ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

In Vitro Cytotoxic Study of *Euphorbia grantii* Oliv. Aerial Parts against MCF-7 and MCF-7^{ADR} Breast Cancer Cell Lines: A Bioactivity-Guided Isolation

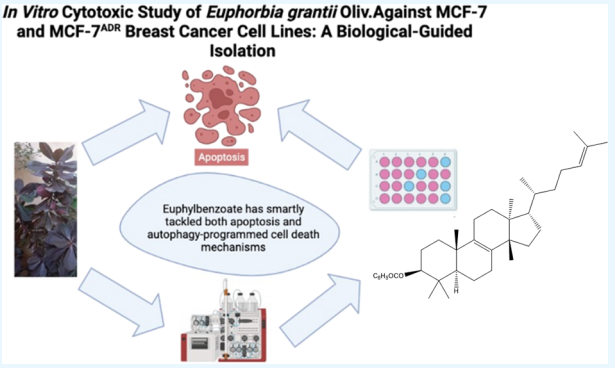
Mai Hussin Radi, Riham A. El-Shiekh, Ali M. El-Halawany, Ahmed M. Al-Abd, and Essam Abdel-Sattar*

Cite This: *ACS Omega* 2023, 8, 18299−18305

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The development of highly efficient and low-toxicity anticancer drugs is one of the most critical problems in the medical field. *Euphorbia grantii* is commonly reported as an antiviral plant; a dilute solution of its latex is used for intestinal worms and to promote blood clotting and tissue healing. Our study evaluated the antiproliferative activity of the total extract, its respective fractions, and the isolated compounds from *E. grantii* aerial parts. A phytochemical study was done by several chromatographic methods, and the cytotoxic activity was assessed using the sulforhodamine B assay. The dichloromethane fraction (DCMF) exhibited promising cytotoxic activity against breast cancer cell lines (MCF-7 and MCF-7^{ADR}), with an IC_{50} of 10.31 and 10.41 μg/mL, respectively. Chromatographic purification of the active fraction revealed the isolation of eight compounds. Among the isolated compounds, euphylbenzoate (EB) exhibited a promising effect with an IC_{50} of 6.07 and 6.54 μM against MCF-7 and MCF-7^{ADR}, respectively, while other compounds showed no activity. Euphol, cycloartenyl acetate, cycloartenol, and epifriedelinyl acetate showed moderate activity (33.27−40.44 μM). Euphylbenzoate has smartly tackled both apoptosis and autophagy programmed cell death mechanisms. These results demonstrated that *E. grantii* aerial parts yield active compounds with significant antiproliferative potential.



1. INTRODUCTION

Cancer is a worldwide health problem impacting all regions and community groups. Breast cancer is the most commonly diagnosed malignancy in women being the main reason why people die from cancer.[1] According to the GLOBOCAN's 2018 estimates, more than 18 million new cases of cancer and 9.2 million more cancer-related deaths were reported. By 2030, it was estimated that the number of cases will reach more 21 million cases worldwide.[2] Nonspecific chemotherapy, less effective generic cytotoxic agents, the toxicity related to the available drugs, and high frequency of tumor recurrence are common drawbacks of cancer treatments.[3] Natural products have been developed as a key basis of several new drugs in the last decades.

The genus *Euphorbia* (family Euphorbiaceae) has been reported to have some promising activities as anti-inflammatory, anticancer, immunoregulation, and fibrinolytic actions.[4] Phytochemical investigation of the family Euphorbiaceae showed the presence of saponins, diterpenes, triterpenes, flavonoids, lectins, phorbol esters, and glycoproteins.[5] *Euphorbia grantii* Oliv. was reported for its antiviral activity against several reported viruses.[6] The activity was correlated to its content of 3-methoxyflavones such as methylquercetin and methylkaempferol.[7]

Triterpenes are biologically active metabolites classified into tetracyclic and pentacyclic triterpenes and have recently caught broad attention. They have been reported as anti-inflammatory, antiviral, antibacterial, sedative, immunoregulatory, blood sugar regulatory, blood pressure-lowering, and antitumor agents.[8] Recent reports indicated that triterpenes could directly inhibit the tumor growth, both *in vivo* and *in vitro via* several mechanisms.[8]

Our study evaluated the antiproliferative activity of the total methanol extract and fractions/subfractions of *E. grantii* aerial parts as well as the isolated compounds from the most active fraction. The most active one was investigated for its mechanisms of action, providing a new insight for further research and investigations on the development of anticancer drugs.

Received: March 29, 2023
Accepted: May 5, 2023
Published: May 15, 2023

2. MATERIALS AND METHODS

2.1. General Experimental Procedures. Solvents were supplied by Piochem Company (Cairo, Egypt) and are analytical grade. ^{1}H NMR (100 MHz) and ^{13}C NMR (400 MHz) spectra were performed on a Bruker high-performance digital NMR spectrometer (Bruker, Karlsruhe, Germany). The mechanisms of cytotoxicity were characterized using an ACEA Novocyte flowcytometer (ACEA Biosciences Inc., San Diego, CA), and the data were calculated using ACEA NovoExpress software (ACEA Biosciences Inc., San Diego, CA).

2.2. Plant Material. *E. grantii* Oliv. aerial parts were collected from El-Orman Botanical Garden, Giza, Egypt, in January 2020. The plant material was kindly authenticated by Therese Labib, a Botanical Specialist and Consultant at El-Orman Botanical Garden. A voucher specimen (No. 4.01.2023) was deposited at the herbarium of the pharmacognosy department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

2.3. Extraction, Fractionation, and Isolation. The air-dried powdered plant material (600 g) was extracted with methanol (3 L × 5) by maceration for 3 days. The collected extract was filtered and evaporated under reduced pressure to give 81.5 g of dry residue. A part of the total methanolic extract (52 g) was fractionated in methanol, water, and dichloromethane (DCM) with a ratio of 1:1:1, to yield two fractions: DCMF (30 g) and the remaining mother liquor (MLF, 22 g).

