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Original Article

Phytochemical and anti-cancer properties of *Euphorbia hierosolymitana* Boiss. crude extracts

[Propiedades fitoquímicas y anticancerígenas de extractos crudos de *Euphorbia hierosolymitana* Boiss.]

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Abstract

Context: *Euphorbia hierosolymitana* is a member of *Euphorbia* species having a restricted use in traditional medicine in eastern Mediterranean countries.

Aims: To phytochemically analyze different extracts of *Euphorbia hierosolymitana* and to investigate their anti-cancer activity against a panel of different cancer cell lines.

Methods: The aerial parts of the plant were extracted by n-butanol and ethyl acetate. Each extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to determine the bioactive compounds. Additionally, the anti-cancer activity of each extract compared to positive control doxorubicin was evaluated against a panel of different cancer and normal cell lines by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: Phytochemical analysis of the different extracts revealed different compounds of alkane hydrocarbons, fatty acids, sterols, phenols, glycosides, alkaloids, indol alkaloids, terpenoids, pyridine derivatives, and desulphosinigrin. Regarding anti-cancer activity, the n-butanol extract exhibited a significant selective concentration-dependent cytotoxicity in the colon cancer cell line (Caco-2) compared to other normal and cancer cell lines. This selective differential was comparable to the positive control, doxorubicin. The ethyl acetate extract, however, showed a significant cytotoxic activity among all the tested cell lines compared to the positive control. This cytotoxicity was in a concentration-dependent manner and weak to normal cell line (Wi38).

Conclusions: The selective differential in anti-cancer activity between different types of extracts is attractive and holds significant promise for the development of new cancer therapies.

Keywords: cancer; *Euphorbia hierosolymitana*; natural products; phytochemical screening.

Resumen

Contexto: *Euphorbia hierosolymitana* es un miembro de la especie *Euphorbia* que tiene un uso restringido en la medicina tradicional en los países del este del Mediterráneo.

Objetivos: Analizar fitoquímicamente diferentes extractos de *Euphorbia hierosolymitana* e investigar su actividad anticancerígena frente a un panel de diferentes líneas celulares cancerosas.

Métodos: Las partes aéreas de la planta se extrajeron mediante n-butanol y acetato de etilo. Cada extracto se sometió a cromatografía de gases-espectrometría de masas (GC-MS) para determinar los compuestos bioactivos. Además, se evaluó la actividad anticancerígena de cada extracto en comparación con la doxorrubicina (control positivo) frente a un panel de diferentes líneas celulares cancerosas y normales mediante bromuro de 3- [4,5-dimetiltiazol-2-il] -2,5-difeniltetrazolio (MTT) ensayo.

Resultados: El análisis fitoquímico de los diferentes extractos reveló diferentes compuestos de hidrocarburos alcano, ácidos grasos, esteroles, fenoles, glucósidos, alcaloides, alcaloides indol, terpenoides, derivados de piridina y desulfosinigrina. El extracto de n-butanol exhibió una citotoxicidad dependiente de la concentración selectiva significativa en la línea celular de cáncer de colon (Caco-2) en comparación con otras líneas celulares normales y cancerosas. Este diferencial selectivo fue comparable a doxorrubicina. El extracto de acetato de etilo, sin embargo, mostró una actividad citotóxica significativa entre todas las líneas celulares probadas en comparación con el control positivo. Esta citotoxicidad fue dependiente de la concentración y débil frente a la línea celular normal (Wi38).

Conclusiones: El diferencial selectivo en la actividad anticancerígena entre diferentes tipos de extractos es atractivo y promete mucho para el desarrollo de nuevas terapias contra el cáncer.

Palabras Clave: cáncer; cribado fitoquímico; *Euphorbia hierosolymitana*; productos naturales.

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INTRODUCTION

The discovery and development of new drugs has for many years depended on medicinal plants. These plants contain diverse active compounds that are considered potentially important for the development of therapies for many human diseases including cancers (Attar et al., 2015). In recent decades, there has been a breakthrough in the use of medicinal plants in the field of oncology. According to a recent review, 47% of the drugs used in the treatment of cancers are either natural products or semi-synthetic derivatives from natural product (Kinghorn et al., 2009). Examples of these drugs are vinca alkaloids (vinblastine and vincristine), camptothecin derivatives (topotecan and irinotecan), epipodophyllotoxin (etoposide and teniposide), and taxanes (docetaxel, paclitaxel and cabazitaxel) (Sharifi-Rad et al., 2019). Despite the great importance of natural products in anti-cancer development, it is estimated that only 2% of the natural products were investigated for anti-cancer (Attar et al., 2015; Sharifi-Rad et al., 2019).

