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Cytotoxicity, Antioxidant Activity and Phytochemical Screening of Four *Euphorbia* Species from Jordan

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ABSTRACT

The current study was designed to reveal the main groups of secondary metabolites in the methanol (M) and acetone (A) extracts from four *Euphorbia* species from Jordan. The extracts of these species were also tested for their total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and cytotoxic effects against human MDA-MB-231 cancer cells. Different groups of secondary metabolites were found in the M and A extracts of *E. hierosolymitana*, *E. aleppica*, *E. petiolata*, and *E. prostrata*. All extracts had moderately high TPC and TFC. Of all species, both extracts of *E. aleppica* had an exceptionally high TFC that far exceeded its content in all other extracts (1462.67 ± 0.04; 1600.44 ± 0.03 mg quercetin equivalents/g of extract, respectively). *E. prostrata* extracts had high amounts of TPC (182.61 ± 0.12; 187.35 ± 0.07 mg gallic acid equivalents/g extract) and TFC (629.78 ± 0.00; 774.22 ± 0.01 mg quercetin/g extract). Extracts obtained from the tested *Euphorbia* species had moderate high antioxidant activity, but the DPPH and ABTS-scavenging activity of the *E. prostrata* extracts was the highest, but were lower than those observed for the the positive controls (range 0.004–0.009 mg/mL). The methanol extract of *E. hierosolymitana* also showed strong DPPH scavenging activity (0.007 ± 0.002 mg/mL). The acetone extract of *E. prostrata* showed much stronger anticancer activity than the positive control drug, doxorubicin (62.5±3.71 mg/mL), against the human breast cancer MDA-MB-231 (HTB-26TM) cell line (IC_{50} value: 58 ± 3.71 µg/mL).

Keywords: *E. prostrata*; *E. heirosolymitana*; *E. aleppica*; *E. petiolata*; Antioxidants; Cytotoxicity; MDA-MB-231 (HTB-26TM).

Introduction

The Euphorbiaceae family is widely distributed and is primarily found in tropical and temperate climates worldwide.[1] Small ephemerals, numerous types of herbaceous plants, cushion sub-shrubs, large shrubs, small trees, and succulent cacti are among the habits of the *Euphorbia* species.[2] A recent study on the taxonomy of the family revealed the detection of 33 species and five genera belonging to the Euphorbiaceae family in Jordan. Of these five genera, *Euphorbia* is the largest one with 26 species, including *E. aleppica*, *E. petiolata*, *E. prostrata*, and *E. hierosolymitana*.[3] These species and other members of the were reported to grow wild in the Mediterranean phytogeographical zone, which extends from Um-Qais in the north to the tops of the Tafela mountains in the south of Jordan.

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Additionally, they were discovered on the slopes of the Ajlun and Tafela Mountains, as well as in the Irano-Turanian region, which encompasses the Saharo-Arabian and Sudanian regions.[4]

Euphorbia plants are generally characterized by their poisonous white, milky latex.[5] Previous reports have shown that latex-bearing plants have been used to treat a wide range of ailments, including diabetes, asthma, dysentery, diarrhea, malaria, and skin conditions. The leaves of these plants have also been eaten to treat constipation and applied externally to treat skin abnormalities like warts.[6,7]

It was recognized that a potential reason for the high cancer rates in northern Iran was attributed to grain harvests containing *Euphorbia* seeds.[8] For hundreds of years, a number of *Euphorbia* species were utilized in traditional medicine on the premise that they were excellent anticancer medicines. Because of their cytotoxicity, antiviral, antibacterial, and antifungal properties, they were also used to treat warts, tumors, migraines, skin conditions, gonorrhea, and intestinal parasites.[9] Species belonging to this genus have been the subject of various phytochemical investigations that have identified a number of groups of secondary metabolites, including phosphatidyl choline, phosphatidyl triterpene, flavonoids, astriacylglycerols, and diterpenoids.[10-19]

The current study was designed to reveal the main groups of secondary metabolites, in addition to evaluating the antioxidant activity, and cytotoxic potentials of four Euphorbia species from the Jordanian flora including *Euphorbia petiolata* Banks & Sol., *Euphorbia hierosolymitana* Boiss. *Ex* Boiss.var. *hierosolymitana*,

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Euphorbia prostrata Ait., and *Euphorbia aleppica* L. The cytotoxic potentials were specifically assessed against the MDA-MB-231 breast cancer cell line. Thorough literature survey revealed that studies related to investigating the anticancer properties, antioxidant levels, and phytochemical screening of these four species were limited.