The DCMF (11 g) was fractionated by chromatography on a Si gel column using *n*-hexane/ethyl acetate (EtOAc) mixtures as an eluent (10−70% EtOAc/*n*-hexane) to get five subfractions as follows: Fr. **1** (2.4 g), Fr. **2** (3 g), Fr. **3** (2 g), Fr. **4** (1.4 g), and Fr. **5** (eluted with 100% methanol, 1.8 g). Fr. **1** was the most active fraction among the five tested fractions as indicated by results of *in vitro* cytotoxic assay on breast cancer cell lines.

Therefore, part of Fr. **1** (2 g) was chromatographed on a Sil gel column (25 × 2.5 cm^2) using *n*-hexane/EtOAc (95:5 v/v) as a mobile phase and fractions of 50 mL each were collected to afford three main subfractions (Subfr **1a**−**c**). Subfraction **1a** (350 mg) was further purified using a medium-pressure liquid chromatography (MPLC) column (3 × 15 cm^2, normal-phase silica) using *n*-hexane/EtOAc (18.5:1.5 v/v) as a solvent system and 5 mL fractions were collected. The column afforded compound **1** (110 mg), compound **2** (11 mg), and compound **3** (8 mg). Subfraction **1b** (850 mg) was purified by chromatography on a Si gel column (1.5 × 20 cm^2) using 5% EtOAc in *n*-hexane, to yield compound **4** (50 mg), compound **5** (19.8 mg), and compound **6** (25 mg). Subfraction **1c** (760 mg) yielded compound **7** (10.8 mg) and compound **8** (20 mg) upon chromatography on a Si gel column (3 × 20 cm^2) using *n*-hexane/EtOAc (99:1 v/v) as a solvent system.

2.4. Cytotoxic Activity. The samples were tested for cytotoxic activity against breast adenocarcinoma (MCF-7) and doxorubicin-resistant breast cancer (MCF-7^{ADR}) cell lines. The two cell lines were obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cell viability was assessed by sulforhodamine B (SRB) assay. The absorbance was measured at 540 nm using a BMGLABTECH-FLUOStar Omega microplate reader (BMG Labtech, Ortenberg, Germany). All assays adopted the method mentioned before.[9]

2.5. Analysis of Cell Cycle Distribution. To assess the effect of EB on the cell cycle distribution of MCF7 and MCF7^{ADR}, cells were treated with the predetermined IC$_{50}$ EB or drug-free media for 48 h. All procedures were described before.[10]

2.6. Apoptosis Assessment. Apoptosis and necrosis cell populations were characterized for EB using an annexin V-FITC apoptosis detection kit (Abcam Inc., Cambridge Science Park, Cambridge, U.K.), adopting a previously described method.[11]

2.7. Autophagy Assessment. To clarify the method of cell death by which cells are killed in response to therapy, autophagic cell death was quantitatively determined using acridine orange coupled with cytometric analysis. Cells were treated with the predefined IC$_{50}$ of EB for 24 h. All procedures were carried out as described before.[11]

2.8. Statistical Analysis. Data are presented as mean ± SD. Analysis of variance (ANOVA) with the LSD test post hoc was used for testing significance using SPSS for windows, version 17.0.0. $P < 0.05$ was taken as being statistically significant.

3. RESULTS AND DISCUSSION

The genus *Euphorbia* is an important source of natural product with a wide range of therapeutically important biological activities: antitumor, cytotoxic, anti-HIV, and anti-inflammatory activity. Terpenoids (diterpenes and triterpenes) represent the most important classes with their interesting structural diversity.[12] Several classes of compounds were identified from the genus *Euphorbia*, in addition to terpenoids such as polyphenolic compounds, organic acids, alkaloids, and fatty acids. These compounds are responsible for the diverse activities reported for the plants such as the antiproliferative, anti-inflammatory, immunoregulation, antiangiogenic, antiparasitic, and antiasthma activities.[13]

Table 1. Cytotoxicity of the Total Methanol Extract, Its Respective Fractions, and the Isolated Compounds of *E. grantii* Aerial Parts[aa]

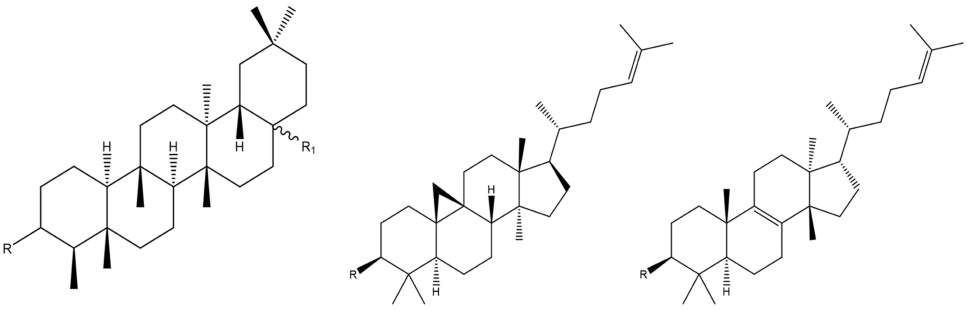
compound	IC$_{50}$, μg/mL (μM)	
	MCF7^{ADR}	MCF7
MeOH extract	19.55	16.47
dichloromethane	10.41	10.31
mother liquor	NA	NA
friedelin (**1**)	NA	NA
3-β-friedelinol (**2**)	NA	NA
epifriedelanol (**3**)	NA	NA
euphol (**4**)	26.25 (61.51)	27.77 (65.08)
cycloartenol (**5**)	25.3 (59.28)	18.56 (43.49)
cycloartenyl acetate (**6**)	23.73 (50.61)	15.6 (33.27)
epifriedelinyl acetate (**7**)	26.18 (55.60)	19.04 (40.44)
euphylbenzoate (**8**)	3.47 (6.54)	3.22 (6.07)

[a]NA: not applicable.