In anti-cancer drug discovery, medicinal plants belonging to the genus *Euphorbia* (*Euphorbiaceae*) have received increasing scientific interest. Globally, many species of *Euphorbia* genus are used as traditional medicines for the treatment of skin disorders, warts, wounds, intestinal parasites, gonorrhea and migraines (Mwine and Van Damme, 2011; Salehi et al., 2019). The plant's biological composition has been shown to exhibit anti-arthritis, anti-convulsion, anti-diabetic, anti-eczema, anti-inflammatory, anti-microbial, antioxidant, anti-spasmodic, and anti-cancer properties. Some *Euphorbia* species show pharmacological activity against certain cancers, including melanoma, squamous cell carcinoma and lung cancer (Salehi et al., 2019). Moreover, several studies report potential cytotoxic activity induced by some *Euphorbia* species against different cell line models (Aleksandrov et al., 2019; Salehi et al., 2019). On this basis, there seems to be an opportunity to find anti-cancer activity among traditionally used medicinal plants.

One member of *Euphorbia* species that has attracted remarkable attention is *Euphorbia hierosolymitana*. It is perennial herb commonly found in Eastern Mediterranean countries, including Jordan (Hand et al., 2015). It is characterized by the presence of milky latex that causes skin and mucosa irritation leading to erythema, buccal irritation, and edema (Baydoun et al., 2015) . In traditional medicine, it was used as a purgative and laxative, and for the treatment of skin disorders such as eczema, psoriasis, and warts. However, the traditional use of *Euphorbia hierosolymitana* was limited to herbalists rather than locals because of its toxicity (Aburjai et al., 2007; Abdelhalim et al., 2017).

There is limited published data on the anti-cancer activity of *Euphorbia hierosolymitana* (Fig. 1). The studies conducted utilized small numbers of cell lines to assess the anti-cancer activity of different types of *Euphorbia hierosolymitana* extracts. These extracts showed various degrees of cytotoxicity against cell lines of hepatocellular carcinoma (HepG2) and breast adenocarcinoma (MCF-7) (Abu-Dahab and Afifi, 2007; El Manawaty et al., 2013). Moreover, only one study dealt with the phytochemical analysis of the *Euphorbia hierosolymitana*. It was found to contain different types of terpenes (α-pinene, verbenone and phytol), flavonoids (kaempferol), flavonoid coumaroyl glycosides (kaempferol 3-O-(4″-O-P-coumaroyl)-glucoside), alkenyl alcohol (1-octen-3-ol), hydrocarbons (longifolene) and pentasaccharide (verbascose) (Al-Hadid et al., 2019). These bioactive components have well-known anti-microbial, anti-inflamma-tory, antioxidant, anti-ulcer, anti-diabetic and anti-cancer biological properties (Riaz et al., 2018; Al-Hadid et al., 2019).

As there are limited studies analyzing the phytochemical and anti-cancer activity of *Euphorbia hierosolymitana*, the aims of the present study are to analyze phytochemically n-butanol and ethyl acetate extracts of *Euphorbia hierosolymitana* and to assess their anti-cancer activity against a panel of normal and cancer cell line models.

MATERIAL AND METHODS

Plant material

The aerial plant parts of *Euphorbia hierosolymitana* were collected at the full flowering stage during April 2019 from surrounding regions of the Mutah, Al-Karak city, Jordan (GPS coordinates: 31.068163, 35.739825). The plant was identified botanically by plant taxonomist, Professor Iman Al-Gohary, Department of Plant Ecology and Range, Desert Research Centre, Cairo, Egypt and a voucher specimen was kept at the Herbarium of the Centre with code number (CAIH-0325S).

Extraction of plant herbs

The aerial parts of the plant were washed and air-dried in the shade at room temperature. The dry parts were ground to a fine powder in a grinder. The crude powder (30 g) was then dissolved in (100 mL) ethyl acetate and fractionated by n-butanol. Each extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to determine the alkaloids and other bioactive compounds.

Phytochemical screening

Samples of different plant extracts were analyzed by the GC-MS (Thermo Scientific TRACE 1310) instrument using a DB5- MS (30 mm × 0.25 mm ID × 0.25 µm, film thickness) (J & W Scientific). Constant flow at 1 mL/min of carrier gas (helium) was used for sample analysis. The injector temperature of the instrument was programmed at 200°C. The oven temperature was set at 40°C and ramped at 5°C/min to 280°C. The withholding time was 7.5 minutes, with injection volumes of 1 µL. The temperature of the ion source was set at 280°C. The ionization of the sample was performed in electron impact mode at an ionization voltage of 70 eV with mass range used from m/z 50-650. Interpretation of GC-MS data was performed using the database of Wiley and Nist libraries.

Cell lines

Human lung cancer (A549), human prostate carcinoma (PC3), human breast cancer (MCF-7), human colon carcinoma (Caco-2), and normal human fetal lung fibroblast (WI-38) were obtained from American Tissue Culture Collection (ATCC) (Manassas, USA).

