

CYTOTOXICITY OF FIVE PLANT EXTRACTS AGAINST DIFFERENT HUMAN CANCER CELL LINES AND THEIR MOLECULAR MECHANISMSALWA EL-HALLOUTY^{1*}, ASHWAQ BATAWI², MANAL SHAFI², ESAM RASHWAN³, EZZELDIN ELHAWARY⁴, NESMA ABDELAAL⁵

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ABSTRACT

Objective: Five methanol plant extracts namely, *Euphorbia hierosolymitana*, *Dracaena marginata*, *Cordyline terminalis*, and *Sapium sebifera* were screened *in vitro* for their cytotoxic effect on human tumor cell lines; namely, PC-3, HepG-2, HCT-116, and MCF-7 (prostate, liver, colon, and breast cancer) and human normal cell lines, namely, BJ-1.

Methods: *E. hierosolymitana* (extract A) selectively inhibited HCT-116 cell proliferation with IC₅₀ of 4.22 µg/ml, both *Dracaena marginata* (extract B) (bark, leaves, and branches) showed moderate cytotoxicity on MCF-7 with IC₅₀ of 22.4 and 48.2 µg/ml, respectively. The remaining two extracts showed minimal to insignificant percentage inhibition on all cell lines.

Results: *E. hierosolymitana* extract was selected for further studying, where the effect of the extract on the HCT116 cells showed a downregulation of her2 and Bcl-2 gene and overexpression of the Bax gene. Flow cytometry analysis was also performed to understand the effect of *E. hierosolymitana* on the apoptosis induction and the cell cycle. The results showed the induction of early and late apoptosis by 5.81 and 10.01%, respectively.

Conclusion: Our results infer that *E. hierosolymitana* has a promising chance in fighting colorectal cancer due to its high cytotoxic effects on HCT116 cancer cells while having minimal effects on the BJ-1 normal cell lines.

Keywords: Anticancer, MTT, Apoptosis, Human Cancer Cell lines, and Plant extract.

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INTRODUCTION

The natural environment has been a source of medicinal agents for thousands of years and is still being used for treating various types of diseases. At present, there are a remarkable number of drugs that have been isolated from natural plant species. *Euphorbia hierosolymitana* (extract A) is a member of the *Euphorbiaceae* (spurge) family. *Euphorbiaceae* family members have been used in alternative medicine for skin disorders, diarrhea, dysentery, and venereal diseases [1].

Dracaena marginata is a species of the *Dracaena* genus which is commonly used as ornamental and medicinal plants. Extract D and extract E are *Dracaena Marginata* (bark) and *Dracaena Marginata* (leaf and branch) respectively. Plants like *D. marginata* alongside with other *Dracaena* species are effective in decreasing the concentration of the volatile organic compound in the air [2]. *Cordyline fruticosa* (extract C) is commonly known as Hawaiian Ti plant. It is an arborescent (tree-like structure) monocotyledon which is classified into the *Agavaceae* family. The leaves were reported to contain tyramine, it is predicted to cause an increase in the systolic blood pressure if ingested in high amounts [3].

Sapium sebifera (extract B) which is also known as *Triadica sebifera* and commonly known as the Chinese tallow tree is a native plant of South and Central China with high fertility. It is a member of the *Euphorbiaceae* family, which is a complex heterogeneous family with around 322 genera and 8900 species [4]. Cancer is characterized by out-of-control cell growth with the potential to invade or spread to other parts of the body. Apoptosis is controlled by a very complex, energy-requiring series of events. There are three main apoptotic pathways: Extrinsic or death receptor pathway, intrinsic or mitochondrial pathway, and

T-cell-mediated cytotoxicity which involves perforin-granzyme-dependent killing [5].

Bcl-2 has been shown to have anti-apoptotic effects by blocking the release of cytochrome c while, in turn, reduce the activity of caspase-9, while Bax (Bcl-2-associated X protein) is a pro-apoptotic protein that belongs to the Bcl-2 family. The overexpression of the Bax results in the promotion of cell death, in other words, the balance between the Bcl-2 and Bax determines the cell fate. However, the mechanism of the Bax is not always related to Bcl-2. Cells, where the Bax gene was knocked out, were still susceptible to apoptosis. However, cells with double-knockout Bax^{-/-}/Bak^{-/-} were almost resistant to all apoptotic stimuli [6].

