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Research Article
In vitro Anticancer, Antioxidant and Antimicrobial Activities of Crude Methanolic Extract of *Euphorbia cactus* Ehrenb Plant

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Abstract

Background and Objective: Euphorbiaceae is a wide spread family of genus *Euphorbia*, from that family, belongs more than 2000 species. The plants of this genus have been used for a long time in traditional medicine. The present investigation is focused on the anticancer and antioxidant potential and antimicrobial activity of the methanolic extract of *Euphorbia cactus* Ehrenb plant (*E. cactus* Ehrenb). **Materials and Methods:** The study was facilitated by collecting the plant sample and subjected to crude methanol extraction. The anticancer activity was examined by MTT assay against three cancer cell lines MCF-7, PC-3 and HEPG2. The antimicrobial activity was determined using the agar diffusion method and MIC-determination. The DPPH radical method was used for the determination of antioxidant activity. **Results:** Interesting cytotoxic activity was observed for the extract against MCF-7 cell line compared to the known anticancer drugs. The antioxidant activity of the extract showed highly inhibitory activity compared with ascorbic acid. In addition, the methanolic extract showed the highest activity against Gram-positive bacteria, *Bacillus subtilis* and *Streptococcus pneumonia* and Gram-negative bacteria, *Escherichia coli*, compared with the standard drug. The extract didn't exert any activity against the pathogenic yeast species (*Candida albicans*) under these screening conditions. **Conclusion:** The current study demonstrated for the first time that *E. cactus* Ehrenb methanolic extract has anticancer effects against cancer cells from 3 types of cancerous cell lines. This may be a promising finding for future cancer treatments and in the prevention of cancer using natural products.

Key words: *Euphorbia cactus* Ehrenb, antioxidant, human cancer cell line, anticancer activity, antimicrobial

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural products, especially medicinal plants, have played a significant role in drug discovery and development of therapeutic agents. Plants contain many biologically active compounds that have potential for development of therapeutic agents. More than 35,000 plant species have been used in various regions around the world for medical purposes[1]. The Euphorbiaceae family comprises about 300 genus and 10,000 species. Euphorbiaceae is among the largest flowering plant families consisting of a wide variety of vegetative forms including trees, succulents and herbaceous plants. Some of the plants from that family are of great importance[2]. Different species of *Euphorbia* grow all over the world either wild or as cultivated. The genus *Euphorbia* is the largest genus of the medicinal plants widely distributed in China, India, Bangladesh and Pakistan[3]. It was reported that plants from Euphorbiaceae family are used in folk medicine against venomous bites and trichiasis and as wart removers. Species of *Euphorbia* are characterized by high ecological amplitudes in tropical, subtropical and warm temperate regions and they are widely spread around the world. They grow in north Africa and in the temperate parts of Asia but mainly in the Mediterranean region[4]. Several plants of Euphorbiaceae family were tested for their anticancer property but most of them have been used in traditional medicine as treatment for various human diseases. Antitumor activity against sarcoma and ascites, leukemia in mice and cytotoxic activity against certain cancer cell lines were also observed[5]. Some species have been used in treatment of dermatosis, paralysis and pain of human body as well as poultice for broken bones ulceration, swelling and hemorrhoids. Several interesting biological activities was also reported such as cytotoxic, hepatoprotective, antispasmodic, anti-inflammatory, antibacterial, antifungal, anti-mutagenic, antiviral, pesticide, molluscicidal and larvicidal activities. Interestingly, the latex of these plants has shown cocarcinogenic and anti-carcinogenic activities[6-8].

E. cactus Ehrenb is a leafless succulent spiny shrub, to about 1 m, with 3-4 angled branches. Flowers in cymes of 2-4 flowers in the upper part of the branches. Fruits capsules, 3-angular, 8-10 mm across, dull red. Seeds subglobose, 2.7 mm diameter, brownish-grey. This species distributed in Africa (Sudan and Eritrea) and South Arabian Peninsula. It has been reported that latex extract of *E. cactus* Ehrenb contain active compounds that have anti-leishmanial activity, which could serve as an alternative agent in the treatment of *Cutaneous leishmaniasis* but further studies would, therefore, be needed to assess the activity of these materials of this plant *in vivo* clinical response and study their toxicity on cell lines[9].

Consequently, the search for natural, stable, safe, anticancer, antioxidant and antimicrobial agents have become a subject of interest as alternatives to artificial compounds. The present study aimed to assess anticancer, antioxidant and antimicrobial efficacies of *E. cactus* Ehrenb methanolic extract.

MATERIAL AND METHODS

Collection of plant material: The current study was carried out in the Department of Biology, Faculty of Science, Jazan University, Jazan, Saudi Arabia from August, 2017-September, 2018. The target plant, *E. cactus* Ehrenb, was collected from different locations from South region of Saudi Arabia and was identified by taxonomist Dr. Yahya Masrahi, Associate Professor, Department of Biology, Faculty of Science, Jazan University, Jazan, Saudi Arabia.