Materials and Methods

Plant specimens

Whole plant material (Table 1) was collected from Irbid governorate, north of Jordan, during the full flowering period of the year 2019, as described in Table 1. The taxonomic identity of all four Euphorbia species was confirmed by Prof. Jamil Lahham from Department of Biological Sciences, Yarmouk University, Irbid, Jordan. The collected plant were allowed to completely dry completely at room temperature and in shade (two weeks). The dried plant material was then grinded to fine powder and stored at -20°C. All four species were treated similarly.

Preparation of the crude extracts

Soxhlet extraction was performed to extract the secondary metabolites from each plant. Briefly, a sample of 60 g of air-dried plant material (powder form) was loaded into a soxhlet extractor and defatting was performed firstly by extraction with petroleum ether. Then, the dried plant residue was subjected to extraction with either acetone (A) or methanol (M) (500 mL, 12 hr) to obtain the required extracts (A and M extracts, respectively). Dried extract residues were then kept at 4 ^{0}C until analysis.

Qualitative phytochemical tests

The methanol (M) and acetone (A) extracts obtained from the different four *Euphorbia* species were subjected to qualitative chemical tests to determine the main classes of secondary metabolites according to the procedures listed in the literature.[20,21]

TFC

The colorimetric aluminum chloride method was used for the determination of the TFC for the two extracts obtained from the four species of *Euphorbia* as described in the literature.[22-27] In summary, four milliliters of distilled water were added to separate ten milliliter volumetric flasks followed by the addition of 1 milliliter (0.5 mg/mL) aliquot of the investigated extract. Each flask was then filled with 0.3 mL of 5% sodium nitrite ($NaNO_2$), and then was allowed to stand for five minutes. Subsequently, 10% aluminum chloride ($AlCl_3$) (0.3 mL) was added, and the mixture was incubated for 6 minutes. After that, 2 mL of 1.0 M sodium hydroxide (NaOH) was added, followed by the addition of 10 mL of distilled water to complete the volume. After fifteen minutes, the absorbance was measured at 510 nm using methanol as the reference. The standard utilized was quercetin. Every measurement was done three times. TFC is given as mg quercetin/g of extract.

TPC

The Folin-Ciocalteu assay was used to determine the TPC in accordance with the protocol outlined in published literature.[22-27] To do this, a 0.5 mL aliquot of extract solution (0.5 mg/mL) was once again treated with 2 mL of 7.5% sodium carbonate (Na_2CO_3) and 2.5 mL of a diluted 10-fold Folin-Ciocalteu reagent. After 15 minutes of room temperature treatment, the mixture's absorbance at 760 nm wavelength was measured using a spectrophotometer. Gallic acid was used as the standard and methanol as the blank to create the calibration curve. Every measurement was done three times. TPC is reported as mg gallic acid/gram of extract.

Evaluation of Antioxidant Activity

The antioxidant activity of each of M and A extracts obtained from the four Euphorbia species was assessed using the DPPH and ABTS radical scavenging test techniques, in accordance with the protocol outlined in the literature.[22-27] The % scavenging activity was calculated according to the following equation:

$$\%\text{Scavenging activity} = \frac{(A_S - A_C)}{A_C} * 100$$

whereas is the extract's absorbance and Ac is the control's absorbance. Plotting the % antiradical activity against the tested extract concentration allowed for the calculation of the IC_{50} values.

DPPH$^{\cdot}$ radical scavenging method

Using a process largely unchanged from the literature, the extracts scavenging capability was calculated and compared to the scavenging activities of the positive controls, ascorbic acid and α-tocopherol, by using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay.[22-28] This procedure involved adding 2 mL of 0.1 mM DPPH solution at various concentrations (0.005, 0.01, 0.05, 0.1, and 0.5 mg/mL) to 2 mL of extract. For thirty minutes, the mixes were incubated in the dark. Next, using methanol as a blank, the absorbance was measured at 517 nm. Every experiment was carried out three times.