METHODS

All the plants were randomly collected from different regions of Egypt. The plant samples, herbs from *Euphorbia hierosolymitana* (extract A) branches of *Sapium sebifera* (extract B) leaves and branches of *Cordyline fruticosa* (extract C); bark of *Dracaena marginata* and leaves and branches of *Dracaena marginata* (extracts D and E), were dried in solar ovens at 40°C then ground into powder. The powder was thoroughly extracted with 95% methanol. The extracts were then freeze-dried and stored at -20°C until needed [7].

Cancer cell lines

In this study, we used human breast adenocarcinoma cell line (MCF-7), the hepatocellular carcinoma cell line (HepG-2), the human colon carcinoma cell line (HCT-116), the human prostate adenocarcinoma cell line (PC-3), and a normal human cell line (BJ-1).

These cell lines were obtained from Karolinska Institute, Department of Oncology and Pathology (Stockholm, Sweden).

Cell preparation

MCF-7, HCT116, PC3, and BJ1 cells were maintained in DMEM medium (Dulbecco's Modified Eagle's Medium, Lonza, Basel, Switzerland). While RPMI medium (in case of HepG2) (Gibco, Gergy Puloisa, France) was used instead of the DMEM and supplemented with 10% fetal bovine serum at 37°C in 5% CO₂ and 95% humidity, cells were subcultured using Trypsin-Versene 0.15%. Skin normal human cell line (BJ-1) immortalized normal foreskin fibroblast cell line was kindly provided by Professor Stig Linder, Oncology and Pathology department, Karolinska Institute, Stockholm, Sweden.

MTT assay

The antiproliferative effects of the plant extracts were tested *in vitro* on the five cell lines, MCF-7, HepG-2, HCT-116, PC-3, and BJ-1, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [8]. The cells were cultured in 96-well plates, where each well contains 1 × 10⁵ cells. A 100 ppm final concentration of the extracts was added in triplicates. The cells were incubated for 48 h. Doxorubicin was used as positive control and 0.5% DMSO was used as a negative control. Cytotoxicity was determined using MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay, as described by Mosmann, 1983 [9].

The media were discarded and 40 µl of MTT (Bio Basic, New York, USA) was added then the plates were incubated for 3–4 h in 5% CO₂ incubator. After the incubation, 140 µl of 1% sodium dodecyl sulfate (SDS) (solubilizing reagent) (ADWIC, Egypt) was added to each well and left for 20 min. The color formed was then measured at 595 nm, on a microplate reader [10]. Using different concentrations ranging from 100, 50, 25, 12.5, 6.25, and 3.125 ppm [11], the IC₅₀ was calculated using the software program SPSS (Statistical analysis software package, v9, Chicago, USA).

$$\text{Percentage inhibition} = 100 - (\text{abs. of treated cells} / \text{abs. of untreated cells}) \times 100.$$

RNA isolation

The effect of the plant extract A (*Euphorbia hierosolymitana*) on the mRNA expression of the Bcl-2, ERBB2/her2, and Bax was determined by a reverse transcription-polymerase chain reaction (RT-PCR) technique. After the treatment (48 h) [12] of the HCT116 cells with the extract, RNA was extracted from the cells using the RNeasy extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Concisely, up to 1 × 10⁶ of the cultured cells were trypsinized, washed with PBS, and collected in an RNase-free centrifuge tube. The sample was then centrifuged at 300 ×g for 5 min, and the supernatant was aspirated off. The pellet was disrupted and homogenized in RLT buffer (buffer RLT is a lysis buffer for lysing cells and tissues before RNA isolation and simultaneous RNA/DNA/protein isolation).

The homogenized lysate was then transferred into a 2 ml collection tube, and 1 ml of 70% ethanol was added to the lysate and mixed

with pipetting. Then, 700 µl of the sample was transferred into an RNeasy spin column placed in a 2 ml collection tube and centrifuged at 10,000 rpm for 15 sec. The flow-through was discarded, and 700 µl of the RW1 buffer (buffer RW1 is a proprietary component of RNeasy kits. Buffer RW1 contains a guanidine salt, as well as ethanol, and is used as a stringent washing buffer that efficiently removes biomolecules such as carbohydrates, proteins, and fatty acids) was added to the spin column and centrifuged at 10,000 rpm for 15 s.

