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جامعة الملك سعود
King Saud University

Unprecedented Insights on Chemical and Biological Significance of *Euphorbia cactus* Growing in Saudi Arabia

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INTRODUCTION

Euphorbia is the third largest genus of flowering plants in the Euphorbiaceae family, with almost 2000 species distributed in tropical and subtropical climate zones. Its species are readily distinguishable by their specialized inflorescences and milky latex. Chemically, diterpenoids are the main components found in *Euphorbia*. *Euphorbia cactus* Ehrenb ex Boiss. (Family; Euphorbiaceae) is widely distributed in central Africa and the southern Arabian Peninsula. The extract of *E. cactus* latex showed antileishmanial activity, whereas the crude methanolic extract of the whole plant has been reported to exhibit antioxidant, antimicrobial, and anticancer activities. To the best of our knowledge, the plant species has not been extensively explored for its chemical and pharmacological potential.

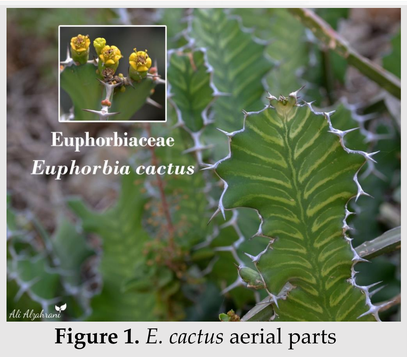


Figure 1. *E. cactus* aerial parts

Family: Euphorbiaceae
Genus: *Euphorbia*
Species: *E. cactus*
Local name in Saudi: Al-Sab

OBJECTIVES

To investigate the phytochemical, antiproliferative and apoptotic properties of aerial parts of *E. cactus* methanolic extract (**ECME)** growing in Saudi Arabia.

Materials and Methods

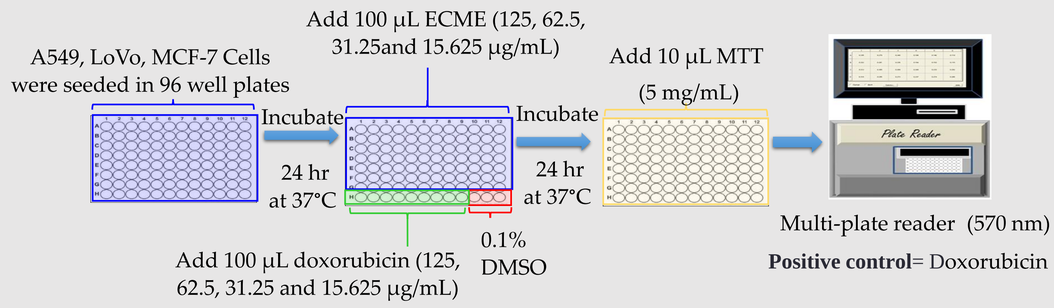
Plant Material:

The aerial parts of *E. cactus* were collected from Fayfa mountains (17°15′01.2″ N, 43°06′40.6″ E) in the southern region of Saudi Arabia in November 2019. and were taxonomically identified by Dr. Ali Mohammed Alzahrani from the Biology Department, Al-Baha University, Saudi Arabia. A voucher specimen (EC-14984) was deposited in the herbarium of the Pharmacognosy Department.

Isolation of Chemical Constituents from (ECME):

Dried aerial parts of *E. cactus* Ehrenb ex Boiss (300 g)
→ Powdered, extracted by MeOH
Viscous residue of *E. cactus* (10.2 g)
→ Diluted with H_2O
water suspension
→ Partition by chloroform
Chloroform layer (5.7 g)
Water suspension layer
→ Extracted by EtOAc
EtOAc layer (2.2 g)
Combined
→ Silica gel column chromatography (CC)
Fr.EC1 (213 mg) → Silica gel CC → Cpd **1** (8.4 mg)
Fr.EC2 (325 mg) → Silica gel CC → Cpd **2** (32.3 mg)
Fr.EC3 &4 (425 mg) → Silica gel CC → Cpd **3** (20.3 mg)
Fr.EC6 (251 mg) → Sephadex CC → Cpd **4** (6.8 mg)

Cytotoxicity Assay:



Cell Cycle Analysis:

A549 cells were plated in 6-well culture plates. After 24 h of incubation, cells were exposed to (20 and 10 µg/mL) concentration of ECME or DMSO as a control. After (48 h), the cells were detached, centrifuged, washed twice with ice-cold PBS and fixed in ice-cold absolute ethanol for 4 h at 4 ◦C. Then it was incubated with a 0.5 mL propidium iodide (PI) staining solution (50 µg/mL PI and 100 µg/mL RNase A) for 30 min in the dark. The cell cycle stages were analyzed using a FACS flow cytometer. CXP software v.3.0 was used for data collection and analysis.