Culturing of cell lines

A laminar airflow cabinet was used to maintain the sterility of the procedure. All cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with 10% Fetal Calf Serum (FCS), 2 mM L-glutamine, and 1 mM sodium pyruvate. These cell lines were routinely maintained as monolayer cultures in 75 cm^2 cell culture treated flasks (T75 flask) (Corning, Amsterdam, Netherlands) in complete media and incubated at 37°C in a humidified atmosphere of 5% CO_2. Culturing and subculturing were performed according to Thabrew et al. (1997).

In vitro cytotoxicity assay

The chemosensitivity of *Euphorbia hierosolymitana* extracts was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Mosmann, 1983). This assay relies on mitochondrial succinate dehydrogenase reduction of the yellow water-soluble tetrazolium salt, MTT, to a purple, water-insoluble formazan salt. The salt was then dissolved in dimethyl sulfoxide (DMSO) and measured on a multi-well scanning spectrophotometer (Mindray-96A, Mindray, Nanshan, Shen-zhen, China). On reaching approximately 70% confluence, cells were detached, and cell suspensions were prepared at a concentration of 1 × 10^4 cell/mL. Cells were seeded in 96-well plates (180 µL/well) and incubated overnight at 37°C in a 5% CO_2 humidified atmosphere. The plant extracts were dissolved in DMSO. Serial dilutions of the dissolved extract were prepared by complete RPMI–1640 medium to give a final concentration of 15.625, 31.25, 62.5, 125, 250, 500 and

1000 µg/mL. Once cells adhered, 0.1 mL of each extract dilution or control (medium) was added to each well, followed by incubation of plates for 96 hours in the dark at 37°C in a 5% CO_2 humidified atmosphere. Following treatment, the supernatant was removed and 200 µL of MTT stock solution (5 mg/mL) was added to each well. Plates were then incubated in the dark for 4 hours at 37°C to allow for the formation of formazan crystals. The supernatant was carefully removed and 150 µL of DMSO was added to dissolve formazan crystals. The mean absorbance of the formazan solutions was determined using a microplate reader (Mindray-96A, China) at 560 nm. The doxorubicin anticancer drug was used as a positive control, and cells without samples were used as a negative control. The chemosensitivity data were calculated from the mean ± SD values of three independent experiments. The inverted microscope (Nikon, 118811) with objective 8 was used to observe the morphological structures of cell lines at varied concentrations of *Euphorbia hierosolymitana* extracts.

Determination of IC_{50} values

The half maximal inhibitory concentrations (IC_{50}) of different plant extracts and doxorubicin (as a positive control), against different cell lines, were calculated by using GraphPad Prism Version 5 software. The percentage of growth inhibition was calculated using the following equation [1]:

$$\text{Percentage(\%)of Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \quad [1]$$

Where OD: Optical density.

Selectivity index

Comparisons of the response of non-cancer cells to cancer cells for each extract were calculated using the selectivity index (SI). It is defined as the IC_{50} for WI-38 cells divided by the IC_{50} for each cancer cell line. Values greater than 1 indicate that extracts have selective activity against cancer, compared to non-cancer cells *in vitro* (Oliveira et al., 2015).

Statistical analysis

All results were presented as mean ± standard deviation. The statistical significance of the data was evaluated by the *t*-test at $p<0.05$ and $p<0.001$ using statistical software (GraphPad InStat, Version 3.10).

RESULTS

Phytochemical screening

The analysis of GC-MS of ethyl acetate and n-butanol extracts revealed the presence of different classes of bioactive secondary metabolites including fatty acids, sterols, phenolic acids, diterpenes, glycosides and alkaloids (Fig. 1 and Table 1). The spectrum shows the main *m/z* peaks found in ethyl acetate extract: pyrrolidine, 1-bicyclo (3,2,1) Oct – 2 – En – 3 – yl – at 18.86 relative abundance (RA = 11.047%); phytol at 27.81 has RA = 4.30% ; palmitic acid, methyl ester at 32.24 has RA = 5.0% ; 9, 12, 15 octadecatrienoic acid, methyl ester at 37.93 with RA = 5.72%; cholesta-4, 6-dien – 3 – OL at 61.18 (RA = 3.38%) and stigmast – 5- En – 3 – OL at 61.18 (RA = 3.38) (Fig. 1A). In n-butanol extract, several compounds with main m/z peaks were found including 12,15 – octadecadienoic acid, methyl ester was located at 12.12 with RA = 3.06%; pyrrolidine, 1-bicyclo (3,2,1) Oct -2 – En – 3 – YL at 18.86 (RA = 3.10%) ; pyrogallol with a higher amount at 21.95 (RA = 33.46%); at 26.76 (RA = 4.3%) for desulphosinigrin and at 28.68 (RA = 2.48%) for stevioside. The obtained results showed the same location of the two peaks for pyrrolidine, 1-bicyclo (3,2,1) Oct – 2 – En – 3 – YL, and palmitic acid, methyl ester compounds at 18.86 and at 32.23, respectively, showing only with different relative abundances (Fig. 1B).