The previous step was repeated 2 times using 500 µl of the RPE buffer (buffer RPE is a mild washing buffer and a proprietary component of RNeasy kits. Its main function is to remove traces of salts, which are still on the column due to buffers used earlier in the protocol) instead of the RW1 buffer to wash the spin column membrane. The collection tube was discarded, and a new 1.5 ml tube was used instead, 40 µl of RNase-free water was added to the center of the membrane and centrifuged at 10,000 rpm for 1 min. The spin column was discarded as the collection tube contains the eluted RNA.

Gene expression analysis

After the RNA extraction, one-step RT-qPCR was performed using iScript™ One-Step RT-PCR kit with SYBR® Green (Bio-Rad Inc., CA, USA) which was added to the Master Mix which contained 25 µl 2x SYBR Green®, 1.5 µl forward primer (10 µM), 1.5 µl reverse primer (10 µM), 11 µl nuclease-free water, and 1 µl ×50 iScript Reverse Transcriptase in a final reaction volume of 50 µl. Two control reactions were created; one lacking the RNA template and the other lacking the reverse transcriptase enzyme.

The one-step RT-qPCR reaction included an incubation step with the complete reaction mix, iScript Reverse Transcriptase enzyme inactivation step, qPCR step (denaturation, annealing, and extension), and dissociation step. The amplification reactions were carried out using the Rotor-Gene Q Real-Time PCR system (Qiagen, Hilden, Germany).

The primers' sequences used to quantify the gene expression of the Her2 are

F: 5'-ATC TGC CTG ACA TCC ACG-3' and R: 5'-GCA ATC TGC ATA CAC CAG TTC-3'.

Bax gene is F: 5'-GTT TCA TCC AGG ATC GAG CAG-3' and R: 5'-CAT CTT CTT CCA GAT GGT GA-3'.

β-Actin gene is F: 5'-TTC CTG GGC ATG GAG TC-3' and R: 5'-CAG GTC TTT GCG GAT GTC-3'.

Bcl-2 gene is F: 5'-TTGTGGCCTTCTTTGAGTTCGGTG-3' and

R: 5'GGTGGCCGGTTCAGGTACTCAGTCA-3'.

Flow cytometry

For the measurement of the cellular DNA content, flow cytometric analysis was carried out using the Annexin V-FITC Apoptosis Detection

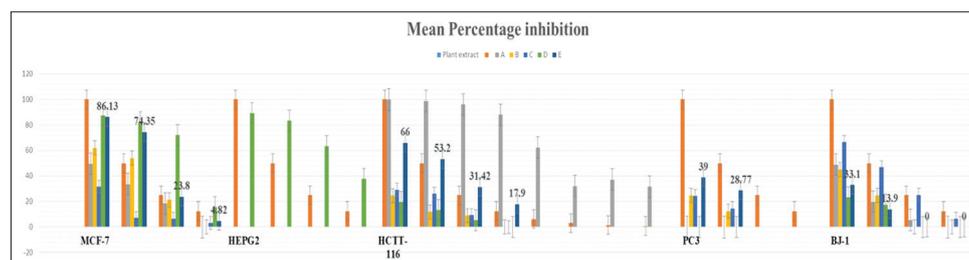


Fig. 1: Schematic representation of the mean cytotoxicity induced by methanol plant extracts on the MCF-7, HEPG2, HCT116, PC3, and BJ1 cells. Four of the five extracts, B, C, D, and E, were tested using four concentrations 100, 50, 25, and 12.5 ppm and the last extract, A, was tested using eight concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 ppm on HCT116 as it had significant inhibitory effects

kit (BioVision, CA, USA). After 48 h of treatment, HCT116 cells were trypsinized and washed with serum-containing media. The solution was then centrifuged at 300×g for 10 min, the supernatant was decanted, and the pellet was resuspended in 500 µl of ×1 binding buffer. Then, 5 µl of Annexin V-FITC and 5 µl of propidium iodide were incubated with the cells for 5 min in the dark. The sample was quantified by BD FACSCalibur (BD Bioscience, CA, USA) [8].