ABTS radical scavenging method

After a slight adjustment, the method described was applied to the radical cation ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) decolorization assay to determine the overall antioxidant activity.[22-28] One milliliter of extract solutions at various concentrations (0.005, 0.01, 0.05, 0.1, and 0.5 mg/mL) was combined with three milliliters of ABTS solution. The absorbance was determined with a UV-VIS spectrophotometer at 734 nm. Every test included a blank, and measurements were collected after at least five minutes. The extract's ability to scavenge ABTS was compared to standards for ascorbic acid and α-tocopherol. Plotting the percentage of antiradical activity against the concentration of the tested extracts or positive controls using a linear regression method allowed for the determination of the IC_{50} values.

Table 1: Plant species used in this study, along with information on their habits, habitats, flowering times, and distribution.[4]

Plant species	Life form	Habitat	Flowering period	Distribution	Voucher spicemen No
E. hierosolymitana	Chamaephyte	Batha and garigue*	March-April	Jarash, Ajlun, Tafilah and Amman	E/Ehv/2019
E. aleppica	Therophyte	Weed among summer crops	May-June	Upper Jordan Valley, Ajlun and Tafilah	E/Eal/2019
E. petiolata	Therophyte	Fallow fields on heavy red soil	May-June	Dead Sea area, Ajlun, Amman and Tafilah	E/Epe/2019
E. prostrata	Therophyte	Road slides &lawns in red soil	May-June	Jarash, Dead Sea area and Ajlun	E/Epr/2019

*Batha (Mediterranean dwarf shrub formation) and garigue (Mediterranean half shrub formation somewhat taller than batha).

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Assay for Cytotoxicity in Cell Culture

The extracts obtained from the four *Euphopia* species were assayed for the cytotoxic activity against human breast cancer cell line MDA-MB-231 (HTB-26TM). Before being used, cell lines were taken out of liquid nitrogen and quickly thawed in a water bath at 37 °C. Following a 70% ethanol disinfection of the vial exteriors, the vials were carefully put into a 15 mL centrifuge tube containing Dulbecco's modified essential medium (DMEM) that had been heated to 37 °C under sterile circumstances. It washed the cells in DMEM at room temperature (23 °C), and then they were grown with 10% fetal bovine serum (FBS), 20 µM L-glutamine, 50 IU/ml penicillin, and 50 µg/mL streptomycin.

The cells were cultured using a trypsin-EDTA solution and maintained at 37 °C in an incubator with 5% CO_2 and 95% humidity.[29]

Seeding of the MDA-MB-231 cell line

The MDA-MB-231 cell line was prepared for seeding in 96-well arrays using a confluent flask. After adding 2 mL of trypsin and washing the flask (75 cm^2) with 6 mL of phosphate buffered saline (PBS), it was incubated for 10 minutes at 37 °C. The 75 cm^2 flask was filled with 6 mL of new medium. The cell suspension was then moved to a sterile tube, and 25 µL was taken out and put into an Eppendorf tube with 25 µL of trypan blue dye. To achieve the necessary final cell density, a suitable volume was taken out of the cell suspension and filled with fresh medium. Using a multichannel pipette, 100µL of the cell suspension was seeded into each of the 96 well ELISA sheets, and the cells were allowed to attach through incubation. 1 × 104 cells/mL were either left untreated (cultured in growth medium alone) or treated separately in growth medium for 48 hours at 37 °C with 1000, 500, 250, 125, 62.5, and 31.25 µg/mL of the 8 plant extracts (Stock of 1 mg/mL).[29]

Cell Viability Assay (MTT)