Cancer is well known of being the second cause of death worldwide. Each year, cancer is diagnosed in about 11 million people, ending with death in 10 million;[14] the number estimated for the year 2030 will top up to 13.1 million cases.[15] Today, about 50% of the clinically available anticancer drugs are derived from natural sources or inspired by natural compounds.[16]

The crude extract and its respective fractions of *E. grantii* (MeOH, DCMF, and MLF) were evaluated for the half-maximal inhibitory concentration (IC$_{50}$) effect in hormone

https://doi.org/10.1021/acsomega.3c02091
ACS Omega 2023, 8, 18299−18305



Compound 1; R=O, R_1= α-CH_3.
Compound 2; R=OH, R_1= β-CH_3.
Compound 3; R=OH, R_1= α-CH_3.
Compound 7; R=$OCOCH_3$, R_1= α-CH_3.

Compound 5; R=OH.
Compound 6; R=$OCOCH_3$.

Compound 4; R=OH.
Compound 8; R= $OCOC_6H_5$.

Figure 1. Structures of the isolated compounds.

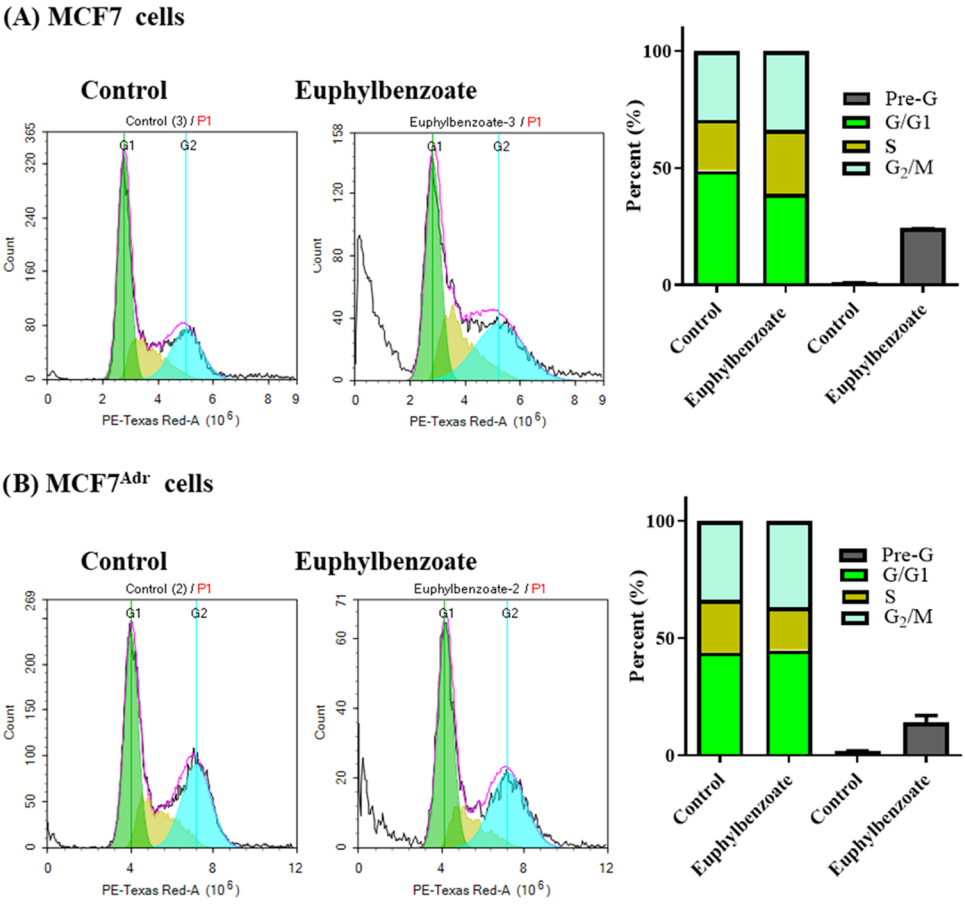


Figure 2. Effect of euphylbenzoate on the cell cycle distribution of MCF7 (A) and $MCF7^{ADR}$ (B) cells. The cells were exposed to euphylbenzoate for 48 h. Cell cycle distribution was determined using DNA cytometry analysis and different cell phases were plotted as percentage of total events. Data is presented as mean ± SD; n = 3.

receptor-positive breast cancer (MCF-7) and doxorubicin-resistant breast cancer ($MCF\text{-}7^{ADR}$) cell lines. Of particular relevance is that the nonpolar fraction (DCMF) showed a potent cytotoxic effect, with IC_{50} values of 10.31 and 10.41 μg/mL against MCF-7 and $MCF\text{-}7^{ADR}$, respectively (Table 1).

