



RESEARCH ARTICLE

# Phytochemical profiling and evaluation of the anticancer activity of *Euphorbia peplus* ethyl acetate extract against prostate cancer cells

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## Abstract

Because of its abundance of bioactive components, *Euphorbia peplus*, sometimes referred to as small spurge, is a herbaceous plant belonging to the Euphorbiaceae family that has long been utilized for a variety of therapeutic uses. This study explores the phytochemical content and anticancer potential of *E. peplus*. After collecting, drying and grinding every part of the plant into a powder, the bioactive components were extracted using a Soxhlet device and ethyl acetate as a solvent. The plant's various chemical components were identified using High-Performance Liquid Chromatography (HPLC). Numerous beneficial substances were also discovered through phytochemical analyses. Salicylic acid, resveratrol, epicatechin and apigenin are among the compounds that have not been previously identified or validated in *E. peplus*, according to a prior literature review. As a result, these substances might be regarded as new or unproven in this species, underscoring the possibility of finding phytochemicals that have not yet been identified. The MTT assay was used to assess the extracts' anticancer efficacy at various doses. With an  $IC_{50}$  value of 162.5  $\mu$ g/mL, the ethyl acetate extract demonstrated notable cytotoxic activity in a concentration- and time-dependent manner, suggesting increased efficacy with time. These results show that *E. peplus* is a good source of bioactive compounds with strong anticancer effects. Such findings highlight the potential of underexplored Euphorbia species growing in Iraq as a promising natural resource for future pharmacological applications. These findings emphasize the importance of further detailed *in vivo* and mechanistic studies to validate the bioactivity and explore therapeutic applications of *E. peplus* in cancer treatment. To support their possible usage in upcoming pharmaceutical applications, more research is advised to extract, identify and assess these components *in vivo*.

**Keywords:** anticancer; apoptosis; bioactive compounds; *Euphorbia peplus*; phytochemicals; prostate cancer

## Introduction

Prostate cancer (PCa) is the second most common solid tumor in men and the fifth main cause of cancer death worldwide, with approximately 1.47 million new cases and 396792 deaths reported in 2022 (1). The occurrence and the mortality rates of PCa are strongly related to location, age, race, family history, as well as environmental factors like obesity (2). PCa is a heterogeneous disease by both genetics and epidemiology and different survival trends across populations correlate with differences in genetics, environment and access to healthcare (3). Moreover, classification of PCa into androgen-sensitive versus androgen-insensitive types is helpful in directing treatment approaches such as androgen deprivation therapy or chemotherapy (4). However, resistance emergence as well as adverse effects to these treatments still represent important clinical obstacles and research on alternative therapeutics is required.

Plants used as drugs have been known for thousands of years to be an abundant resource that supplies bioactive substances. Plants have been used in traditional medicine for thousands of years to cure diseases, in addition to their use as

prophylactics and food preservers (5). Chemical compositions of such plants are responsible for their biological activities and mainly include flavonoids, terpenoids and phenolic compounds, presenting a wide spectrum in pharmacology.

The genus *Euphorbia* has more than 2000 species and is one of the largest genera of flowering plants, commonly called spurges and of family Euphorbiaceae (6). These are plants that secrete a toxic milky latex as a chemical defense against herbivores. Members of this genus are characterised by a broad range of chemical profiles such as steroids, flavonoids, sesquiterpenoids, glycerols and cerebrosides, with different types of biological activities and also commercial uses (7). *E. peplus*, naturalized from Europe and Western Asia, occurs in Somalia (Som-800520) and the Western Himalaya (7). Phytochemical investigations have revealed that *E. peplus* contains sterols, diterpenes, C- and O-glucosides, triterpene alcohols, cerebrosides, dihydroflavonol 3-O-monoglycosides, rutin, quercetin, kaempferol and myricetin, compounds associated with cytotoxic and pharmacological effects (2, 3). Historically, *Euphorbia* spp. has been used for a variety of conditions including gonorrhea, intestinal parasite infection,

migraines, skin diseases and as healing agents (4). Recent reports have also shown antihyperglycemic effects in animal models and a wide range of therapeutic potential (5).