Extraction of plant material: The dried *E. cactus* Ehrenb whole plant (150 g) was ground and extracted with methanol (200 mL) for 24 h using Soxhlet apparatus. The methanol was removed under reduced pressure to yield a viscous and will kept in refrigerator until use. The extract was dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. The final DMSO concentration was below 1% of total volume of the medium in all treatments and controls[10].

Determination of anticancer activities

Cell culture: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT and Sanford, ME, USA). Human breast cancer cell line (MCF-7), prostate cell line (PC-3), human hepatocellular carcinoma cell line (HEPG2) and normal breast epithelial cell line (MCF-10A) were included. The human foreskin fibroblast cell line, Hs68, was also included in the cell panel. These cell lines were obtained from the National Cancer Institute, Cairo University. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U mL^{-1} of penicillin and 100 µg of streptomycin/ml in a humidified incubator with 5% CO_2 at 37°C.

Cell viability assay: For determination of cytotoxic activity of extracts, viable cell numbers were determined using the trypan blue exclusion method. Trypan blue dye exclusion was

added to all cultures in a ratio of 1:1. Then, extract treatments and preparations were examined under the standard light microscope at 100X magnification. The ratio of live cells to dead cells (cell viability) was also determined. Standard curves were prepared and 50% cytotoxic concentrations of extracts (IC_{50}), which caused a 50% decrease in cell viability, were derived.

Trypan blue exclusion assay was performed to assess the effect of the methanolic extract of *E. cactus* Ehrenb on the viability of MCF-7, PC-3, HEPG2, MCF-10A and Hs68 cells. Approximately 7.5×104 cells mL^{-1} was seeded in a six-well tissue culture plate and different concentrations of the extract was added after 24 h. For the determination of growth rate, smaller aliquots were collected in 0.5 mL tubes, trypan blue (0.4%) was added to the cell suspension and the number of cells (viable-unstained and non-viable-blue) was counted using a hemocytometer. The media were not changed during the induction period. Each experiment has repeated a minimum of 3 times[11].

MTT assay: The methanolic extract of *E. cactus* Ehrenb was subjected to a screening system for evaluation of its anticancer activity against breast carcinoma (MCF-7), prostate (PC-3), hepatocellular carcinoma (HEPG2), normal breast epithelial (MCF-10A) and foreskin fibroblast cell line, Hs68, in comparison to the known anticancer drugs: 5-FU and DOX. Cell viability was determined by colorimetric assay using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to evaluate the antiproliferative activities of the tested extract against the cancer cell lines. The extract was tested on the four cell lines in 4 different concentrations (5, 12.5, 25 and 50 μM). Exponentially growing cells were plated in triplicate in 96 well sterilized plates at a density of 1×104 cells/well. After 24 h, cells were treated with escalating doses of the extract and incubated in 5% CO_2 atmosphere with high humidity. After 48 and 72 h of the extract exposure, the cells were incubated with MTT (0.5 mg mL^{-1}) for another 4 h at 37°C. The blue MTT formazan precipitate was then solubilized in detergent (50% final concentration of N, N-dimethylformamide and 10% of sodium dodecyl sulfate) and incubated for an additional 2 h. Absorbance was measured at 570 nm on a multiwell ELISA plate reader. The mean absorbance of medium control was blank and was subtracted. The IC_{50} values were estimated after 72 h exposure to the extract. The absorbance of control cells was taken as 100% viability and the values of treated cells were calculated as a percentage of control. The 5-fluorouracil and doxorubicin anticancer drugs were used as positive control and cells without sample were used as negative control. The relation between surviving fraction and extract concentration was plotted to get the survival curve of the three cancer cell lines with the specified extract. DMSO is the vehicle used for dissolution of extract[12].

Cell division analysis: The medium was discarded from the T75 flask, the cells were washed with 5 mL of PBS and then discarded. The cells were detached by applying 1 mL of trypsin, incubated for 3 min and then the trypsin was deactivated by applying 9 mL of complete medium. The cells were collected in 15 mL Falcon tube and 50 μL of the cell suspension in Eppendorf tube. The Falcon tube was centrifuged at 1000 rpm for 3 min and cell counting was carried out. The supernatant was discarded from the Falcon tube and the cells were re-suspended in 10 mL of complete medium. The required number of cells for seeding 6 wells plate was transferred to a new Falcon tube. The Falcon tube was centrifuged at 1000 rpm for 3 min and the supernatant was discarded. The cells were stained by applying 1 mL of CFSE (carboxyfluorescein succinimidyl ester) working solution and incubated at 37°C and 5% CO_2 for 10 min. The staining process was stopped by applying 4 mL of complete medium, vortexing, centrifuging for 3 min at 1000 rpm and discharging the supernatant. The cells were washed with 5 mL of PBS, centrifuged for 3 min at 1000 rpm and the supernatant was discarded. The cells were re-suspended in complete medium (1 mL/well) and seeded in a 6 well plate. After 24 h incubation, the cells were treated with IC_{50} and $2\times IC_{50}$ of the extract and incubated for 48 h at 37°C and 5% CO_2. The medium was discarded from the wells, the cells were washed with 5 mL of PBS and then it was discarded. Afterwards, the cells were detached by using 0.5 μL of trypsin and incubation for 3 min at 37°C and 5% CO_2. The trypsinization was stopped by adding 1 mL of complete medium in each well and cells were collected in 15 mL Falcon tube from each well by pipette tip harvesting technique. The Falcon tubes were centrifuged at 1000 rpm for 3 min and the supernatant was discarded. The cells were washed with 1 mL of PBS, centrifuged, supernatant was discarded and then, re-suspended in 1 mL of PBS. The cells were analyzed by flow cytometry[13].