RESULTS

Cytotoxicity assay

Data were pooled from the MTT assays where five methanol plant extracts were tested on five human cancer cell lines. The mean percentage inhibition and the IC₅₀ of the extracts on each cell line are represented below. Probit analysis was performed using the SPSS software program.

All tested extracts did not have any cytotoxic effects on the HepG-2 or PC-3 cell line.

Extracts D and E showed relatively high percentage inhibition (87.29% and 86.13%) and (81.78% and 74.35%), respectively, on the MCF-7 cells when used in high concentrations (100 and 50 ppm) while having minimal to moderate effects (72.06% and 23.80%) and (15.5% and 4.82%), respectively, when used in low concentrations (25 and 12.5 ppm). IC₅₀ and IC₉₀ values of extracts D on the MCF-7 cell line were 22.4 and 84.7 while IC₅₀ and IC₉₀ values of extracts E were 48.2 and 92.4 ppm. Three of the tested extracts, B, C, and D, did not show any significant inhibitory effects on the HCT-116 cells. Whereas, extract E showed moderate inhibitory effects on the cells at concentrations of 100 and 50 ppm. On the other hand, A showed very high cytotoxicity on the cells at 12.5 ppm concentration, so it was further tested with concentrations of 6.25, 3.125, 1.56, and 0.78 ppm.

The IC₅₀ and IC₉₀ were calculated for extracts A and E. The values of IC₅₀ and IC₉₀ for extract A were 4.22 and 17.6 ppm, respectively, while the values for extract E were 62.04 and 17.9 ppm.

According to the National Cancer Institute Guidelines, extracts with IC₅₀ values <20 µg/ml are considered active. Thus, the *Euphorbia hierosolymitana* (extract A), which has an IC₅₀ of 4.22 µg/ml in HCT116 cell line, showed minimal to insignificant effects on the BJ1 cells with 5.57 and 0%; mean inhibition at 25 and 12.5 ppm, respectively, was selected for further *in vitro* testing which is discussed (Table 1 and Fig. 1).

Gene expression quantitation

The effects of IC₅₀ (4.22 ppm for HCT116) using methanol extract of *Euphorbia hierosolymitana* on the gene regulation was investigated by qRT-PCR by measuring the level of expression of *her2*, *Bax*, and *Bcl-2* genes in HCT-116 cells. Gene expression changes are represented as fold changes and were calculated using 2^{-Ct} method

$$Ct_{-2}Ct \text{ (treated cells)} - Ct \text{ (control cells)} \quad (2)$$

DMSO and the *β-actin* gene were used as internal control; as it is a highly conserved gene that is commonly used as an internal control for gene expression analysis [13].

After comparing the *Her2*, *Bax*, and *Bcl-2* gene expression levels of the treated and untreated cells, there was a very slight down expression in the *her2* gene by 0.09 folds, overexpression in the *Bax* gene by 14.37 folds, and almost no change in the expression of *Bcl-2* gene by 0.95 folds (Fig. 2).

Apoptosis induced by *Euphorbia hierosolymitana*

The typical morphological features of apoptosis are disruption of the cell membrane permeability, cellular shrinkage, and nuclear granulation [10]. The effects of the extract on the cell cycle profile were

Table 1: The mean percentage inhibition values for extracts A, B, C, D, and E on MCF-7, HEPG2, HCT-116, PC3, and BJ1 cell line

Plant extract	MCF-7					HEPG2					HCTT-116					PC3					BJ-1									
	100	50	25	12.5	6.25	100	50	25	12.5	6.25	100	50	25	12.5	0.78	1.56	3.125	6.25	12.5	25	50	100	50	25	12.5	100	50	25	12.5	
A	49.61	33.6	18.4	0							99.7	98.6	96	87.91	62.26	32.18	37.18	31.63	0	0	0	0	0	0	0	48.9	19.8	5.57	0	0
B	61.92	53.9	21.3	0							24.7	11.95	8.94	0					24.9	12.4						44.9	24.8	0	0	0
C	31.55	7.32	6.57	3.37							29.2	26.15	9.31	0					24.4	14.7					66.6	46.9	25.3	6.56	0	0
D	87.29	81.8	72.1	15.5	89	83.25	63.21	37.76			19.7	13.55	5.54	0					0	0					23.3	17.4	0	0	0	0
E	86.13	74.4	23.8	4.82							66	53.2	31.4	17.9					39	28.8					33.1	13.9	0	0	0	0

characterized by cellular DNA staining with PI and observed using flow cytometry. After the HCT116 cells were treated with extract A, there was a decrease in the proportion of the cells in the G0-G1 phase and the S phase, and an increase in the G2/M phase and the pre-G1 phase (Table 2).