Cytotoxicity assay

Data were pooled from the MTT assays where anti-cancer activity of n-butanol and ethyl acetate crude plant extracts at different concentrations (15.625, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL) were evaluated on one normal human cell line and four human cancer cell lines. The complete concentration-response curves and IC_{50} values for each type of extract on different cell lines were generated. Based on U.S. National Cancer Institute (NCI)

and Geran protocol (Sidambaram et al., 2011; Srisawat et al., 2013), the criteria used to categorize the cytotoxicity of plant extracts against cancer cell lines, were as follows: IC_{50} ≤ 20 µg/mL (highly cytotoxic), IC_{50} = 21 - 200 µg/mL (moderately cytotoxic), IC_{50} = 201 - 500 µg/mL (weakly cytotoxic) and IC_{50} >501 µg/mL (no cytotoxicity).

Results showed a significant concentration-dependent decrease in cell viability after 72 hours of exposure to plant extracts. Interestingly, the moderate cytotoxic activity of the n-butanol extract was against Caco-2 cells with IC_{50} of 152 µg/mL, while the normal human cell line (WI-38) exhibited weak cytotoxicity with IC_{50} of 212 µg/mL. This decrease was significant when compared to doxorubicin ($p<0.001$) and in a concentration-dependent manner (Fig. 2). In contrast, no cytotoxicity was observed in other cancer cell lines when treated with n-butanol extract compared to doxorubicin ($p<0.001$). The IC_{50} obtained for the inhibitory effects of this extract on PC3, MCF-7 and A549 cells was more than 500 µg/mL (Table 2) (Fig. 3).

Regarding the ethyl acetate crude extract, there was a significant concentration-dependent decrease in cell viability in all the cell lines compared to doxorubicin ($p<0.05$) (Fig. 4). The highest cytotoxic activities among the cell lines were observed in MCF-7, PC3 and A549 cell lines, with the IC_{50} of 93, 100 and 103 µg/mL, respectively. Other cell lines exhibited various degrees of cytotoxic activities with IC_{50} of 125.1 µg/mL for Wi38 and 170 µg/mL for Caco-2 (Table 2).

A microscopic examination of the different cell lines treated with 500 µg/mL of plant extracts for 72 hours was done. The analysis revealed that the treatment of n-butanol extract caused Caco-2 cells to shrink, become rounded, and detached in comparison with untreated control cells. Other cell lines exhibited no change in morphology. In contrast, all cells treated with ethyl acetate crude extract were shrunken and become rounded and detached in comparison with untreated control cells (Fig. 5).

Selectivity of cytotoxic effect

The results of the selectivity for *Euphorbia hierosolymitana* extracts are presented in Table 3. The n-butanol extract exhibited more selectivity than doxorubicin in Caco-2 cell line (1.45 times), while no selectivity was seen against MCF-7, PC-3 and A549 (selectivity ≤1.0). By contrast, ethyl acetate extract showed more selectivity than doxorubicin in MCF-7, PC-3 and A549 cell lines and no selectivity for Caco-2 cell line.

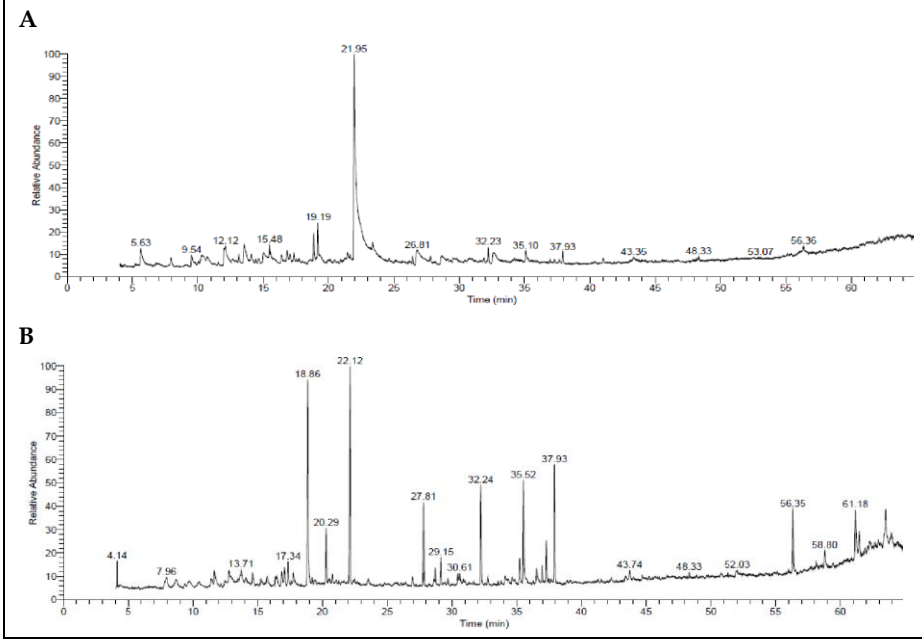

Figure 1. Gas chromatography-mass spectrometry spectra of *Euphorbia hierosolymitana*. **(A)** Ethyl acetate extract and **(B)** n-butanol extract.