DPPH radical scavenging assay: The free radical scavenging activity was tested according to Mensor *et al.*[14]. Various concentrations of the extract were mixed with 80 mM of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. Then, the solution was incubated for 30 min at room temperature. Ascorbic acid was used as positive control. Absorbance of

the solution was measured at 517 nm by a double-beam spectrophotometer. The DPPH radical scavenging activity was calculated using the equation:

$$\text{Inhibition (\%)} = \frac{AB - AA}{AB} \times 100$$

Where:
AB = Absorption of blank sample
AA = Absorption of test sample

The percentage of DPPH radical scavenging activity was calculated. The 50% inhibitory concentration (IC_{50}) was expressed as the quantity of the extract necessary to react with one half of DPPH radicals[15].

Antimicrobial bioassay by using the agar diffusion cylinder method: The methods of Akujobi *et al.*[16] and Esimone *et al.*[17] were adopted. The methanolic crude extract were dissolved in 30% dimethylsulphoxide (DMSO) and further diluted to obtain 500, 250, 200, 150, 100 and 50 mg mL^{-1} concentrations. All microbial strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The *E. cactus* Ehrenb methanolic extract was tested *in vitro* against different types of bacteria, *Streptococcus pneumoniae* and *Bacillus subtilis* as examples of Gram-positive bacteria and *Pseudomonas aeruginosa* and *Escherichia coli* as examples of Gram-negative bacteria. Fungi, as well as bacteria, were used for testing the antifungal activity of the methanolic plant extract. *Aspergillus fumigates* and *Candida albicans* were used as example of fungi and yeast, respectively. The plates were incubated at 37°C for 24 h for bacteria and yeast and for 48-72 h for fungi. Tetracycline was used as the standard antibacterial drug while amphotericin B was used as the standard antifungal drug. The diameters of the inhibition zones (mm) were measured and used as criterion for the antimicrobial activity.

At the end of incubation, the plates were collected and zones of inhibition that developed were measured. The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of extract against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC values[17,18].

Statistical analysis: Statistical analysis was performed using SPSS (v.16). All statistical comparisons were made by means of the one-way analysis of variance (ANOVA) test followed by Tukey's test post hoc analysis. A p-value less than 0.05 was considered significant.

RESULTS

Cell viability: The effects of *E. cactus* Ehrenb methanolic extract on cell viability of different cell lines is shown in Fig. 1. Cells were incubated with three different concentrations (5, 10 and 20 μg mL^{-1}) of the extract. The results showed that the methanolic extract of *E. cactus* Ehrenb exhibits a moderate to strong growth inhibition of MCF-7 cell line in comparison to the other two cell lines.

The relationship between surviving fraction and extract concentration was plotted to obtain the survival curve of each of the three cell lines. The response parameter calculated was the IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability.

The methanolic extract of *E. cactus* Ehrenb showed cytotoxicity against the breast carcinoma cell line (MCF-7), hepatocellular carcinoma cell line (HEPG2) and prostate carcinoma cell line (PC-3) with IC_{50} values of 17.11±0.73, 24.1±0.65 and 27.01±0.80 μg mL^{-1}, respectively versus 13.35±0.21 and 14.70±1.1 μg mL^{-1} for 5-fluorouracil and doxorubicin, respectively (Fig. 2).