Utilizing the MDA-MB-231 cell line and the cell viability (MTT assay), the cytotoxicity effects of plant extracts were investigated. The MTT assay (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, USA) was used to check how alive cells were after being incubated with different amounts of plant extract. In this experiment, doxorubicin (EBEWE Pharma, Austria) served as the positive control, while the whole media served as the negative control. The cancer cell line was grown in 96-well tissue culture plates (Greiner, Germany) for 48 hours at 37 °C, 5% CO_2, and 95% humidity. Four hours before the incubation ended, 20 µL of phosphate buffered saline (PBS) containing 5 mg/mL MTT was added. Following the completion of incubation, culture plates were centrifuged, and 100 µL of solubilization solution (absolute dimethyl sulfoxide, or DMSO) was added after the supernatant was removed. Using a plate spectrophotometer (sunrise) set to absorbance at 570 nm, the developed color was measured. The following formula determined the percentage of cells that survived:

$$\%\text{Viability} = \frac{(\text{OD Negative control (Media)} - \text{OD for sample})}{(\text{OD negative control} - \text{OD positive control (Doxorubicin))}} * 100$$

To calculate the cell cytotoxicity percentage, deduct the viability percentage from 100. The levels at which each cell line exhibits a 50% inhibition of growth are known as IC_{50} values.[30]

Statistical analysis

The averages ± SD of the findings from three separate studies with comparable patterns make up the given data. In each of the three separate studies, each concentration was examined in triplicate. The GraphPad Prism 6 software's two-way ANOVA was used for statistical analysis.

Results and Discussion

Phytochemical screening

The results obtained from the chemical tests performed on acetone (A) and methanol (M) extracts of *E. petiolata*, *E. hierosolymitana*, *E. prostrata*, and *E. aleppica* are shown in Table 2. The investigated extracts were found to be rich in various types of secondary metabolites, containing mainly flavonoids, tannins, saponins, and glycosides. Alkaloids, anthraquinones, and phobatannins were completely absent.

Determination of the total phenolic content (TPC) and total flavonoid content (TFC).

Results for the TPC and TFC are shown in Table 3. Among the different species investigated, the M and A extracts of *E. prostrata* had the highest amounts of TPC (182.606 ± 0.12 mg gallic acid/g dry extract; 187.35 ± 0.07 mg gallic acid/g dry extract, respectively). The A and M extracts of *E. aleppica* had remarkably high TFC (1600.44 ± 0.03; 1462.67 ± 0.04 mg quercetin/gry extract, respectively) that far exceeded the content observed for all other extracts of the different species. Table 3 results showed that there was a statistically significant difference ($p<0.05$) in each plant between the methanol and acetone extracts.

Antioxidant Activity

Many plant extracts, particularly those containing flavonoids and phenolic acids, have strong antioxidant qualities.[30] The DPPH and ABTS test techniques were used to assess the free radical scavenging activity of the M and A extracts derived from *E. hierosolymitana*, *E. aleppica*, *E. petiolata*, and *E. prostrata*. Results for the DPPH and ABTS antioxidant activities are listed in Table 4. Statistical analysis revealed no significant differences in the antioxidant activity of both M and A extracts for four plant species ($P > 0.05$). The antioxidant activity of M and A extracts as determined by the two methods was concentration dependent (Figures 1 and 2).

Table 2: Significant phytochemical groups were found in the crude extract fractions of *E. petiolata*, *E. hierosolymitana*, *E. aleppica*, and *E. prostrata*.

Test	*E. hierosolymitana*		*E. aleppica*		*E. petiolata*		*E. prostrata*	
	M	**A**	**M**	**A**	**M**	**A**	**M**	**A**
Flavonoids	+*	+	+	+	+	+	+	+
Tannins	+	-	-	-	+	-	+	+
Saponins	+	-	+	+	+	-	+	-
Alkaloids	-	-	-	-	-	-	-	-
Reducing sugers	+	+	-	-	-	-	-	+
Anthraquinones	-	-	-	-	-	-	-	-
Glycosides	+	-	+	-	+	-	+	-
Phobatannins	-	-	-	-	-	-	-	-
Proteins	-	+	-	+	-	+	-	+

* The phytoconstituents are shown by positive (+) and negative (-) signs, respectively, indicating their presence or absence.