Triterpenes are divided into linear ones: tetracyclic and pentacyclic.[15] They are the major secondary metabolites detected in the genus including tirucallane, euphane, cycloartane, and oleanane subclasses and dominated in the DCMF active fraction. They were previously assessed for *in vitro* cytotoxic activities against estrogen-sensitive cells (MCF-7).[17] In particular, 3-β,25-dihydroxycycloart-23-(E)-ene, 3-β,25-dihydroxycycloart-23-(Z)-ene, (24 R)-tirucalla-8,25-diene-3 β,24-diol, lupenone, and 11 β-hydroperoxyeupha-8, 25-diene-3 β-ol exhibited significant activities against MCF-7 cells.[17]

The phytochemical studies of the DCMF of the methanolic extract of *E. grantii* led to the isolation of eight triterpenes (Figure 1). The isolated compounds were identified as friedelin (**1**), 3-β- friedelinol (**2**), epifriedelanol (**3**), euphol (**4**), cycloartenyl acetate (**5**), cycloartenol (**6**), epifriedelinyl acetate (**7**), and euphylbenzoate (**8**) using ^{1}H and ^{13}C NMR (see the Supporting Information). The structures of the

https://doi.org/10.1021/acsomega.3c02091
ACS Omega 2023, 8, 18299−18305

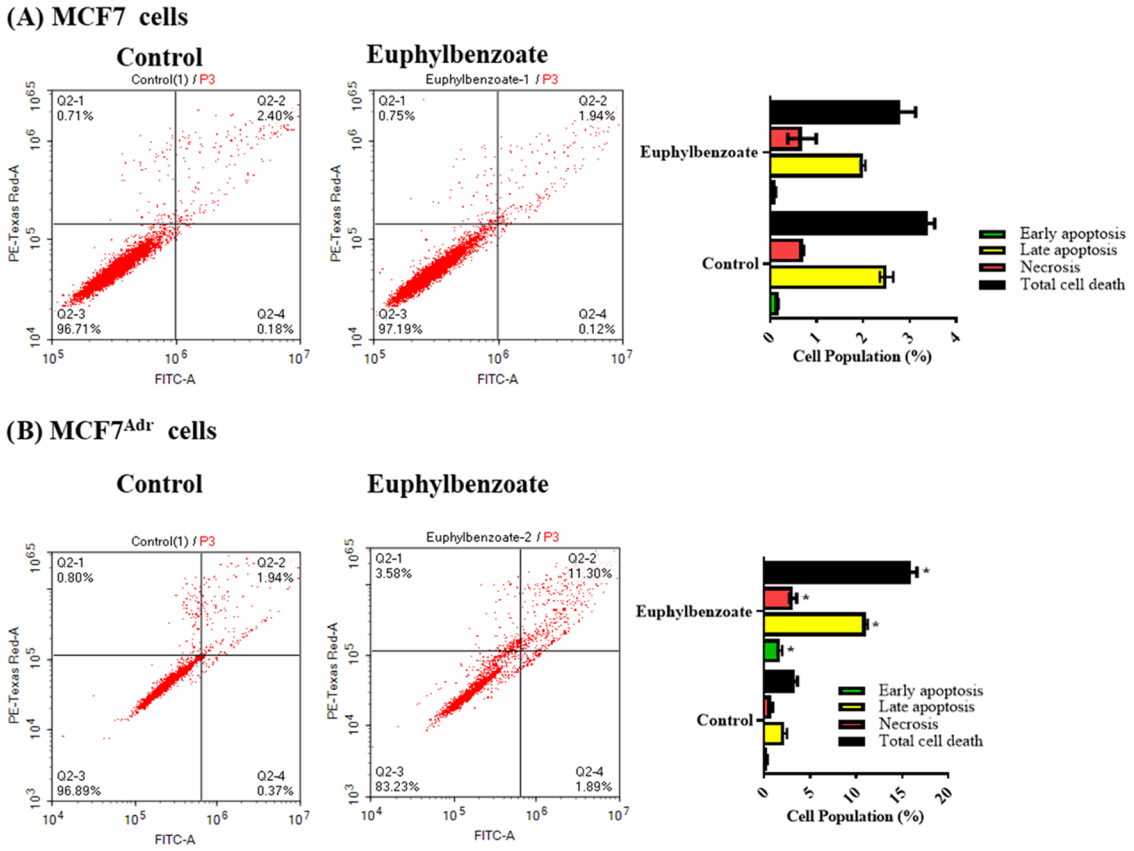


Figure 3. Induction of programmed cell death (apoptosis) by euphylbenzoate in MCF7 (A) and MCF7^{ADR} (B) cells. The cells were exposed to euphylbenzoate for 48 h. Cells were stained with annexin V-FITC/PI, and different cell populations were plotted as a percentage of total events. Data are presented as mean ± SD; n = 3. *Significantly different from the corresponding control at $P < 0.05$.

isolated compounds were elucidated by comparison of their physical and spectral data (^{1}H and ^{13}C NMR) with those mentioned in the literature.[18−24]

The ^{13}C NMR showed 30 carbons, indicative of a triterpenoid typical of the friedelane skeleton in compounds **1**, **2**, **3**, and **7**, which were reliable with the reported values of friedelin, 3-β- friedelinol, epifriedelanol, and epifriedelinyl acetate, respectively. The C-3 signal of the carbon bearing a carbonyl group at δ_C of 213.25 in compound **1** was observed. Furthermore, the C-3 signal of the carbon bearing a hydroxyl group at δ_C 72.22 and 72.77 could easily be correlated with the chemical shift of H-3 at δ_H 3.76c and 3.40 in compounds **2** and **3**, respectively. Furthermore, the C-3 signal of the carbon bearing a hydroxyl group at a δ_C 74.61 in addition to carbon signals of the acetyl group (δ_C 21.12, 170.92) could be correlated with the chemical shift of H-3 at δ_H 2.04 (OAc, 1H, s). In the four compounds, the interpretation nearly matched and is characteristic of the tertiary methyl 8 carbons resonated at δ_C 11.31−35.03 (C-23-30); these carbons exhibited coupling with protons at δ_H 0.75−1.15.