Cell division analysis: Of the three cancer cell lines investigated, MCF-7 was the most sensitive to *E. cactus* Ehrenb methanolic extract. The effect of plant extract on cell division of MCF-7 cell line could be determined by CFSE assay. Breast carcinoma cell line (MCF-7) cell division was slightly affected by treating with the methanolic plant extract. The IC_{50} showed a slight reduction in the cell division from 6-11%

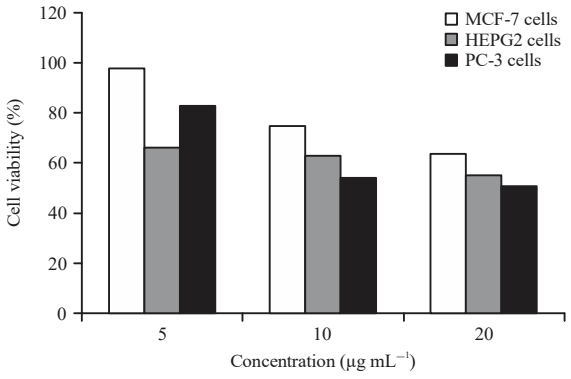

Fig. 1: Effect of methanolic extract of the *E. cactus* Ehrenb on the viability of the three cancer cell lines used

Table 1: Scavenging effect (%) and IC_{50} for methanolic extract of *E. cactus* Ehrenb and ascorbic acid

	Scavenging effect (%) at different concentrations*	
Concentrations of extract (μg mL^{-1})	Methanolic extract	Ascorbic acid
50	5.0±0.13	11.7±0.71
100	29.1±0.21	30.0±0.41
150	48.0±0.25	44.7±0.23
200	54.1±0.34	66.4±1.2
250	91.3±0.35	90.0±0.48
500	93.7±0.45	94.8±0.61
IC_{50}	7.98	5.63

*Mean values of 3 replicates±SD

Table 2: *In vitro* antimicrobial activity of the different concentrations of methanolic crude extract of *E. cactus* Ehrenb

	Zones of inhibition (mm)*					
Concentrations of extract (mg mL^{-1})	*Bacillus subtilis*	*Streptococcus pneumoniae*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus fumigatus*
250	8.4[c]	9.5	11.9[a]	10.1[a]	NI	NI
200	5.6[d]	7.9	9.8[a]	11.3[c]	NI	NI
150	NI	8.5	8.0[a]	8.2[a]	NI	NI
100	NI	5.6	5.8[a]	6.1[a]	NI	NI
50	NI	7.8	NI	NI	NI	NI

*Values are means of 3 replicate readings, NI: No inhibition, [a,b,c]Values with different superscripts on the same row are significantly different (p = 0.05)

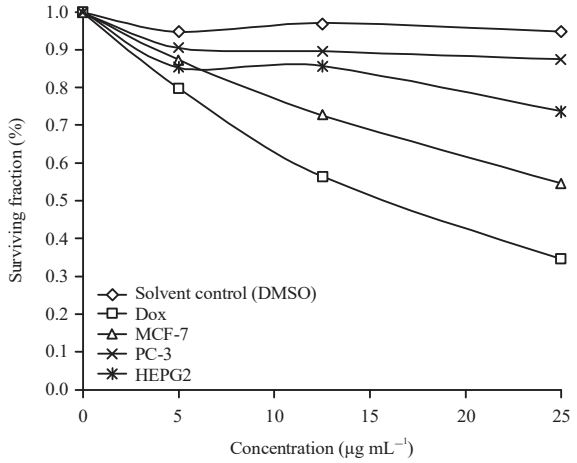

Fig. 2: Cytotoxic activity of the methanolic extract of *E. cactus* Ehrenb against the cancer cell lines used

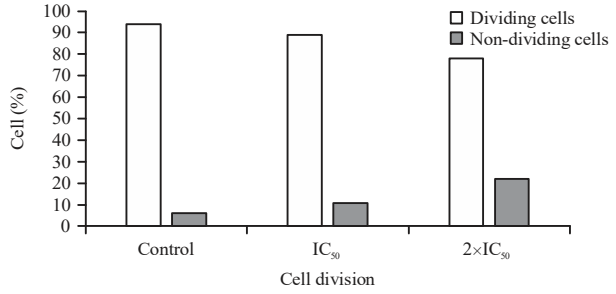

Fig. 3: Proliferation inhibition potential of the methanolic plant extract on the breast carcinoma cell line (MCF-7)

and by doubling the IC_{50} concentration, the cell division was further reduced to 22%. These data are presented in Fig. 3.

Antioxidant activity: Methanolic extract of *E. cactus* Ehrenb plant showed effective free radical scavenging activity as determined by DPPH assay. The results are presented in Table 1. Crude methanolic extract has excellent free radical scavenging with IC_{50} 7.89 μg mL^{-1} which is comparable to ascorbic acid (IC_{50}, 5.63 μg mL^{-1}), a very important antioxidant compound.

Table 1 showed that by increasing concentration of *E. cactus* Ehrenb methanolic extract, the scavenging activities also increasing (50<100<150<200<250< 500 μg mL^{-1}). Ascorbic acid was used as a reference antioxidant compound and similar results were observed by the same series of concentrations (50<100<150<200<250 μg mL^{-1} and 500 μg mL^{-1}).