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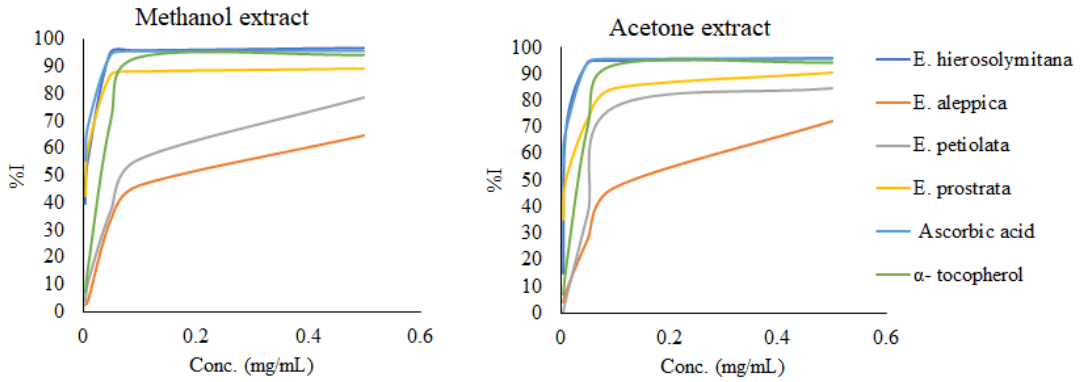

Figure 1: Antioxidant activities of crude extracts from *E. hierosolymitana*, *E. aleppica*, *E. petiolata* and *E. prostrata* and positive controls by using DPPH. Values expressed mean of three parallel measurements. Mean values are not significantly different (P > 0.05).

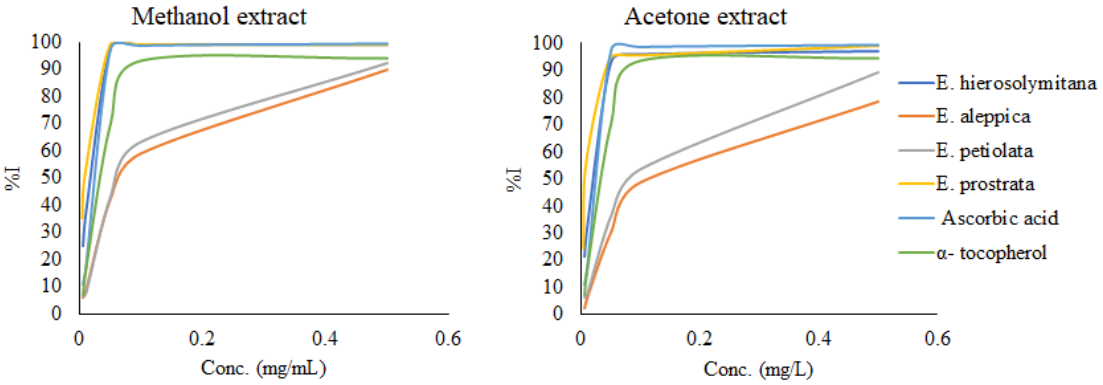

Figure 2: Antioxidant activities of the methanol (M) and acetone (A) extracts obtained from *E. hierosolymitana, E. aleppica*, *E. petiolata* and *E. prostrata* and positive controls evaluated by ABTS assay method. Values expressed mean of three parallel measurements. Mean values are not significantly different (P > 0.05).

Both extracts (M and A) obtained from *E. prostrata* had the highest levels of DPPH (IC_{50} 0.004 ± 0.000; 0.006 ± 0.004 mg/mL) and ABTS (0.007 ± 0.002; 0.009 ± 0.002 mg/mL) when compared to the positive controls. This was in agreement with the observed high TPC and TFC for *E. prostrata*. Interestingly, *E. aleppica* showed only moderate antioxidant activity in both assay methods, despite its highest TFC, which far exceeded all other extracts. It's worth mentioning that *E. hierosolymitana* showed interestingly high DPPH antioxidant activity that was slightly lower than those observed for *E. prostrata* (0.005 ± 0.0007; 0.009 ± 0.0009 mg/mL) that could also be correlated with its high TPC and TFC (Table 3). Results shown in Table 4 demonstrated no statistically significant variations in the antioxidant activity of methanol and acetone extracts across the four plant species (P > 0.05).