Table 1. Chemical constituent identification of different extracts of *Euphorbia hierosolymitana* by GS-MS.

No.	Compounds	M. F.	M. W.	Ethyl acetate		n-Butanol	
				RT (min)	RA (%)	RT (min)	RA (%)
1.	**Alkane hydrocarbon**						
	Decane, 2,5,6-trimethyl-	C13H28	184			7.96	2.05
2	**Fatty acid**						
	12,15-Octadecadienoic acid, methyl ester	C19H34O2	294			12.12	3.06
	Octadecadiynoic acid, 2,6-dimethyl, dimethylester	C12H18O4	179			17.07	1.00
	5,8,11,14Eicosatrienoic acid, methyl ester	C20H34O2	306			37.93	1.33
	2-Myristynoyl acid pantethene	C25H44N2O5S	484	29.70	0.40	16.40	1.3
	Palmitic acid, methyl ester	C17H34O2	270	32.24	5.01	32.23	1.94
	Ethyl linoleate	C20H36O2	308	37.30	2.20		
	9,12,15-Octadecatrienoic acid, methyl ester	C19H32O2	292	37.93	5.70		
	Eragost-22en-3-ol	C24H38O3	374	43.74	0.75		
	3-Oxo-9b-lanosta-7-en-26,23-olide.	C30H46O3	454	61.90	0.46		
3	**Sterols**						
	Cholesta-4,6-dien-3-Ol, (3á)-	C27H44O	384	56.35	3.75		
	Stigmast-5-EN-3-ol	C29H50O	414	61.18	3.38		
4	**Phenolics**						
	Pyrogallol	C6H6O3	126			21.95	33.46
5	**Glycosides**						
	Stevioside	C38H60O18	804			14.10	5.90
6	**Alkaloids derived**						
	Pyrrolidine,1-bicyclo[3,2,1]Oct-2 -En-3-Yl-	C12H19N	177	18.86	11.04	18.86	3.10
	Thieno(3,4C)pyridine,1,3,4,7-tetraphenyl.	C31H21NS	439	58.80	1.32		
7	**Indol alkaloid**						
	O-Acetylaminovincinine	C23H28N204	396	48.33	0.30		
8	**Diterpene**						
	Phytol	C20H40O	296	27.81	5.47		
	Others						
9	Desulphosinigrin	C10H17NO6S	279	4.11	0.51	15.03	4.38
10	Pyrazole-5-carboxylic acid,3-methyl-	C5H6N2O2	126			23.38	1.7

M. F.: Molecular formula; M. W.: Molecular weight; RT: Retention time; RA: Relative abundance.

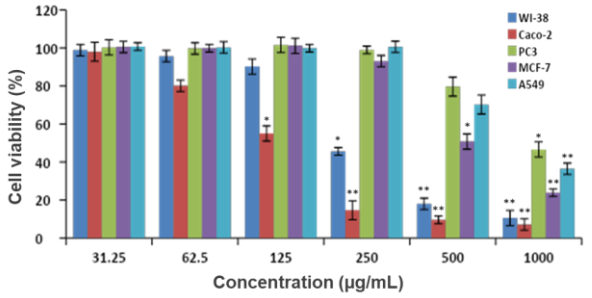

Figure 2. Cytotoxic effects of n-butanol crude extract of *Euphorbia hierosolymitana* against different types of cell lines.

These cell lines were treated with serial concentrations of n-butanol plant extract for 72 hours. A significant concentration dependent cytotoxicity was observed in Caco-2 cells compared with WI-38 cells (control). Data are shown as mean ± SD from three independent experiments. *p<0.05 represents the statistical differences respect to control; **p<0.001 symbolizes high significant difference from control.