Antimicrobial screening: As shown in Table 2, the extract displayed variable *in vitro* antibacterial and antifungal activities. From the screening results, it can be noted that the methanolic extract showed the highest activity against Gram-positive bacteria *Bacillus subtilis* and *Streptococcus pneumonia* and Gram-negative bacteria *Escherichia coli*, compared with the standard drug. Regarding the activity of the extract against the tested filamentous fungus *Aspergillus fumigatus* and the pathogenic yeast species (*Candida albicans*) the extract didn't exert any activity under these screening conditions.

DISCUSSION

Historically, plants, herbs and spices are a folkloric source of medicinal agents. Medicinal plants drug discovery

continues to provide new and important leads against various pharmacological targets, including cancer, HIV, malaria and pain and remains an important route to new pharmaceuticals[15,19-21]. Many chemo-preventive agents have been associated with anti-proliferative and apoptotic effects on cancer cells because of their high antioxidant activity, targeting signaling molecules and preventing or protecting cells from further damage or transformation into cancer cells[22,23].

Free radical scavenging is generally the accepted mechanism for antioxidants to inhibit lipid oxidation. Antioxidants (inhibitors of lipid peroxidation) are important not only for preservation of food but also for the defense of living cells against oxidative damage[24]. The preferred method for evaluation of the scavenging free radicals' activities is the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test[25,26]. The screening of herbal extracts and their components by the DPPH scavenging assay has become a routine parameter for testing their antioxidant efficacy[27,28]. The inhibition of free radical DPPH is one of the oldest and most frequently used methods for total antioxidant potential/capacity of food and biological extracts. It is based on the ability of an antioxidant to give hydrogen radical to synthetic long-lived nitrogen radical compound DPPH, which can evaluate the scavenging free radical activities in a relatively short time compared to other methods[29].

Several species of *Euphorbia* have been tested for their anticancer and cytotoxic activity. Chi *et al.*[30] have evaluated the cytotoxicity of ethanol extract of *E. hirta* against K562 (human leukemia) and A549 (lung cancer) cell lines. From their results, the ethanol extract exhibited a weak activity against A549 cells and it was inactive against K562 cells. Patil and Magdum[31] studied antitumor activity of *E. hirta*. They reported that the extracts enhanced mean survival time and reduced solid tumor mass in mice. Ping *et al.*[32] investigated the genotoxic effect of methanol extract of *E. hirta* using Allium cepa Assay. The tested extracts decreased mitotic index and increased chromosome aberrations as the concentrations of *E. hirta* extract increased. This result confirmed that *E. hirta* methanol extract (1000 μg mL^{-1}) exerted a significant genotoxic and mitodepressive effect[32].

Munro *et al.*[33] showed the anticancer properties of latex extract from *E. tirucalli*. They found that it exhibited dose and time dependent cytotoxic effects against a significant number of cell lines, with most prominent effects against oesophageal squamous cell and pancreatic cell carcinomas. Munro *et al.*[33] examined the *in vitro* activity of extracts of *E. tirucalli* on human pancreatic cancer cell line Mia-PaCa-2. They worked with methanolic and aqueous extracts of *E. tirucalli*. They concluded that toxicity of *E. tirucalli* methanolic extract was dose dependent and cell viability was decreasing by increasing the extract concentration. The methanol extract exerted a significant decrease in cell viability even in the low dose[33].

Hsieh *et al.*[34] used water extract from *E. formosana* and they assessed its anticancer and cytotoxic activity on human lung carcinoma cell line A-549, human bladder papillary transitional cell carcinoma, cell line BFTC-905, human monocytic leukemia-derived cell line THP-1 and human promyelocytic leukemia cell line HL-60. They concluded that both leukemic cell lines, THP-1 and HL-60 had dose-dependent growth inhibition. The high concentration did not inhibit growth of the lung carcinoma line A-549 and the bladder carcinoma line BFTC-905. Their results showed that the extract selectively inhibited the growth of leukemic cancer cells; solid human cancer cells are not sensitive to the extract and it had low toxicity in normal cells[34].

The extracts of *E. helioscopia* L. effectively inhibited the growth of human hepatocellular carcinoma lines SMMC-7721, BEL-7402, HepG2, gastric carcinoma cell line SGC-7901 and colorectal cancer[35] cell line SW-480. According to Mai *et al.*[36], the terpenoid compounds from *E. helioscopia* are potent inhibitors of P-glycoprotein (ABCB1) and they have also exhibited cytotoxic activity against MDA-MB-231 cell lines.

Prakash and Gupta[5] examined the cytotoxic activity of *E. helioscopia* ethanolic extract (EHE) against colon cancer cell line (Colon HT-29, SW-20, SiHa), liver cancer cell line (Hep-2), breast cancer cell line (T-47D), cervix cancer cell line OVCAR-5 and prostate cancer cell line (PC-3). These authors concluded that ethanol extract of *E. helioscopia* inhibited the growth of three human cancer cell lines: Hep-2, T-47D and PC-5. Wang *et al.*[37] evaluated the anticancer effects of *E. helioscopia* extracts on 5 different human cancer cell lines. In their study they showed that the extract significantly inhibited the proliferation of SMMC-7721 cells in a time and dose-dependent manner. The extract treatment arrested cell cycle in G1 phase. After extract treatment at higher concentrations, the percentage of apoptotic cells was increased. Based on this data, Wang *et al.*[37] concluded that extract of *E. helioscopia* could have chemopreventive potential against the human cancer.