Anticancer activity (MTT assay)

The triple-negative MDA-MB-231 breast cancer cell line is one of the most aggressive cell lines that can only be treated with chemotherapy. Thus, the extracts of the four different *Euphorbia* species were screened for their cytotoxic activity against this type of cancer cell line to unveil their antitumor potentials.

A test called the MTT assay was used to see how well the methanol and acetone extracts from the four species of *Euphorbia* killed MDA-MB-231 breast cancer cells in a lab setting. Different concentrations of the two extracts (1000, 500, 250, 125, 62.5, and 31.2 µg/mL) were treated with the cells. The effect on cells changed with increasing concentrations; the effect got stronger as the tested extracts' concentrations went up (Figure 3), mostly between 58 and 262 µg/mL (Table 5). Based on the obtained results, the tested *Euphopia* plants were consolidated into three groups according to their anticancer activity. In general, the acetone extract of all tested plants had higher antitumor activity as compared to the methanol extract, except for *E. aleppica*. The acetone extract of *E. prostrata* had the highest cytotoxic activity against MDA-MB-231 cell lines (IC_{50} 54 µg/mL) which was slightly higher than that observed for the positive control doxorubicin (IC_{50} value is: 62.5± 3.71 µg/mL). This could be attributed to its high TFC. It was noticed that two extracts showed moderate antitumor activity of comparable values, including the methanol extract of *E. petiolata* (IC_{50} 135 ± 2.02 µg/mL) and the acetone extracts of *E. hierosolymitana* (IC_{50} 135 µg/mL). The remaining extracts showed interestingly moderate activity at concentration levels exceeding 200 µg/mL with the lowest activity observed for the methanol extract of *E. aleppica* (262 ± 2.02 µg/mL). However, the strong antitumor activity observed for *E. prostrata* could be attributed to its strong antioxidant activities, which are correlated with its high TPC and TFC.

The results of the current investigation revealed that *Euphorpia* species had a wide spectrum of secondary metabolites that included flavonoids, tannins, saponins, and glycosides. The studied extracts were principally rich in phenolics and flavonoids as could be deduced from their TPC and TFC. Previous phytochemical investigations of the alcoholic extract obtained from the aerial parts of *E. prostrata*, *E. milii*, *E. hirta*, *E. helioscopia*, and *E. caducifolia* indicated the detection of alkaloids, steroids, terpenoids, flavonoids, tannins, and terpenoids.[31-37] In addition to these classes, the aqueous extract of *E. heterophylla* contained saponins.[38] This wide spectrum of secondary metabolites contributes greatly to minimizing oxidative stress, cancer, impotency, cardiac dysfunction, and microbial inhibition.[39]

Phenolic compounds and flavonoids are characterized for their diverse biological activities, especially as antioxidants.[40]

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Table 3: Results for the crude extract fractions of *E. petiolata*, *E. hierosolymitana*, *E. prostrata* and *E. aleppica* were obtained for TPC (mg gallic acid/g dry extract) and TFC (mg quercetin/g dry extract).

Plant	TPC (mg gallic acid/g dry extract)		TFC (mg quercetin/ g dry extract)	
	M	A	M	A
E. hierosolymitana	102.10 ± 0.10	116.00 ± 0.10	516.44 ± 0.01	560.89 ± 0.02
E. aleppica	51.60 ± 0.06	34.46 ± 0.02	1462.67 ± 0.04	1600.44 ± 0.03
E. petiolata	38.57 ± 0.00	41.50 ± 0.03	253.78 ± 0.01	352.67 ± 0.04
E. prostrata	182.606 ± 0.12	187.35 ± 0.07	629.78 ± 0.00	774.22 ± 0.01

A $p < 0.05$ value was considered statistically significant.