Annexin V-FITC/PI Apoptosis Detection:

The protocol was followed according to the manufacturer's instructions (Biolegend,USA). In brief, at 48 h after treatment, floating cells and adherent cells were collected, and the pelleted cells were washed with PBS. Thereafter, the pelleted cells were resuspended in Annexin binding buffer (100 µL) and transferred to cytometer tubes. Cells staining was performed by the addition of 5 µL from both dyes (5 µL of FITC Annexin V and 5 µL propidium iodide) and incubated (10–15 min) in the dark. This was followed by the addition of 0.4 mL of incubation buffer, and the cells were analyzed immediately on FACS flow cytometer (Cytomics FC 500; Beckman Coulter, Brea, CA, USA).

RT-PCR and Western Blot:

A549 cells were treated either with the vehicle or plant extract at 10 and 20 µg/mL concentrations. After 24 h, total RNA and total protein were prepared from the vehicle and treated cells. RT-PCR was used to demine the gene expression levels of Bax, Bcl-2 and caspase-3 while western blot was employed to estimate the protein levels as described previously.

RESULTS

Isolation of Chemical Constituents:

Phytochemical investigation of *E. cactus* methanol extract (ECME) aerial parts resulted in the isolation and characterization of four secondary metabolites (Figure 2), which are reported for the first time from this plant species. Their chemical structures were established using NMR, IR, and MS. The isolated compounds were identified as glutinol (1), catechin (2), kaempferol-3-*O*-α-L-rhamnopyranoside (3) and quercetin-3-*O*-α-L-rhamnpyranoside (4). These isolated compounds were isolated previously from different *Euphorbia* species.

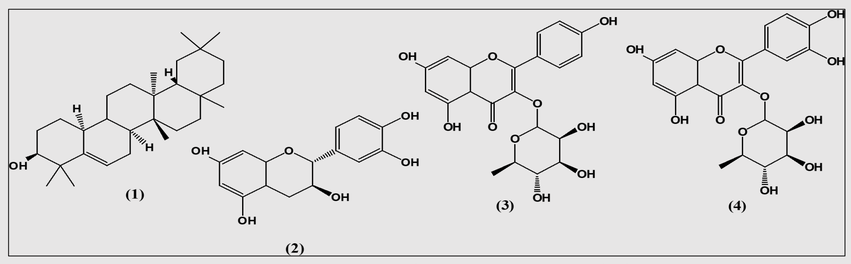


Figure 2. Chemical structures of isolated compounds from ECME

Antiproliferative and Apoptotic Activity of ECME:

Using MTT assay, we found that *E. cactus* methanolic extract (ECME), exerted a promising antiproliferative activity against different cancer cell lines (Table 1) and the A549 lung cancer cells were the most sensitive with an IC_{50} value of 20 µg/mL.

Table 1. IC_{50} of ECME against cancer (A549, LoVo, and MCF-7) and normal (HUVEC) cell lines as measured by MTT assay.

	Cell lines and IC_{50} (µg/mL)			
	A549	LoVo	MCF-7	HUVEC
ECME	20.1 ± 0.5	53.2 ± 0.4	58.80 ± 1.83	65.26 ± 2.80
Doxorubicin	1.20 ± 0.02	1.30 ± 0.05	1.40 ± 0.02	4.1 ± 0.2

We next determined the cell cycle phases of lung cancer cells after their treatment with ECME extract for 24 h. The flow cytometric data revealed a higher percentage of cells in the G2/M phase (Figure 3a). To explore the apoptosis induction, annexin V/PI staining and cells were performed. As shown in (Figure 3b), ECME induced A549 apoptosis in a dose-dependent manner.

RT-PCR and western blot analysis were also performed to validate the apoptosis induction induced by ECME on A549 cell line . Upon treatment, downregulation of anti-apoptotic Bcl-2 on the gene and protein level were observed (Figure 4 a,b). This downregulation of Bcl-2 was coupled to an upregulation of the pro-apoptotic Bax and executioner caspase 3 in gene and protein level (Figure 4 a,b) confirming apoptosis induction.