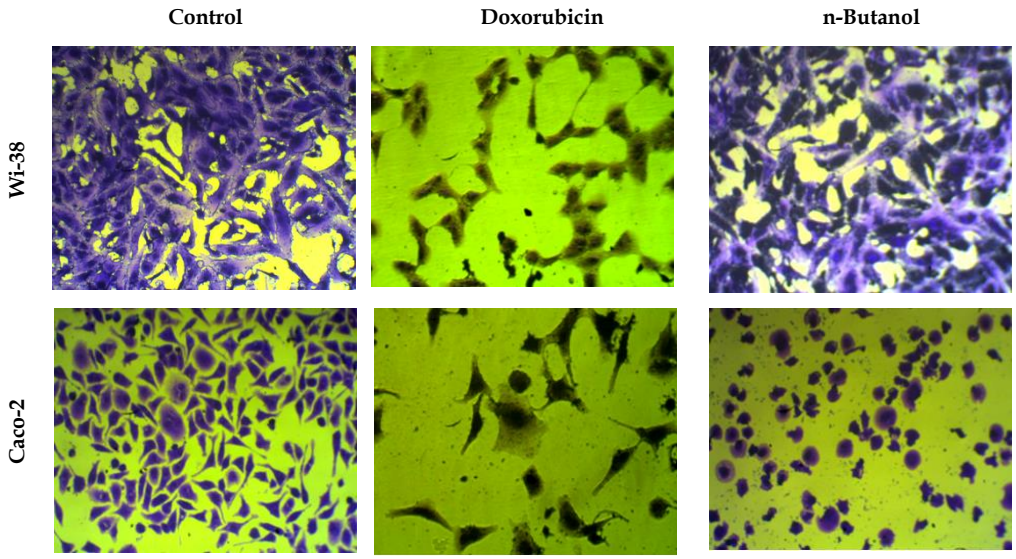

Figure 3. Anti-cancer effects of n-butanol crude extract of *Euphorbia hierosolymitana* on normal (Wi-38) and colon cancer cell lines (Caco-2).

These cell lines were treated with 152 µg/mL of plant extract (IC_{50} for Caco-2 cell line) and 83 µg/mL doxorubicin (IC_{50} for Caco-2 cell line) for 72 hours. Selective cytotoxic effect was seen between Caco-2 and Wi-38 cells. Caco-2 cells exhibited cytotoxicity while no effect observed on Wi-38 cells. Doxorubicin showed cytotoxicity on both cell lines.

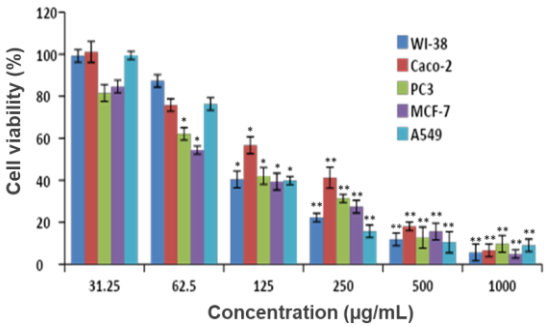

Figure 4. Cytotoxic effects of ethyl acetate crude extract of *Euphorbia hierosolymitana* against different types of cell lines.

These cell lines were treated with serial concentrations of ethyl acetate plant extract for 72 hours. A significant concentration dependent cytotoxicity was observed in all cell lines. Data are shown as mean ± SD from three independent experiments. *p<0.05 represent the statistical differences respect to control; **p<0.001 symbolizes high significant difference from control.

Table 2. IC_{50} values of different crude extracts of *Euphorbia hierosolymitana* on normal and cancer cell lines.

Treatment	IC_{50} (µg/mL)				
	Wi38	**Caco-2**	**PC3**	**MCF-7**	**A549**
n-Butanol extract	212.0 ± 6.3**	152 ± 5.9**	965 ± 43.9**	654.2 ± 28.3**	822.8 ± 34.7**
Ethyl acetate extract	125.1 ± 2.5**	170.4 ± 20.2*	100.0 ± 8.0*	93.0 ± 4.5**	103.9 ± 2.1*
Doxorubicin	79.7 ± 2.5	82.7 ± 0.6	80.3 ± 4.9	58.4 ± 7.5	85.1 ± 7.8

IC_{50}: The half maximal inhibitory concentration in µg/mL. Each value represents mean ± SD from three independent experiments. *p<0.05 represent the statistical differences respect to doxorubicin; **p<0.001 symbolizes high significant difference from doxorubicin.