Since many types of cancer require anticancer medications with high therapeutic efficacy, minimal side effects and both preventive and curative properties, new therapeutic approaches are required. Extracted phytochemicals from various plants serve as a significant source of naturally occurring compounds with cytotoxic properties. As such, the development of novel plant-based anticancer drugs in various regions of the world is often difficult. Small spurge's applications and health benefits have been extensively studied, but there is currently a dearth of information on the pharmacological and biological properties of unique chemical components (polyphenolic compounds) found throughout the entire plant. Only the effect of crude plant extract as a cytotoxic agent has been studied; the effect of polyphenolic plant extract has not been fully explored. Additionally, no research on the polyphenol plant component in the human prostate carcinoma cell line (PC-3) has been conducted in Iraq. To fill this void, this research describes the methodology for examining the first report on extract using a sequential extraction technique, detecting polyphenols using high-performance liquid chromatography and evaluating its cytotoxic impact on PC-3 prostate cancer cells.

## Materials and Methods

### Chemicals and reference standards

Acetonitrile (HPLC grade, 99.3 %) and methanol (HPLC grade) were purchased from Sigma-Aldrich (Germany). Ethyl Acetate (99.5 %) was obtained from Scharlab S.L. (Spain) and hexane (99 %) from BDH Limited (England). MTT Stain was purchased from Bio-World (USA). Reference standards used were apigenin, chlorogenic acid, benzoic acid and ferulic acid (Picasso, China); caffeic acid and syringic acid (MACKLIN, China); resveratrol (Hangzhou Hyper Chemicals Ltd., China); and epicatechin (Picasso, China).

### Instruments

Cell culture plates were obtained from (Santa cruz Biotechnology, USA). The following instruments were used: chiller (Ultratemp 2000, Julabbo F30, Buchi, Germany), digital caliper (Ingco China), electrical sensitive Balance (ADAM AFP-360L), high-performance liquid chromatography (SYKAMN, Germany; column C18, Ministry of Science and Technology, Department of Environment and Water), incubator and micropipettes (Cypress Diagnosis, Belgium), inverted microscope (Nikon, Japan), Laminar flow hood (K&K Scientific supplier, Korea), microtiter reader (Gennex Lab, USA), Rotary evaporator (Evaporation of solvent was carried out under reduced pressure utilizing IKARV 10 D S99 R) and water bath (MEMMERT, Germany).

### Plant materials

Whole fresh plants of *E. peplus* (leaves, stems, flowers and roots) were collected from Babylon Governorate, Iraq, in April 2024. The plant was identified by comparison with the available specimens in the University of Baghdad Botanical Library, Faculty of Science, Department Biology. A voucher specimen (BUH No. 86039) was deposited (8, 9). The plants were washed with distilled water and air dried in the shade for 7–10 days, ground to fine powder by an

electronic blender (CEER-220, Retsch, Germany). The ground material was stored in closed containers at room temperature until the extraction (10).

### Extraction of *E. peplus*

Sequential Soxhlet extraction was performed. Approximately 500 g of powdered plant material was weighed into a Soxhlet thimble (Sigma-Aldrich, USA). Extraction was carried out with n-hexane (1000 mL, HPLC) to eliminate the non-polar fraction over 6 hr. The extract was filtered under reduced pressure and concentrated by rotary evaporator (Heidolph, Germany) to obtain 6 g of n-hexane extract (11, 12). The extraction of residual plant material was performed with ethyl acetate (1000 mL, HPLC grade) for 12 hr, after which the ethyl acetate fraction was evaporated in vacuum to give 8.2 g (8.2 % w/w) dry extract, stored at 4 °C until use (13). The extraction process is illustrated in Fig. S1.

### Phytochemical analysis by HPLC

The qualitative and quantitative analysis of polyphenols in the ethyl acetate extract was performed at Environmental and Water Research Department, Ministry of Science and Technology (14). Resveratrol, salicylic acid, syringic acid, ferulic acid, benzoic acid, chlorogenic acid, caffeic acid, epicatechin and apigenin were employed as standards. Sample peaks ( $R_t$ ) were compared with standards for qualitative reference. HPLC conditions are summarized in Table S1 (15-17). Quantitation was carried out with the aid of calibration curves (AUC vs. standard concentrations) and the linear regression line:

$$Y = aX + b$$

where a is slope, b is y-intercept, X is concentration and Y is peak area. The retention time values of standards and samples are summarized in Table S2.