Table 4: IC_{50} (mg/mL) for the antioxidant activity of the methanol (M) and acetone (A) extracts obtained from *E. hierosolymitana*, *E. aleppica*, *E. petiolata* and *E. prostrata* and the positive controls

	IC_{50} (mg/mL)			
	DPPH		ABTS	
	M	A	M	A
E. hierosolymitana	0.005 ± 0.0007	0.009 ± 0.0009	0.013 ± 0.001	0.015 ± 0.0006
E. aleppica	0.118 ± 0.003	0.120 ± 0.007	0.042 ± 0.03	0.054 ± 0.04
E. petiolata	0.088 ± 0.006	0.060 ± 0.001	0.063± 0.006	0.085 ± 0.001
E. prostrata	0.004 ± 0.000	0.006 ± 0.004	0.007 ± 0.002	0.009 ± 0.002
Ascorbis acid	$(1.79 \pm 0.06) \times 10^{-3}$		$(1.89 \pm 0.06) \times 10^{-3}$	
α-tocopherol	$(2.33 \pm 0.04) \times 10^{-3}$		$(1.81 \pm 0.01) \times 10^{-3}$	

Values expressed mean ± SD of three parallel measurements. SD: Standard deviation; Mean values are not significantly different (P > 0.05).

Table 5: MTT assay IC_{50} (µg/mL) values for the plant's extracts.

MDA-MB 231 IC_{50} (µg/mL)*				
Extracts	***E. hierosolymitana***	***E. aleppica***	***E. petiolata***	***E. prostrata***
Methanol	260 ± 3.19	262 ± 2.02	135 ± 2.02	250 ± 2.07
Acetone	150 ± 2.13	210 ± 2.69	253 ± 1.28	58 ± 2.69[A]
Doxorubicin		62.5 ± 3.71		

Values expressed are means ± SD of two parallel measurements. SD: Standard deviation, [A] not significantly different from the positive control (P > 0.05)

The results of current investigation revealed higher TPC and TFC in the extracts of the four *Euphorbia* species as compared to the work listed in the literature, [39, 41-44] especially for the TFC observed for *E. aleppica* (1462.67 ± 0.036; 1600.44 ± 0.026 mg of quercetin/g, respectively).

In the current study, the methanol extracts of *E. prostrata* and *E. hierosolymitana* had the highest DPPH antioxidant activities of almost comparable values (Table 4). The acetone extract of *E. prostrata* showed almost the same strong antioxidant power as determined by the ABTS assay method, indicating a variable composition that can scavenge radicals in different mechanisms. Previous work on the different extracts of several *Euphorbia* species revealed interesting antioxidant activities. The methanol extract of *E. prostrata* collected from Pakistan showed interesting DPPH scavenging activity reaching 50.25 % at a concentration level of 250 g/mL.[39] The methanolic extract of *E. cactus* Ehrenb. had strong DPPH scavenging activity (IC_{50} is 7.89 µg/ mL) as compared to ascorbic acid (IC_{50} is 5.63 µg/ ml).[45] Also, the aqueous extract of *E. heterophylla* had a moderately strong DPPH scavenging power (IC_{50}: 141.11 ± 4.23 µg/mL).[38] The most active extract was discovered to have antioxidant activity, and it matched that of α-tocopherol in the ethanol extract of *E. acanthothamnos* from Turkey.[42] At doses higher than 0.2 mg/mL, *E. golondrina* was shown to have stronger ABTS scavenging activity than butylated hydroxytoluene (BHT).[46]

The high antitumor activity results observed for the four *Euphorbia* species, especially for *E. prostrata*, could be attributed not only to their wide spectrum of secondary metabolites but also to their high TPC, TFC, that caused strong antioxidant power. Several *Euphorbia* species have been previously tested for their anticancer and cytotoxic activity, especially against MCF-7 cancer cell lines. [47-49] The methanol extract of *E. cactus* showed high cytotoxicity activity against MCF-7 cell line with an IC_{50} value comparable to that observed for the positive control doxorubicin (17.11 ± 0.73 µg/mL; 14.70 ± 1.1 µg/mL, respectively).[45] Furthermore, the Diyarbakir-obtained *E. fistulosa* extract exhibited potent cytotoxic effects on the MCF-7 breast cancer cell lines (IC_{50}: 14.04 ± 0.04). When gathered from Malatya and Van in Eastern Turkey, the aerial portions of *E. macroclada* showed excellent anticancer activity against MCF-7 (IC_{50}: 42.88 ± 0.19).[48] The results of this particular investigation revealed promising antitumor activity of *E. fistulosa* and *E. macroclada* against breast cancer cells.[48] In another study, the dichloromethane extract obtained from *E. macroclada* showed strong cytotoxic activity against MDA-MB-468 breast cancer cell lines (IC_{50} is 30 µg/mL).[49] Furthermore, studies by Asadi-Samani et al. demonstrate that *E. szovitsii* is a valuable source of natural products for the synthesis of anti-breast cancer medications, as it has the ability to induce cycle arrest and apoptosis in the MDA-MB-231 cell line (IC_{50}: 59.52 µg/mL).[50] The results of the current study revealed an interesting cytotoxic effects of the extracts obtained from the four *Euphorbia* species', particularly the methanolic extract of *E. prostrata* against the highly aggressive MDA-MB 231 breast cancer cell line (IC_{50}: 58± 3.71 µg/mL).