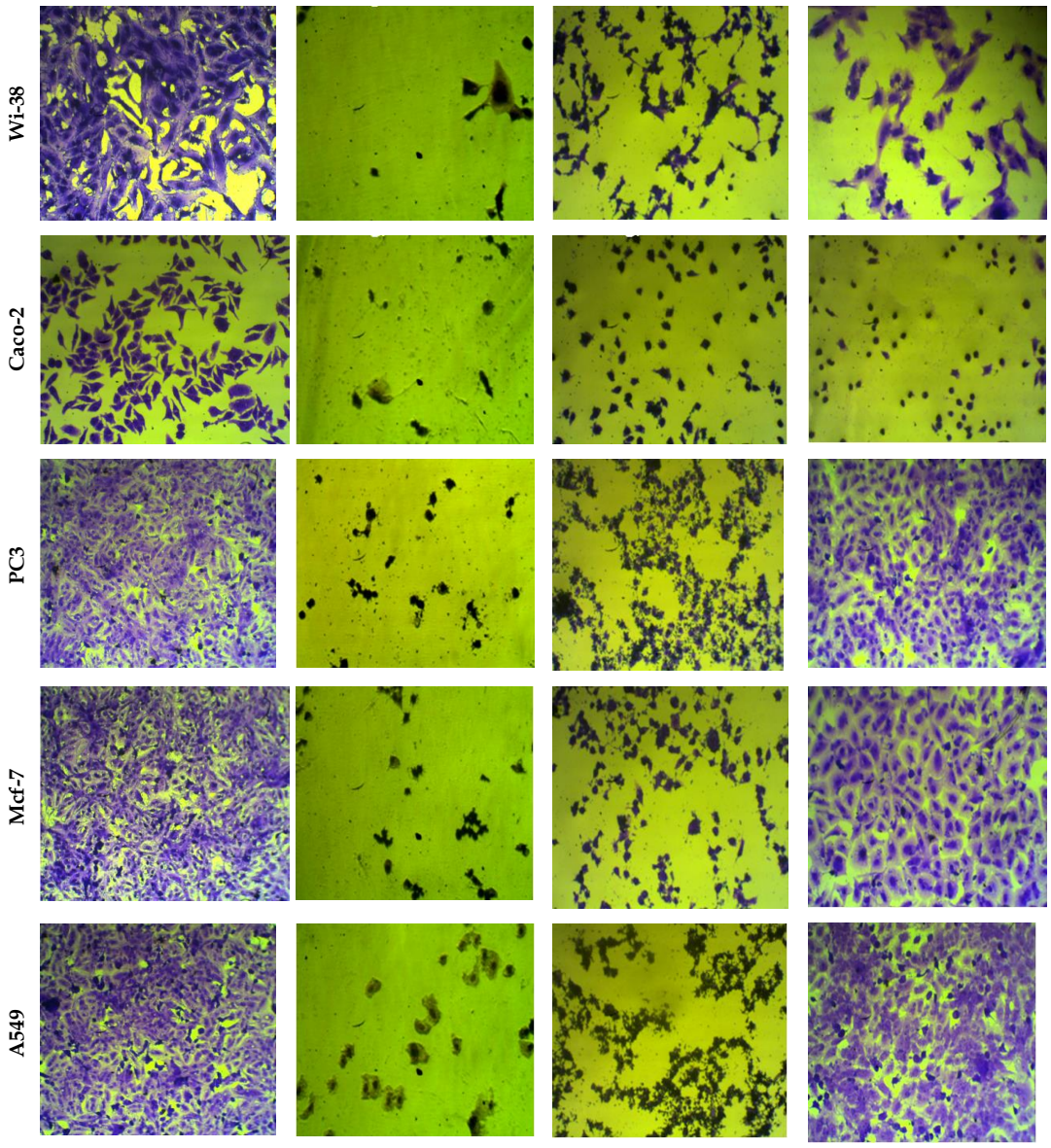

Figure 5. Anti-cancer effects of different extracts of *Euphorbia hierosolymitana* on normal and cancer cell lines.

The cell lines were treated with 500 µg/mL of plant extracts and doxorubicin (positive control, 500 µg/mL) for 72 hours. Doxorubicin exhibited cytotoxicity on all tested cell lines. All cell lines treated with ethyl acetate crude extract showed cytotoxic effect and cells become rounded and detached. The n-butanol crude extract showed selective cytotoxicity in Caco-2 cells while minimum effect seen on other normal and cancer cell lines. Caco-2 cells were shrunk, become rounded, and detached in comparison with untreated control cells.

Table 3. Selectivity index of extracts and positive control of the Caco-2, MCF-7, PC-3, and A549 cancer cell lines.

Treatment	Selectivity ratio			
	Caco-2	**PC3**	**MCF-7**	**A549**
n-Butanol extract	1.4	0.21	0.32	0.025
Ethyl acetate extract	0.73	1.25	1.35	1.2
Doxorubicin	0.96	0.99	1.65	0.94

The selectivity index is defined as the ratio of IC50 values for WI-38 divided by the IC50 for each cancer cell line. Values greater than 1 represent compounds that are preferentially active against cancer cells compared to non-cancer WI-38 cells.

DISCUSSION

Many medicinal plants possessing the ability to inhibit or halt the progress of cancer have been traditionally used. Although their mode of action is unknown, several modern technologies can help in finding new effective anti-cancer drugs derived from plant sources with an elucidated mechanism of action (Sidambaram et al., 2011). Therefore, for the first time, the present study investigates the phytochemical compositions of the n-butanol and ethyl estate crude extracts of *Euphorbia hierosolymitana* plant. Moreover, the evidence is presented of the anti-cancer activity of *Euphorbia hierosolymitana* against a panel of different normal and cancer cell lines.