The apoptosis marker, phosphatidylserine (PS), is exposed to the surface of the plasma membrane when the cell enters apoptosis. This marker is detected by Annexin V, which is a Ca²⁺-dependent phospholipid-binding protein that has an affinity to PS, to investigate the apoptotic capacity of extract A in HCT116 cells. Flow cytometry revealed that the extract induced early and late apoptosis by 5.81% and 10.01%, respectively (Table 3 and Fig. 3).

DISCUSSION

In this study, different plants were screened to find whether they have anticancer activity on multiple human cancer cell lines. Three of the five examined extracts showed some anti-proliferative activity. The extract

with the most promising inhibitory effect was selected for further tests to recognize the mechanism of these effects.

The extracts D and E demonstrated moderate anticancer effects on some of the cancer cell lines.

Extract A showed high cytotoxicity on the HCT-116 cell line (IC₅₀ of 4.22 ppm) while having minimal effects on the normal BJ-1 cells. This indicates that extract A has selective toxicity for cancer cells specifically colon cells. However, it is still unknown if the extract will also affect normal colon cells. Interestingly, despite that previous experiments [4,14] showed that *E. hierosolymitana* exhibited very high cytotoxicity in HepG-2 cells (99.87%), the extract did not show any anti-proliferative effects on the HepG2 cells. No previous data were found on the effects of *E. hierosolymitana* extract in HCT-116 cells [4].

Although some extracts were said to have compounds that induce high anticancer activity, they showed inactive or marginal effects on some of the cell lines. Whereas, extracts B and C were inactive in all cancer cell lines.

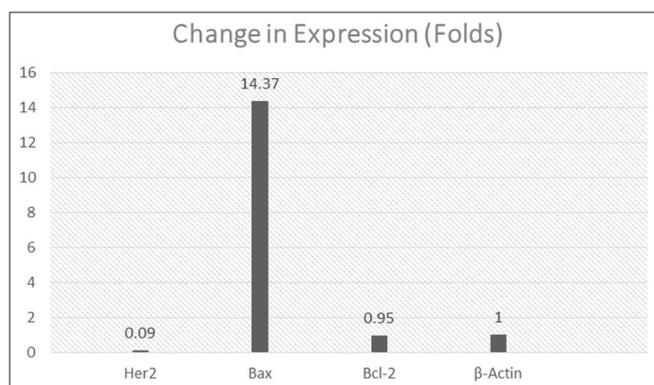


Fig. 2: Schematic representation of the gene expression profile for the Her2, Bcl-2, and Bax genes. The gene expression levels were compared with normal/untreated cells at the same conditions

Table 2: Effects of extract A on the cell cycle of HCT116 cells after 48 h treatment

Sample data		Result			
Sample code	Used conc. ug/ml	% G0/G1	% S	% G2/M	% Pre-G1
Treated	4.22	34.41	20.4	45.19	17.39
Control		65.13	30.53	4.52	0.77

Table 3: Apoptotic effects of extract A on the HCT116 cells

Sample data	Apoptosis			Necrosis (%)
	Total (%)	Early (%)	Late (%)	
Treated	17.39	5.81	10.01	1.57
Control	0.77	0.14	0.22	0.41

