sub>50</sub> = 13.3 & 10 µg/ml in HepG-2 and LC<sub>50</sub> = 22 & 16 µg/ml in HT-29 cell lines, respectively. Therefore, avocado and cherimoya represent very promising sources for anticancer drugs at least for liver and colon cancers.

In the second approach, we used plant system to determine the effect of AFWE and CLWE on mitotic index (MI), micronuclei (MN) formation rate and chromosomal abnormalities. MI, MN and chromosomal abnormalities are well recognized markers to measure the mitodepressive and genotoxic properties of a test substance using plant cells. To date, there are no published data assessing the effect of avocado or cherimoya on *Vicia faba* root tip cells. Roots of *Vicia faba* plants were treated with 100, 1250, 2500, 5000, 10000, 20000 µg/ml of AFWE or ALWE for 4 and 24 h. We have noticed a decrease in mitotic index values within root-tips treated with AFWE or ALWE, as compared with the untreated control (Table 3). This decrease was more pronounced in case of AFWE treatments (Table 3). This indicates that both extracts are mitodepressive. Mitodepressive effect had been assumed to result from the inhibition of cells access to mitosis (Badr & Ibrahim, 1987) which is likely attained by preventing DNA biosynthesis or / and microtubule and chromatin organization (Yüzbaşıoğlu, Aœnal, Sancak, & Kasap, 2003). This will lead into a slower progression of cells from S (DNA synthesis) phase to M (mitosis) phase of the cell cycle (Blakemore, Boes, Cordell, & Manson, 2013).

The percentages of different mitotic phases (Prophase, metaphase, and ana-telophase) were decreasing in a dose dependant manner in AFWE (4 h) and CLWE (4 and 24 h) treated plants (Figures. 1-a, c & d). When plants treated with AFWE for 24 h, the prophase cell percentage was progressively increasing in a dose dependant manner (Figure 1b). Thus, it can be assumed that AFWE may have arrested cell division via interfering with DNA biosynthesis rather than affecting other stages in cell cycle.

The induction of micronuclei (MN) had been used in many studies as an indicator of genotoxicity (Cavas & Ergene-Gozukara, 2005). In this work, the genotoxic effect of AFWE and CLWE was determined using the frequency of micronuclei (MN) formation. This will give us an idea about the safety of using these extracts in cancer therapies in future applications. We observed that the decrease in the mitotic index was associated with a significant increase in micronuclei (MN) formation rate in ALWE treated plants at all concentrations used (Table 4). We also observed the formation of two micronuclei per cell (Figure 2 a) and nuclear bud (Figure 2 b) at higher concentrations of CLWE (10000 and 20000 µg/ml). Interestingly, no micronuclei were detected in plants treated with all concentrations of AFWE.

Chromosomal stickiness was the only abnormality observed at a significant level in AFWE treated plants. This gives an indication that the mitodepressive effect of avocado is more potent to an extent that it may had prohibited further cell division. On contrary, the clastogenic effect of cherimoya included chromosomal stickiness, bridges, laggards and disturbed metaphase. In this connection, the presence of micronuclei is commonly associated with structural and numerical chromosomal aberrations induced by clastogenic agents that cause chromosomal breaks and aneugenic agents that disturb microtubule (Bellini et al., 2006; Bellini et al., 2006; Benfenati et al., 2009; Dufour, Kumaravel, Nohynek, Kirkland, & Toutain, 2006). This result suggests that both extracts interfered with the cell cycle. It can also be assumed that CLWE may be affecting chromatin and microtubule organization as indicated by micronuclei formation and chromosomal breaks. The mode of action of AFWE and CLWE as Mitodepressive agents needs further investigation.

## 5. Conclusion

This study highlighted the value of avocado fruit and cherimoya leaves to be used as promising sources for anticancer drugs. Both extracts showed potent cytotoxic activity toward cancer cells. However, avocado should be given more attention in this respect due to less chromosomal abnormalities associated with its treatment as compared to cherimoya.

This study can also be used to compare between the efficiency of MTT cancer cell viability and root tip assay in determining the cytotoxic properties of a plant extract toward cancer cells. Our results indicated that both bioassays can lead into the same conclusion. Thus, *Vicia faba* root tip assay can be used as an initial screening step particularly when large number of extracts is involved. The initial screening can then be followed by extra analysis for the promising extracts to determine the effective dose, selectivity, ..... etc using animal models and human cell lines. When it comes to plant bioassay, we found that reduction in mitotic index may indicate the cytotoxic effect of a plant extract on cancer cells. However, MN frequency and chromosomal abnormalities should be considered to validate the bio safety of the extract under investigation.

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