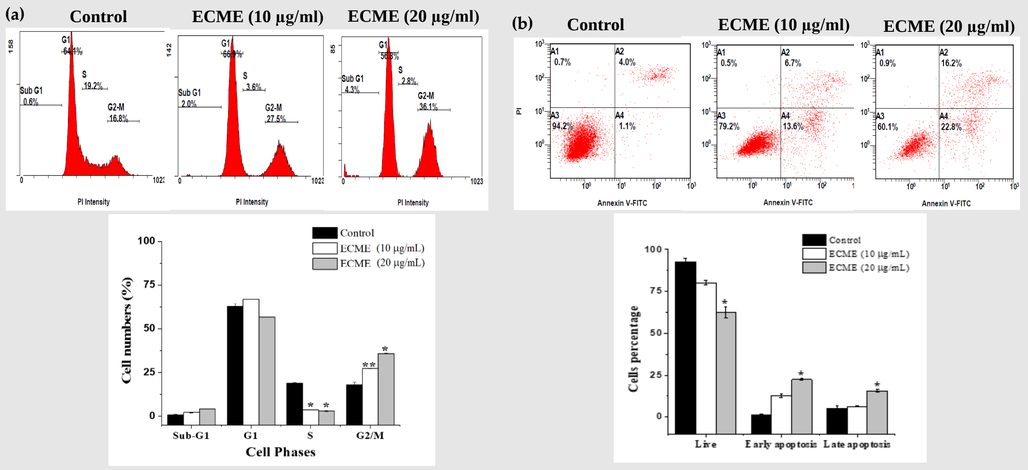


Figure 3: Effects of different concentrations of ECME on the cell cycle distribution and apoptosis induction of A549 determined by flow cytometry (a) Representative flow cytometric histograms cell cycle phases (G1, S and G2-M) and their relative percentage population (**b**) Histogram revealing the live, early and late apoptotic phase and their relatively quantified population. Data are represented in triplicates as mean ± SD. * $p < 0.05$, ** $p < 0.01$.

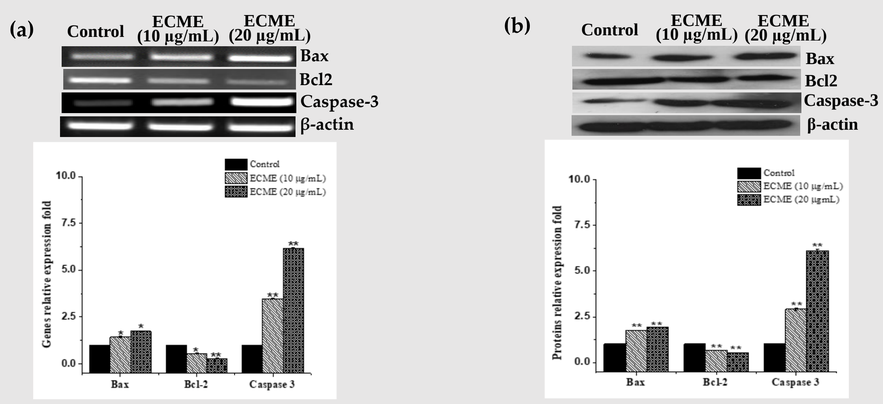


Figure 4: Effects of of different concentrations of ECME on gene and protein expression levels in A549 lung cancer cells. (**a**) Gene expression of Bax, Bcl2 and caspase 3 and its quantitative analysis in comparison to β-actin, an internal control. (**b**) Protein expression of Bax, Bcl2 and caspase 3 and its quantitative analysis in comparison to β-actin, an internal control.* $p < 0.05$, ** $p < 0.01$ indicate the significant differences from the control

CONCLUSION

Phytochemical investigation of ECME resulted in one triterpenoid along with three flavonoids which have been reported from *E. cactus* for the first time. Furthermore, ECME was found to display an antiproliferative activity towards various cancer cells, especially the A549 lung cancer cells, possibly via induction of apoptosis and G2/M cell cycle phase arrest. Apoptosis mediating by ECME was potentially through modulating of Bax, Bcl-2, and caspase-3 proteins. These findings are the primary insights to demonstrate the antiproliferative mechanism of *E. cactus*, suggesting that it might be a promising natural source for developing novel therapeutics against cancer.

REFERENCES

This work was published as: Al-Hamoud, G.A.; Fantoukh, O.I.; Amina, M.; Nasr, F.A.; Musayeib, N.M.A.; Ahmed, M.Z.; Noman, O.M.; Al-Sharidah, R.E.; Alasmari, F.; Alqahtani, A.S. Unprecedented Insights on Chemical and Biological Significance of *Euphorbia cactus* Growing in Saudi Arabia. *Plants* 2022, 11, 681. https://doi.org/10.3390/plants11050681.