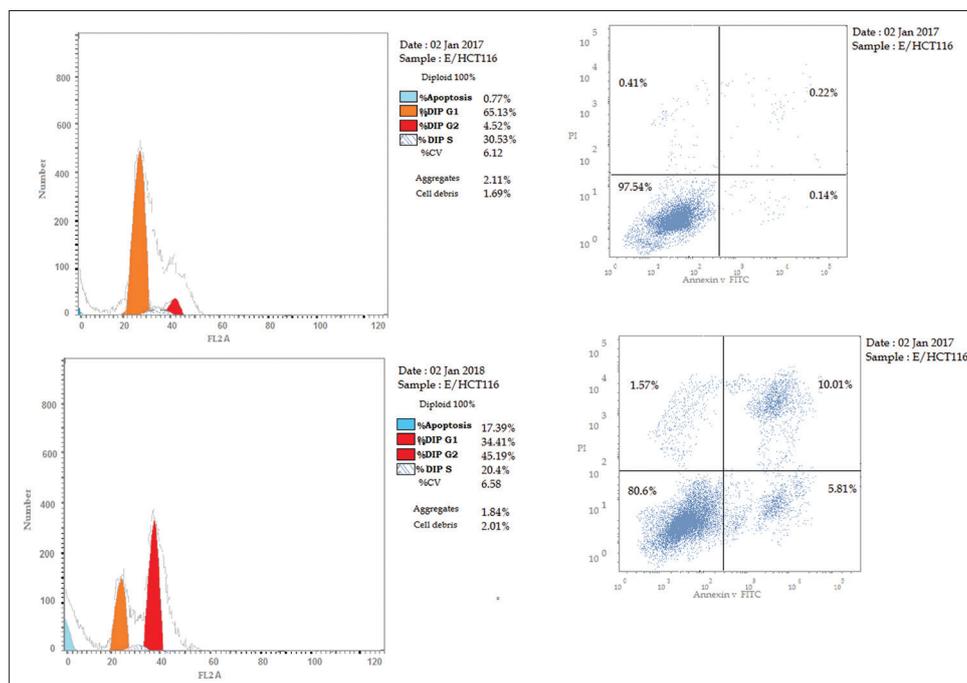


Fig. 3: Schematic representation of the apoptotic activity of extract A on the HCT116 cell lines. I represents the cell count in stages of the cell cycle (untreated control); II represents the cell count in stages of the cell cycle (treated with 4 µg/ml of extract A for 48 h); III Annexin V/PI (untreated control); Annexin V/PI (treated with 4 µg/ml of extract A for 48 h)

It was demonstrated that extract A possesses antiproliferative activity and induces apoptosis of HCT-116 cells. The cell cycle analysis showed that extract A affected the distribution of the cells in the cell cycle. The proportion of the cells in the G0-G1 phase and the S phase decreased, while it increased in the G2/M phase and the pre-G1 phase. These data suggest a perturbation of the cell cycle, arresting the cell at the G2/M phase. There was no previous cell cycle analysis made on *E. hierosolymitana*. However, Kwan et al. [8] stated that *Euphorbia hirta*, a plant from a different *Euphorbia* species, induced apoptosis, and resulted in DNA fragmentation in MCF-7 cells.

Another study on the *Euphorbia dendroides* L. presented that the herbs of the plant induced tumor apoptosis both in 2-D and 3-D of HCT-116 and hTERT-RPE1 cultured cells [5].

Based on the flow cytometry results (Table 3), extract A induced 5.81% in early apoptosis and 10.01% in late apoptosis. Furthermore, it induced necrosis with 1.57% compared to 0.41% of the untreated cells. *E. hierosolymitana* extract exhibited high apoptosis-inducing ability while maintaining a low necrosis induction. Therefore, the extract is considered promising for a potential anticancer drug.

It was demonstrated that the treatment of HCT-116 cells with extract A resulted in a change in the balance of Bcl-2/Bax RNA expression level [12], indicating that the extract can shift the anti-apoptotic/pro-apoptotic balance of the cells toward undergoing apoptosis (Table 3). This indicates that an increase in the balance between Bax/Bcl-2 is another mechanism of apoptosis in HCT-116 cells induced by extract A. her2/EBB2 gene is a member of the human epidermal growth factor receptor family [15].

The overexpression of this gene plays a significant role in the development and progression of a more aggressive form of cancer like in breast cancer. Several studies evaluated the her2 expression profile in colorectal cancer, and the overexpression rates varied widely from 0 to 84% [16]. Thus, extract A was used in the treatment of HCT-116 cells and the her2 gene expression was quantified (Table 3).

The results showed a downregulation of the her2 gene by 0.09 folds. The downregulation of her2 gene often results in the induction of apoptosis in human cancer cells [12]. No previous her2 expression analysis related to *E. hierosolymitana* was found. Based on the gene expression results, *E. hierosolymitana* extract showed the high capability of inducing apoptosis by downregulating her2 genes and stimulating the overexpression of pro-apoptotic proteins like Bax, making this plant a promising anticancer drug.