For the first time, the proliferative effects and anticancer activity of *E. cactus* Ehrenb extract were investigated on human breast cancer cell line (MCF-7), prostate cell line (PC-3), human hepatocellular carcinoma

cell line (HEPG2), normal breast epithelial cell line (MCF-10A) and human foreskin fibroblast cell line (Hs68). Since some relationships between cytotoxicity and antioxidant activity have been substantiated[38], the antioxidant potential of euphorbia extract was examined, through DPPH scavenging test. Of the three cancer cell lines investigated, MCF-7 was the most sensitive to *E. cactus* Ehrenb methanolic extract. Moreover, cell proliferation upon exposure to plant extract decreased in distinct dose and time-dependent manners. Interestingly, no effect on normal foreskin fibroblast cell line (Hs68) viability was recorded, suggesting that the methanolic extract was not cytotoxic to non-cancerous cells.

Several species of *Euphorbia* have been tested for their antiviral activity. Among 10 species tested, 3 showed potent antiviral activity [39-41]. Sudhakar *et al.*[42] suggested that *E. hirta* exhibited activity against both Gram-positive and Gram-negative organisms which are *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. aureus.* Antibacterial activity has also been reported for different *Euphorbia* sp.[43] and this agrees with the present study.

Since no antimicrobial investigation has been carried out on *E. cactus* Ehrenb, it is hard to attribute the antiviral activity of *E. cactus Ehrenb* to any specific type of compound(s). Therefore, the present study was carried out to investigate the antimicrobial activity of *E. cactus* Ehrenb so that the justification for the isolation for the isolation of its antiviral components could be achieved.

Most extracts or pure compounds obtained from plants act as antiviral via two mechanisms including exhibiting their effects on viral particles prior to attachment to host cell or after the virus enters the host cell. The bioactive component(s), namely, protein and some derived polyphenolic compounds such as polysaccharides, lignins and bioflavonoids, were reported to act principally by binding to the protein coat and thus arrest absorption of the virus[44].

The greater resistance of Gram-negative bacteria to plant extracts has been reported previously[45-47] and it is supported by this study. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to the many environmental substances including antibiotics.

CONCLUSION

To the best of our knowledge, this is the first investigation about cytotoxic, antioxidant and antimicrobial effects associated with *E. cactus* Ehrenb plant extract. Results of this study indicated that this plant has potential as a source of anticancer agents for breast cancer treatments. Further studies are required to assess the active ingredients of *E. cactus* Ehrenb, involved in the antiproliferative or cytotoxic effects of this plant. The type(s) of effects applied by the plant component(s) involved in its activity must be further analyzed by molecular or other relevant techniques. Many other plants are waiting to be examined for any possible therapeutic effects and if present they may represent a convenient and cheap means to manage with many types of cancer.

SIGNIFICANCE STATEMENT

The present study reports on the anticancer, antioxidant and antimicrobial activities of methanolic extract of *E. cactus* Ehrenb sampled from Jazan region, Saudi Arabia. It aimed to expand upon the already existing knowledge regarding Euphorbia genus activities. The current study demonstrated for the first time the high capacity of the extract to has anticancer effects against cancer cells from 3 types of cancerous cell lines. This may be a promising finding for future cancer treatments and in the prevention of cancer using natural plants.

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REFERENCES

1. Al-Faifi, Z.I.A., Y.S. Masrahi, M.S. Aly, T.A. Al-Turki and T. Dardeer, 2017. Evaluation of cytotoxic and genotoxic effects of *Euphorbia triaculeata* Forssk. extract. Asian Pac. J. Cancer Prev., 18: 771-777.
2. Gupta, N., G. Vishnoi, A. Wal and P. Wal, 2013. Medicinal value of *Euphorbia tirucalli*. Syst. Rev. Pharm., 4: 40-46.
3. Nyeem, M.A.B., M.S. Haque, M. Akramuzzaman, R. Siddika, S. Sultana and B.M.R. Islam, 2017. *Euphorbia hirta* Linn. A wonderful miracle plant of Mediterranean region: A review. J. Med. Plants Stud., 5: 170-175.
4. Shaaban, M., M. Ali, M.F. Tala, A. Hamed and A.Z. Hassan, 2018. Ecological and phytochemical studies on *Euphorbia retusa* (Forssk.) from Egyptian habitat. J. Anal. Methods Chem., Vol. 2018. 10.1155/2018/9143683.
5. Prakash, E. and D.K. Gupta, 2013. Cytotoxic activities of extracts of medicinal plants of euphorbiacae family studied on seven human cancer cell lines. Universal J. Plant Sci., 1: 113-117.