Compound **4** is a tetracyclic triterpene identified as euphol, where compound **8** was identified as its benzoyl ester. Both compounds exhibited signals mostly appearing at the upfield region and are typical of the triterpenoid signals. The more peculiar ones in the H NMR spectra are an olefinic proton (δ_H 5.02, 1H, m) and an axial proton on an oxygen-bearing carbon (δ_H 3.14−3.18, m). Furthermore, both compounds exhibited seven singlet signals characteristic of tertiary methyl groups (δ_H 0.77−1.61) and a secondary methyl group, δ_H of 0.82 (H-21, 3H, d, J = 6), which form the eighth methyl groups in the skeleton. The ^{13}C NMR spectrum of **4** exhibited four carbon signals (δ_C 134.02, 133.54, 125.21, and 130.88) characteristic of four olefinic carbons of C-8, C-9, C-24, and C-25, respectively, indicating that compound **4** contains two double bonds. The carbon signal (δ_C 79.01) was identified as carbon bearing a hydroxyl group of C-3. These data are in agreement with the structure of euphol,[21] where compound **8** showed protons and carbons of the benzoyl moiety.[24]

The ^{1}H NMR spectra of compounds **5** and **6** revealed the presence of a geminal olefinic proton resonate at δ_H 5.09 (H-24) and 5.28 (H-24), respectively, a cyclopropyl group at δ_H 0.81 (H-30) and 0.78 (H-30), respectively, and two allylic methyl at δ_H 1.61 (H-26) and 1.69 (H-26), respectively, with five other methyl groups as well as hydroxy methine at δ_H 3.67 (H-3). The presence of a cyclopropyl group of C-19 with C-9 and C-10 was in agreement with the data reported in the literature of both compounds.[22] Thus, the structure of compound **5** as cycloartenol and compound **6** as acetate ester of **5** was elucidated by comparison with the data in the literature;[22] in addition, compound **6** showed signals due to the acetate group. ^{13}C NMR: δ_C 21.12, 170.92, and ^{1}H NMR: δ_H 2.05 (3H, s).[22]

Triterpenes are phytocompounds that display a broad spectrum of biological activities, antimicrobial, anti-inflammatory, antioxidative, antiviral, and antifungal properties, in addition to anticancer and chemopreventive actions. They are able to inhibit the life of neoplastic cell lines, in addition to inducing apoptosis of cancer cells and causing their "suicidal" death, without any threats to normal cells of the body.[15] *In vitro* cytotoxic activity of the isolated compounds revealed that

https://doi.org/10.1021/acsomega.3c02091
ACS Omega 2023, 8, 18299−18305

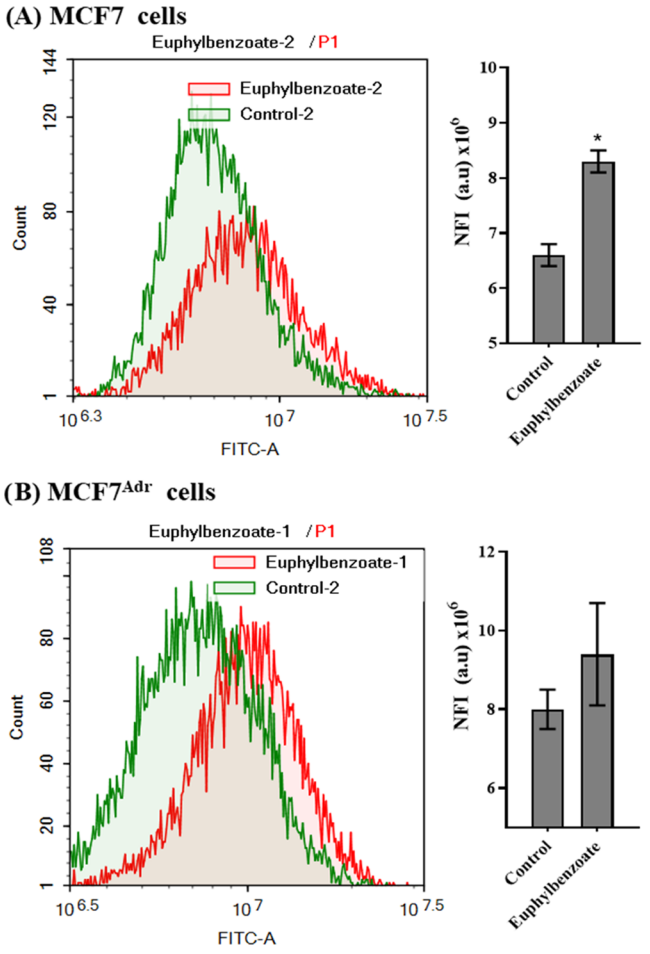


Figure 4. Induction of programmed cell death (autophagy) by euphylbenzoate in MCF7 (A) and MCF7^{ADR} (B) cells. The cells were exposed to euphylbenzoate for 48 h and then were stained with acridine orange. Average net fluorescence intensity (NFI) was plotted and compared to control cells. Data are presented as mean ± SD; n = 3. *Significantly different from the control at $P < 0.05$. **Significantly different from the corresponding single treatment (5-FU or GCB) at $P < 0.05$.

compounds **1**, **2**, and **3** showed no activity in both cell lines, where compounds **4**−**7** had a moderate activity with IC_{50} ranging from 15.60 to 27.77 μg/mL against MCF-7 and 23.73 to 26.25 μg/mL against MCF-7^{ADR}, respectively. It is worth noting that compound **8**, namely euphylbenzoate (EB), which was isolated for the first time from nature but previously reported as a synthesized compound from euphol (**4**),[24] showed potent activity with an IC_{50} of 3.22 and 3.47 against MCF-7 and MCF-7^{ADR} cells, respectively (Table 1).