CONCLUSION

The effects of *Euphorbia hierosolymitana* on cell proliferation and apoptosis could include different mechanisms, and it was demonstrated that extract A changed the cell cycle and affected the gene expression of the her2, Bax, and Bcl-2. However, the molecular mechanism that regulates the equilibrium between cellular proliferation and apoptosis requires further investigations. Due to the capacity of *E. hierosolymitana* to induce high cytotoxicity *in vitro* in the colon cancer cell line, It should be further. It should be further studied phytochemically and pharmacologically to isolate and identify active compounds responsible for the anticancer activity. Other extracts from *Dracaena marginata* plant showed moderate cytotoxic effects on one or more cancer cell lines and should be considered for future studies.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, and interpretation of data.

AUTHORS' FUNDING

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REFERENCES

1. Abbotta IA, Shimazub C. The geographic origin of the plants most commonly used for medicine by Hawaiians. *J Ethnopharmacol* 1985;14:213-22.
2. Dela Cruz M, Christensen JH, Thomsen JD, Müller R. Can ornamental potted plants remove volatile organic compounds from indoor air? A review. *Environ Sci Pollut Res* 2014;21:13909-28.
3. Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N. Global medicinal uses of *Euphorbia* L. (*Euphorbiaceae*). *J Ethnopharmacol* 2015;24:90-101.
4. El Manawaty M, Fayad W, El-Fiky M, Nabaweya M, Wassel G, El-Menshawi B. High-throughput screening of 75 *Euphorbiaceae* and *Myrtaceae* plant extracts for *in-vitro* antitumor and pro-apoptotic activities on human tumor cell lines, and lethality to brine shrimp. *Int J Pharm Pharm Sci* 2013;5:178-83.
5. Fayad W, EL-Hallouty S, El-Manawaty M, Mounier M, Soliman A, Youstry A, et al. A systematic multicellular spheroids screening approach lead to the identification of antineoplastic activity in three different plant extracts from the Egyptian flora. *JAPS* 2017;7:13-22.
6. Ilodibia CI, Ugwu RU, Okeke CU, Akachukwu EE, Ezeabara EE. Phytochemical evaluation of various parts of *Dracaena arborea* Link and *Dracaena mannii* Bak. *Afr J Plant Sci* 2015;9:287-92.
7. Ilodibia CV, Ugwu RU, Okeke CU, Akachukwu EE, Ezeabara CA. Phytochemical evaluation of various parts of *Dracaena arborea* Link. and *Dracaena mannii* Bak. *Afr J Plant Sci* 2015;9:287-92.
8. Kwan YP, Saito T, Ibrahim D, Al-Hassan FM, Oon CE, Chen Y, et al. Evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells evaluation of the cytotoxicity, cell-cycle arrest, and apoptotic induction by *Euphorbia hirta* in MCF-7 breast cancer cells. *Pharm Biol* 2016;54:1223-36.
9. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983;16:55-63.
10. Lifiani R, Harahap U, Hasibuan PA, Satria D. Anticancer effect of African leaves (*Vernonia amygdalina* Del.) to T47D cell resistant. *Asian J Pharm Clin Res* 2018;11:4-7.
11. Ruksiriwanich W, Akazawa H, Jantrawut P. Selective anticancer activity of *Curcuma zanthorrhiza* fermented juices. *Asian J Pharm Clin Res* 2018;11:439-44.
12. Elmore S. Apoptosis: A review of programmed cell death. *Toxicol Pathol* 2007;35:495-516.
13. Roh H, Pippin J, Drebin JA. Down-regulation of HER2/neu expression induces apoptosis in human cancer. *Adv Brief* 2000;60:560-65.
14. Ruixin G, Zushang SZ, Yin Y, Sun L. Germplasm, chemical constituents, biological activities, utilization, and control of Chinese tallow (*Triadica sebifera* (L.) Small). *Biol Invasions* 2016;18:809-29.
15. Tai W, Mahato R, Cheng K. Down-regulation of HER2/neu expression induces apoptosis in human cancer cells that overexpress HER2/neu. *Cancer Res* 2000;1:560-5.
16. Suma S, Ummerali SK. HER2/neu expression in colorectal cancers. *IJCMR* 2017;4:1240-3.