Plants contain various chemicals that may have the capacity to change the physiological functions of cells and thus act as anti-cancer drugs, arresting the growth of cancer cells. In this investigation, several important bioactive compounds demonstrating anti-cancer activity were found by phytochemical analysis. One of these attractive compounds is phytol, the precursor of synthetic vitamin E and vitamin K. This compound was shown to be cytotoxic against breast cancer cell lines (MCF7) (Sheeja et al., 2016). Moreover, phytol was proven to exhibit antioxidant and antinociceptive effects (Santos et al., 2013). Additionally, phytosterols, such as α-sitosterols, β-sitosterols and stigmasterols, were found to prevent cancer progression through targeting different mechanisms leading to cancer. They were able to arrest angiogenesis by increasing antioxidant enzymes and inhibiting reactive oxygen species production and oxidative stress (Woyengo et al., 2009). These sterols even blocked inflammatory cytokines and induced apoptosis (Woyengo et al., 2009; Grattan, 2013).

Other bioactive compounds were also found to have anti-cancer properties through different mechanisms. These include stevioside, pyrogallol, deaulphosinigrin, vincinine and pyridine derivatives. Stevioside (diterpene glycoside) is a derivative of a steriol and rubusoside. This glycoside was shown to demonstrate anti-cancer activity in various *in vitro* anti-cancer studies by acting as a chemopreventive agent for chemical carcinogenesis. The glycoside also exhibited anti-inflammatory and immunomodulatory therapeutic benefits (Takasaki et al., 2009; Chen et al., 2018). Pyrogallol exhibited anti-cancer properties against several cancer cell lines, as shown using MTT cell viability assay (Chew et al., 2014). Desulphosinigrin, an anti-cancer drug target, was used as a ligand, and its inhibitory activity towards cyclin-dependent kinase was observed by means of docking and simulation studies by Krishnaveni (2015). Moreover, vincinine derivative (O-acetyl amino vincinine) was shown to possess potent anti-cancer effects in various tumor models. Through a docking study, pyridine derivatives (thieno (3, 4-c) pyridine 1, 3, 4, 7-tetraphenyl, pyrrolidine, 1-bicyclo (3,2,1) Oct-2-En-3 yl and pyrazol-5-carboxylic acid, 3 methyl) were reported as anti-tumor agents (El-Zahar et al., 2011; Abdelaziz et al., 2018).

There is an urgent need to develop anti-cancer drugs that potentially target tumor cells without affecting normal cells (Sidambaram et al., 2011). In this study, for the first time, this selectivity in cytotoxic activity was observed between normal (WI-38) and colon cancer cells (Caco-2) treated with the n-butanol crude extract. Moreover, the anti-cancer activity of the n-butanol crude extract was only observed in the colon cancer cell line (Caco-2), while other cancer cell lines exhibited minimal cytotoxicity. It is worth testing this type of extract on normal colon cancer cell lines.

The cytotoxic activity was further assessed using ethyl acetate crude extract. Selective cytotoxic activity was only observed against MCF-7, PC-3 and A549 cell lines. The greatest potential was achieved at certain optimal concentrations of the extract. This finding contradicts other studies where the methanolic and ethanolic extract of *Euphorbia hierosolymitana* exhibited minimal cytotoxicity against HepG2 and MCF-7 cell lines, respectively (Abu-Dahab et al., 2007; El Manawaty et al., 2013). It seems that the extraction method of *Euphorbia hierosolymitana* plays an important role in anti-cancer activity. There was also a differential in the selectivity of anti-cancer activity of extracts against all the cell lines tested. This selectivity in anti-cancer activity is particularly interesting and

is a promising finding for the development of potential anti-cancer drugs.

CONCLUSIONS

Based on the data presented, it seems that *Euphorbia hierosolymitana* consists of one or more cytostatic chemicals, which demonstrate selective anti-cancer activity against cancer cell lines, particularly human colorectal cancer cell line. The use of *Euphorbia hierosolymitana* as a potential anti-cancer agent is therefore promising as the isolation of cytotoxic compounds from crude extracts and the use of such compounds can prevent or stall the progression of cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Concepts or ideas	x	x	x				x
Design	x	x	x		x		
Definition of intellectual content	x	x	x	x	x	x	x
Literature search	x	x	x				x
Experimental studies	x		x	x			
Data acquisition	x		x	x	x	x	x
Data analysis	x	x				x	x
Statistical analysis		x			x		
Manuscript preparation	x		x	x		x	
Manuscript editing	x	x					x
Manuscript review	x	x	x	x	x	x	x

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