Cell cycle distribution using DNA flow cytometry was characterized to explain the cytotoxic/antiproliferative effect of EB against MCF7 and MCF7^{ADR} cells. Treating MCF7 cells with the IC_{50} of EB significantly increased cell populations in both the S-phase and G_2/M-phase from 21.7 ± 1.4 to 27.3 ± 0.7% and from 29.4 ± 1.1 to 33.7 ± 0.3%, respectively. Reciprocally, the nonproliferating cell fraction (G_0/G_1-phase) significantly decreased from 48.8 ± 0.5 to 39.0 ± 0.4% (Figure 2A). On the other hand, EB marginally enhanced cells in the G_2/M-phase from 22.9 ± 1.7 to 22.9 ± 1.7% in the resistant MCF7^{ADR} cells with no other significant influence on any other cell population (Figure 2B). However, EB significantly increased the pre-G cells in both naïve (MCF7) and resistant (MCF7^{ADR}) breast cancer cells from to 1.4 ± 0.1 to 24.4 ± 0.1% and from 1.8 ± 0.1 to 14.0 ± 1.8%, respectively (Figure 2A,B). The increased Pre-G cell population is indicative of cell death rather than antiproliferative properties for EB. Still, the exact mechanism of cell death is not well defined.[25]

To establish the exact mechanism of cell death (programmed or not programmed), both naïve (MCF7) and resistant (MCF7^{ADR}) cells were evaluated by annexin-V/FITC staining coupled with flowcytometric analysis after exposure to the IC_{50} of EB for 48 h. Still, no considerable apoptosis or necrosis was noticed in MCF7 cells after 48 h of exposure to EB (Figure 3A). However, exposure of the resistant MCF7^{ADR} cells to EB for 48 h resulted in significant apoptosis (5-fold) and necrosis (4-fold) compared to the control untreated cells (Figure 3B).

MCF7 cells showed minimal or no apoptosis response compared to MCF7^{ADR} cells after treatment with EB. On the other hand, the pre-G cell population in MCF7 was significantly increased, which is indicative of detectable cell death. Still, alternative pathways (rather than apoptosis/necrosis) are expected to result in cell death in the MCF7 cell line. It is worth mentioning that MCF7 cells are defective in the caspase-3 expression, and consequently, apoptosis is expected to participate minimally in the cell death pathway of this cell line.[26] Autophagy is an alternative programmed cell death pathway; however, it has complicated roles and is debated in the cancer cell death.[27] In this, we performed autophagy using acridine orange lysosomal stain coupled with flowcytometric analysis.

In MCF7^{ADR} cells, no significant autophagy was detected upon treatment with EB for 48 h (Figure 4A). On the other hand, autophagy was the predominating method of cell death in MCF7 cells in response to EB with a significant increase of the acridine orange-induced autophagosome fluorescence signal (Figure 4B). From the literature, it is well known that MCF7 cells are lacking caspase-dependent apoptosis[27] and autophagy in this cell line is a pro-death mechanism rather than pro-survival apoptosis escape mode.[28,29]

To the best of our knowledge, this feature is not reported yet in MCF-7^{ADR}. Still, it is very interesting how a steroidal compound such as euphylbenzoate behaves smartly and tackles both apoptosis and autophagy programmed cell death mechanisms to induce cell killing as noticed in the form of elevated pre-G cell population (Figure 2A,B).

This study suggests that euphylbenzoate may have a unique mode of action compared to other compounds that induce cell death through a single mechanism. The ability of the compound to target multiple pathways may also make it a promising candidate for the treatment of cancer or other diseases where programmed cell death is involved. However, it is important to note that further research is needed to confirm these findings and fully understand the mechanism of action of euphylbenzoate. Further *in vitro* studies are needed, such as western blot analysis to examine the expression of proteins associated with apoptosis and autophagy or fluorescence markers to visualize the cellular changes associated with these pathways. *In vivo* experiments on tumor growth and programmed cell death in the animal model could be of benefit to determine whether euphylbenzoate has the same effects *in vivo* as it does *in vitro*.

This study further confirmed that the *Euphorbia* species could be a possible source of bioactive metabolites for exploring new anticancer drugs. Furthermore, EB could be a

https://doi.org/10.1021/acsomega.3c02091
ACS Omega 2023, 8, 18299−18305

lead compound to synthesize structurally related compounds to be examined for their cytotoxicity.

From the results in Table 1 and structures in Figure 1, it was indicated that acylation is required for the activity, as seen for compound **3** (inactive), which upon acetylation gave the moderately active compound **7** with an IC_{50} of 19.04 and 26.18 μg/mL against MCF-7 and MCF-7^{ADR} cells, respectively. In addition, the activity of compound **5** was marginally increased upon acetylation to compound **7**. On the other hand, benzoylation of compound **4** to compound **8** increased the activity by 7.5- and 8.6-fold against MCF-7^{ADR} and MCF-7 cells, respectively.

Oxidation of the OH group at C-3 shows no relation to the activity (see compounds **1**, **2**, and **3**), while the extended double bond conjugation to the carbonyl at C-3 results in an increase of the activity, as seen in quinone-methide triterpenes commonly present in the family Celastraceae.[30,31] Euphylbenzoate is isolated for the first time in the nature and showed potent cytotoxic activity.