6. Frohne, D. and H.J. Pfander, 2005. Poisonous Plants: A Handbook for Doctors, Pharmacists, Toxicologists, Biologists and Veterinarians. 2nd Edn., Timber Press, Portland OR., USA., ISBN-13: 9780881927504, pp: 183.
7. Kumar, S., R. Malhotra and D. Kumar, 2010. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses and pharmacological activities. Pharmacogn. Rev., 4: 58-61.
8. Pascal, O.A., A.E.V. Bertrand, T. Esaie, H.A.M. Sylvie and A.Y. Eloi, 2017. A review of the ethnomedical uses, phytochemistry and pharmacology of the *Euphorbia* genus. Pharma Innov. J., 6: 34-39.
9. Al-Hajj, M.M.A., H.A. Al-Shamahy, B.Y. Alkhatib and B.A. Moharram, 2018. *In vitro* anti-leishmanial activity against cutaneous leishmania parasites and preliminary phytochemical analysis of four Yemeni medicinal plants. Universal J. Pharm. Res., 3: 48-54.
10. Ramezani, M., J. Behravan, M. Arab and S.A. Farzad, 2008. Antiviral activity of *Euphorbia helioscopia* extract. J. Biol. Sci., 8: 809-813.
11. Strober, W., 2015. Trypan blue exclusion test of cell viability. Curr. Protoc. Immunol., 111: A3.B.1-A3.B.3.
12. Van Meerloo, J., G.J.L. Kaspers and J. Cloos, 2011. Cell sensitivity assays: The MTT assay. Methods Mol. Biol., 731: 237-245.
13. Kaluderovic, G.N., T. Krajnovic, M. Momcilovic, S. Stosic-Grujicic, S. Mijatovic, D. Maksimovic-Ivanic and E. Hey-Hawkins, 2015. Ruthenium(II) *p*-cymene complex bearing 2,2-dipyridylamine targets caspase 3 deficient MCF-7 breast cancer cells without disruption of antitumor immune response. J. Inorg. Biochem., 153: 315-321.
14. Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. dos Santos, C.S. Coube and S.G. Leitao, 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127-130.
15. Cragg, G.M. and D.J. Newman, 2005. Plants as a source of anti-cancer agents. J. Ethnoharmacol., 100: 72-79.
16. Akujobi, C., B.N. Anyanwu, C. Onyeze and V.I. Ibekwe, 2004. Antibacterial activities and preliminary phytochemical screening of four medical plants. J. Applied Sci., 7: 4328-4338.
17. Esimone, C.O., M.U. Adikwu and J.M. Okonta, 1998. Preliminary antimicrobial screening of the ethanolic extract from the *Lichen usnea subfloridans* L. J. Pharm. Res. Dev., 3: 99-101.
18. Osadebe, P.O. and S.E. Ukwueze, 2004. A comparative study of the phytochemical and anti-microbial properties of the eastern Nigerian specie of African mistletoe (*Loranthus micranthus*) sourced from different host trees. Bio-Research, 2: 18-23.
19. Balunas, M.J. and A.D. Kinghorn, 2005. Drug discovery from medicinal plants. Life Sci., 78: 431-441.
20. Tan, G., C. Gyllenhaal and D.D. Soejarto, 2006. Biodiversity as a source of anticancer drugs. Curr. Drug Targets, 7: 265-277.
21. Jones, W.P., Y.W. Chin and A.D. Kinghorn, 2006. The role of pharmacognosy in modern medicine and pharmacy. Curr. Drug Targets, 7: 247-264.
22. Khan, N., V.M. Adhami and H. Mukhtar, 2008. Apoptosis by dietary agents for prevention and treatment of cancer. Biochem. Pharmacol., 76: 1333-1339.
23. Khan, N., V.M. Adhami and H. Mukhtar, 2010. Apoptosis by dietary agents for prevention and treatment of prostate cancer. Endocr. Relat. Cancer, 17: R39-R52.
24. Barbaste, M., B. Berkee, M. Dumas, S. Soulet and J.C.L. Delaunay *et al*., 2002. Dietary antioxidants, peroxidation and cardiovascular risks. J. Nutr. Health Aging, 6: 209-223.
25. Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.
26. Mahdi-Pour, B., S.L. Jothy, L.Y. Latha, Y. Chen and S. Sasidharan, 2012. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac. J. Trop. Biomed., 2: 960-965.
27. Mothana, R.A.A., S.A.A. Abdo, S. Hasson, F.M.N. Althawab, S.A.Z. Alaghbari and U. Lindequist, 2010. Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some yemeni medicinal plants. Evidence-Based Complement. Alternat. Med., 7: 323-330.
28. Jung, M.J., H.Y. Chung, S.S. Kang, J.H. Choi, K.S. Bae and J.S. Choi, 2003. Antioxidant activity from the stem bark of *Albizzia julibrissin*. Arch. Pharm. Res., 26: 458-462.
29. Gil-Izquierdo, A., M.I. Gil, F. Ferreres and F.A. Tomás-Barberán, 2001. *In vitro* availability of flavonoids and other phenolics in orange juice. J. Agric. Food Chem., 49: 1035-1041.
30. Chi, S.M., Y. Wang, Y. Zhao, J.X. Pu and X. Du *et al*., 2012. A new cyclopentanone derivative from *Euphorbia hirta*. Chem. Nat. Compounds, 48: 577-579.
31. Patil, S.B. and C.S. Magdum, 2011. Phytochemical investigation and antitumour activity of *Euphorbia hirta* Linn. Eur. J. Exp. Biol., 1: 51-56.
32. Ping, K.Y., I. Darah, U.K. Yusuf, C. Yeng and S. Sasidharan, 2012. Genotoxicity of *Euphorbia hirta*: An allium cepa assay. Molecules, 17: 7782-7791.
33. Munro, B., Q.V. Vuong, A.C. Chalmers, C.D. Goldsmith, M.C. Bowyer and C.J. Scarlett, 2015. Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. Antioxidants, 4: 647-661.
34. Hsieh, Y.J., C.J. Chang, C.F. Wan, C.P. Chen, Y.H. Chiu, Y.L. Leu and K.C. Peng, 2013. *Euphorbia formosana* root extract induces apoptosis by caspase-dependent cell death via Fas and mitochondrial pathway in THP-1 human leukemic cells. Molecules, 18: 1949-1962.