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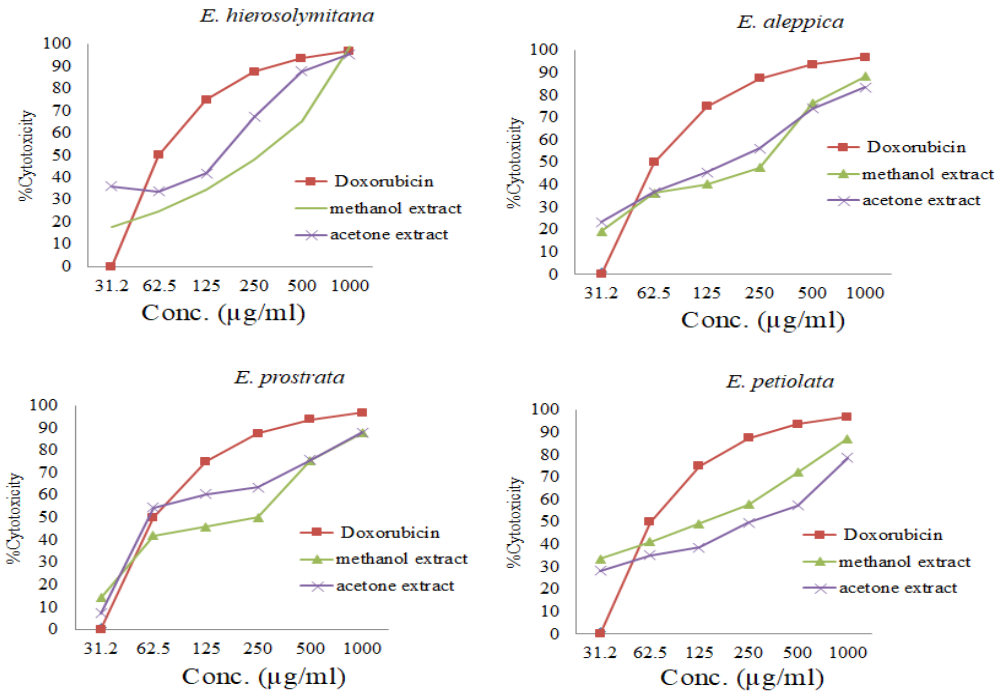

Figure 3: % Cytotoxicity of the methanol and acetone extracts of *E. hierosolymitana*, *E. aleppica*, *E. petiolata* and *E. prostrata* extracts compare to the positive control Doxorubicin.

Conclusion

In principle, the current study was designed to shed light on the phytochemical composition, TPC and TFC of the methanol and acetone extracts obtained from four different *Euphorbia* species from Jordan. Additionally, the antioxidant activity (DPPH and ABTS scavenging power) and cytotoxic effects (against the MDA-MB-231 breast cancer cell line) of these extracts were also measured. While all investigated *Euphorbia* species showed interestingly high TPC and TFC that were correlated with the observed interesting antioxidant and cytotoxic activities, one particular species, *E. protrata*, was exceptionally interesting. The results of the current study suggest further investigation to isolate and characterize the active constituents of these species, especially *E. prostrata*. More studies are required to reveal the antioxidant and cytotoxic mechanisms behind the observed activities.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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