4. CONCLUSIONS

The *Euphorbia* species have a powerful history of ethnomedicinal uses as well as ethnopharmacological uses in drug discovery from ancient times to the present. *E. grantii* Oliv. (Euphorbiaceae) showed potent cytotoxicity against the MCF7 breast cancer cells and their resistant variant MCF-7^{ADR} cells, which indicated that it could be a source for novel anticancer drugs to fight breast cancer. The phytochemical investigations of the plant resulted in the isolation of eight triterpenes from its active DCM fraction, where euphylbenzoate (EB) was the most potent isolate. EB resulted in significant apoptosis and necrosis compared to the control untreated cells against MCF7^{ADR}.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02091.

^{1}H NMR data of the isolated compounds, ^{13}C NMR data of the isolated compounds, and ^{1}H NMR and ^{13}C NMR charts of the isolated compounds (C1−C8) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Essam Abdel-Sattar − *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt;* orcid.org/0000-0002-0617-1420; Phone: 23639307; Email: essam.abdelsattar@pharma.cu.edu.eg; Fax: +2023628426

Authors

Mai Hussin Radi − *Herbal Department, Egyptian Drug Authority (EDA), 12511 Giza, Egypt*

Riham A. El-Shiekh − *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt;* orcid.org/0000-0002-3179-3352

Ali M. El-Halawany − *Pharmacognosy Department, Faculty of Pharmacy, Cairo University, 11562 Cairo, Egypt;* orcid.org/0000-0002-5864-1674

Ahmed M. Al-Abd − *Pharmacology Department, Medical and Clinical Research Institute, National Research Centre, 12622 Cairo, Egypt;* orcid.org/0000-0001-7872-4867

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c02091

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Therese Labib, a Botanical Specialist and Consultant at El-Orman Botanical Garden for authentication of the plant materials.

■ REFERENCES

(1) Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA, Cancer J. Clin.* **2018**, *68*, 394−424.

(2) Boyle, P. Triple-negative breast cancer: epidemiological considerations and recommendations. *Ann. Oncol.* **2012**, *23*, vi7−vi12.

(3) Safarpour, D.; Tavassoli, F. A. A targetable androgen receptor−positive breast cancer subtype hidden among the triple-negative cancers. *Arch. Pathol. Lab. Med.* **2015**, *139*, 612−617.

(4) Campos, A.; Vendramini-Costa, D. B.; Longato, G. B.; Zermiani, T.; Ruiz, A. L. T. G.; de Carvalho, J. E.; Pandiella, A.; Cechinel Filho, V. Antiproliferative effect of Synadenium grantii Hook f. stems (Euphorbiaceae) and a rare phorbol diterpene ester. *Int. J. Toxicol.* **2016**, *35*, 666−671.

(5) Balakrishnan, N. P.; Chakrabarty, T. *The Family Euphorbiaceae in India: A Synopsis of Its Profile, Taxonomy and Bibliography*; Bishen Singh Mahendra Pal Singh: Dehradun, 2007.

(6) Van Hoof, L.; Berghe, D. A. V.; Hatfield, G. M.; Vlietinck, A. J. Plant antiviral agents; VI. 1 3-Methoxyflavones as potent inhibitors of viral-induced block of cell synthesis. *Planta Med.* **1984**, *50*, 513−517.

(7) Vlietinck, A. J.; Pieters, L.; Apers, S.; Cimanga, K.; Mesia, K.; Tona, L. The value of central-African traditional medicine for lead finding: some case studies. *J. Ethnopharmacol.* **2015**, *174*, 607−617.

(8) (a) Zhang, W.; Men, X.; Lei, P. Review on anti-tumor effect of triterpene acid compounds. *J. Cancer Res. Ther.* **2014**, *10*, 14−19. (b) Abbas, S. E.; Barsoum, F. F.; Georgey, H. H.; Mohammed, E. R. Synthesis and antitumor activity of certain 2,3,6-trisubstituted quinazolin-4 (3H)-one derivatives. *Bulletin of Faculty of Pharmacy, Cairo University* **2013**, *51*, 273−282. (c) Elkhayat, E. S. Chemical constituents from Gazania linearis cultivated in Egypt. *Bulletin of Faculty of Pharmacy, Cairo University* **2016**, *54* (2), 257−261.

(9) Abdel-Mohsen, H. T.; El Kerdawy, A. M.; Omar, M. A.; Petreni, A.; Allam, R. M.; El Diwani, H. I.; Supuran, C. T. Application of the dual-tail approach for the design and synthesis of novel Thiopyrimidine−Benzenesulfonamide hybrids as selective carbonic anhydrase inhibitors. *Eur. J. Med. Chem.* **2022**, *228*, No. 114004.

(10) Soliman, F. M.; Fathy, M. M.; Salama, M. M.; Al-Abd, A. M.; Saber, F. R.; El-Halawany, A. M. Cytotoxic activity of acyl phloroglucinols isolated from the leaves of Eucalyptus cinerea F. Muell. ex Benth. cultivated in Egypt. *Sci. Rep.* **2014**, *4*, No. 5410.

(11) Bashmail, H. A.; Alamoudi, A. A.; Noorwali, A.; Hegazy, G. A.; Ajabnoor, G. M.; Al-Abd, A. M. Thymoquinone enhances paclitaxel anti-breast cancer activity via inhibiting tumor-associated stem cells despite apparent mathematical antagonism. *Molecules* **2020**, *25*, 426.

(12) Vasas, A.; Hohmann, J. Euphorbia diterpenes: isolation, structure, biological activity, and synthesis (2008−2012). *Chem. Rev.* **2014**, *114*, 8579−8612.