35. Cheng, J., W. Han, Z. Wang, Y. Shao and Y. Wang *et al*., 2015. Hepatocellular carcinoma growth is inhibited by *Euphorbia helioscopia* L. extract in nude mice xenografts. BioMed Res. Int., Vol. 2015. 10.1155/2015/601015.
36. Mai, Z.P., G. Ni, Y.F. Liu, L. Li and G.R. Shi *et al*., 2017. Heliosterpenoids A and B, two novel jatrophane-derived diterpenoids with a 5/6/4/6 ring system from *Euphorbia helioscopia*. Scient. Rep., Vol. 7. 10.1038/s41598-017-04399-w.
37. Wang, Z.Y., H.P. Liu, Y.C. Zhang, L.Q. Guo, Z.X. Li and X.F. Shi, 2012. Anticancer potential of *Euphorbia helioscopia* L extracts against human cancer cells. Anatomical Record, 295: 223-233.
38. Grigalius, I. and V. Petrikaite, 2017. Relationship between antioxidant and anticancer activity of trihydroxyflavones. Molecules, Vol. 22. 10.3390/molecules22122169.
39. Abdelgaleil, S.A.M., S.M.I. Kassem, M. Doe, M. Baba and M. Nakatani, 2001. Diterpenoids from *Euphorbia paralias*. Phytochemistry, 58: 1135-1139.
40. Madureira, A.M., J.R. Ascenso, L. Valdeira, A. Duarte, J.P. Frade, G. Freitas and M.J.U. Ferreira, 2003. Evaluation of the antiviral and antimicrobial activities of triterpenes isolated from *Euphorbia segetalis*. Nat. Prod. Res., 17: 375-380.
41. Tanaka, T., K. Kasubuchi, S. Kita, H. Tokuda, H. Nishino and S. Matsunaga, 2000. Bioactive steroids from the whole herb of *Euphorbia chamaesyce*. J. Nat. Prod., 63: 99-103.
42. Sudhakar, M., C.V. Rao, P.M. Rao, D.B. Raju and Y. Venkateswarlu, 2006. Antimicrobial activity of *Caesalpinia pulcherrima*, *Euphorbia hirta* and *Asystasia gangeticum*. Fitoterapia, 77: 378-380.
43. Annapurna, J., I.P. Chowdary, G. Lalitha, S.V. Ramakrishna and D.S. Iyengar, 2008. Antimicrobial activity of *Euphorbia nivulia* leaf extract. Pharm. Biol., 42: 91-93.
44. Jassim, S.A.A. and M.A. Naji, 2010. *In vitro* evaluation of the antiviral activity of an extract of date palm (*Phoenix dactylifera* L.) pits on a pseudomonas phage. Evid. Based Complement. Altern. Med., 7: 57-62.
45. Paz, E.A., R.N. Lacy and M. Bakhtian, 1995. The β-Lactum Antibiotics Penicillin and Cephalosporin in Perspective. Hodder Strong, London, Pages: 324.
46. Vlietinck, J., L. van Hoof, J. Totte, A. Lasure, D.V. Berghe, P.C. Rwangabo and J. Mvukiyumwami, 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. J. Ethnopharmacol., 46: 31-47.
47. Kudi, A.C., J.U. Umoh, L.O. Eduvie and J. Gefu, 1999. Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol., 67: 225-228.