(13) Munhoz, A. C.; Minozzo, B. R.; Cruz, L. S.; Oliveira, T. L.; Machado, W. M.; Pereira, A. V.; Fernandes, D.; Manente, F. A.; Vellosa, J. C.; Nepel, A.; et al. Chemical and pharmacological investigation of the stem bark of Synadenium grantii. *Planta Med.* **2014**, *80*, 458−464.

(14) Ferlay, J.; Ervik, M.; Lam, F.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. *Global Cancer Observatory: Cancer Today*; International Agency for Research on Cancer: Lyon, 2020.
(15) Chudzik, M.; Korzonek-Szlacheta, I.; Król, W. Triterpenes as potentially cytotoxic compounds. *Molecules* **2015**, *20*, 1610−1625.
(16) Rezadoost, M. H.; Kumleh, H. H.; Ghasempour, A. Cytotoxicity and apoptosis induction in breast cancer, skin cancer and glioblastoma cells by plant extracts. *Mol. Biol. Rep.* **2019**, *46*, 5131−5142.
(17) Magozwi, D. K.; Peter, X.; Langat, M. K.; Mhlanga, R.; Vukea, N.; de la Mare, J.-A.; Siwe-Noundou, X.; Krause, R. W.; Tembu, V. J. In vitro cytotoxic effects of chemical constituents of Euphorbia grandicornis Blanc against breast cancer cells. *Sci. Afr.* **2021**, *14*, No. e01002.
(18) Mann, A.; Ibrahim, K.; Oyewale, A. O.; Amupitan, J. O.; Fatope, M. O.; Okogun, J. I. Antimycobacterial friedelane-terpenoid from the root bark of Terminalia avicennioides. *Am. J. Chem.* **2011**, *1*, 52−55.
(19) Kiem, P. V.; Minh, C. V.; Huong, H. T.; Nam, N. H.; Lee, J. J.; Kim, Y. H. Pentacyclic triterpenoids fromMallotus apelta. *Arch. Pharmacal Res.* **2004**, *27*, 1109−1113.
(20) Kannathasan, K.; Senthilkumar, A.; Venkatesalu, V. Crystal structure and antibacterial evaluation of epifriedelinol isolated from Vitex peduncularis Wall. ex Schauer. *Arab. J. Chem.* **2019**, *12*, 2289−2292.
(21) Nyigo, V. A.; Peter, X.; Fouche, G.; Mabiki, F.; Malebo, H. M.; Mdegela, R. H. Isolation and identification of euphol and β-sitosterol from the dichloromethane extracts of Synadenium glaucescens. *J. Phytopharmacol.* **2016**, *5*, 100−104.
(22) De, P. T.; Urones, J.; Marcos, I.; Basabe, P.; Cuadrado, M. S.; Moro, R. F. Triterpenes from Euphorbia broteri. *Phytochemistry* **1987**, *26*, 1767−1776.
(23) Sukumar, E.; Rao, R. B.; Kundu, A. A friedelane triol from the roots of Pristimera grahamii. *Phytochemistry* **1990**, *29*, 3044−3046.
(24) Romero-Morán, L. J.; Ramírez-Apan, M. T.; Hernández-Ortega, S.; Martínez-Otero, D.; Delgado, G. Tris nor-Euphane-Type Triterpenoid and Other Constituents Isolated from Euphorbia tanquahuete Sessé & Moc.: Preparation and Cytotoxic Evaluation of Semisynthetic Derivatives of Euphol. *ACS Omega* **2022**, *7*, 35077−35082.
(25) Mohamed, G. A.; Al-Abd, A. M.; El-Halawany, A. M.; Abdallah, H. M.; Ibrahim, S. R. New xanthones and cytotoxic constituents from Garcinia mangostana fruit hulls against human hepatocellular, breast, and colorectal cancer cell lines. *J. Ethnopharmacol.* **2017**, *198*, 302−312.
(26) Jänicke, R. U. MCF-7 breast carcinoma cells do not express caspase-3. *Breast Cancer Res. Treat.* **2009**, *117*, 219−221.
(27) Hippert, M. M.; O'Toole, P. S.; Thorburn, A. Autophagy in cancer: good, bad, or both? *Cancer Res.* **2006**, *66*, 9349−9351.
(28) Bashmail, H. A.; Alamoudi, A. A.; Noorwali, A.; Hegazy, G. A.; AJabnoor, G.; Choudhry, H.; Al-Abd, A. M. Thymoquinone synergizes gemcitabine anti-breast cancer activity via modulating its apoptotic and autophagic activities. *Sci. Rep.* **2018**, *8*, No. 11674.
(29) Towers, C. G.; Thorburn, A. Therapeutic targeting of autophagy. *EBioMedicine* **2016**, *14*, 15−23.
(30) Abdel Sattar, E. Quinone-method triterpenes from tissue cultures of catha edulis. *Saudi Pharm. J.* **1998**, *6*, 242−245.
(31) Hernandes, C.; Miguita, L.; de Sales, R. O.; Silva, E. P.; Mendonça, P. O. R.; Lorencini da Silva, B.; Klingbeil, M. F. G.; Mathor, M. B.; Rangel, E. B.; Marti, L. C.; et al. Anticancer activities of the quinone-methide triterpenes maytenin and 22-β-hydroxymaytenin obtained from cultivated Maytenus ilicifolia roots associated with down-regulation of miRNA-27a and miR-20a/miR-17-5p. *Molecules* **2020**, *25*, 760.

https://doi.org/10.1021/acsomega.3c02091
ACS Omega 2023, 8, 18299−18305