### Cell culture

PC-3 human prostate carcinoma cells (ATCC, Manassas, VA, USA) were cultured in MEM supplemented with 10 % fetal bovine serum (FBS, Capricorn-Scientific, Germany), 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were maintained at 37 °C in 5 % CO<sub>2</sub>, subcultured at 70 %-80 % confluence and used in exponential growth phase for experiments (18).

### Cytotoxicity assay (MTT)

Cell viability was assessed using the MTT assay (19, 20), in which determination of the formazan content at a specific wavelength, 570 nm, was made. There is a direct correlation between the number of living cells and the amount of formazan generated. As a consequence, the ethyl acetate extract treatment reduces formazan formation, which indicates cytotoxicity via lowering absorbance. The half-maximal inhibitory concentration (IC<sub>50</sub>), which shows how much ethyl acetate extract is needed to inhibit 50 % of the cells, can be found more easily using the dose-response curve. The MTT cytotoxicity assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is a commonly used technique for assessing cell viability and cytotoxicity. Using mitochondrial dehydrogenases, living cells convert yellow MTT into purple formazan crystals, which is the basis for this chromatic assay. For the MTT experiment, cells are usually arranged on a 96-well plate and exposed to various doses of the ethyl acetate extract (21). MTT is added to every well and incubated once more following the incubation period. Insoluble formazan is produced from MTT by viable cells. When a chemical reduces cell viability by

50 %, its absorbance is measured by a spectrophotometer. PC-3 cells were seeded at  $1 \times 10^4$  cells/well in 96-well microplates (NEST Biotech, China), incubated for 24 hr and treated with *E. peplus* ethyl acetate extract at 31.25, 62.5, 125, 250, 500 and 1000 µg/mL for 72 hr. Following treatment, 28 µL of MTT solution (2 mg/mL in PBS, Elabscience, China) was added per well and incubated for 3 hr. Formazan crystals were dissolved in 100 µL DMSO and absorbance was measured at 492 nm using a microplate reader (BioTek, USA). As shown in the (Table S3), Cytotoxicity percentage was calculated as:

$$\text{Cytotoxicity \%} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

IC<sub>50</sub> values were obtained from dose-response curves using GraphPad Prism 8 software (22).

### Morphological observation

Apoptotic changes in treated PC-3 cells were observed under a 40× inverted microscope (Optika, Italy) after 48 hr, documenting features like cell shrinkage, membrane blebbing and apoptotic bodies.

### Statistical analysis

All experiments were performed in triplicate. Data were presented as mean ± SD. Differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism 8 software. Significance was set at  $p < 0.05$  (8).

## Results

### Summary of key findings

The ethyl acetate extract of *E. peplus* contains a variety of phenolic acids and flavonoids, as revealed by RP-HPLC analysis. The extract exhibited dose-dependent cytotoxicity against PC-3 prostate cancer cells, with an IC<sub>50</sub> of 162.5 µg/mL. To our knowledge, this is the first report from Iraq describing both the phytochemical composition and cytotoxic potential of *E. peplus*.

### Extraction methodology

The whole plant (leaves, stems, flowers and roots) was selected to extract as much as possible of potential bioactive constituents. Sampling was carried out in peak flowering season (April 2024) to capture the maximum concentration of bioactive compounds. The ethyl acetate fraction was chosen owing to its intermediate polarity, which allows extraction of a wide spectrum of bioactive compounds, including phenolic acids and flavonoids.

### Phytochemical profile

RP-HPLC identified nine polyphenolic compounds: phenolic acids (salicylic acid, syringic acid, ferulic acid, benzoic acid, caffeic acid), a polyphenol ester (chlorogenic acid), a stilbenoid (resveratrol) and flavonoids (apigenin, epicatechin). Benzoic acid was the most abundant, followed by chlorogenic acid (Table S4). The HPLC chromatograms of the ethyl acetate extract and the corresponding standard compounds are presented in Fig. S2-S11. Each chromatogram represents the retention behavior and peak matching between the extract and its respective standard, confirming the identity of the detected polyphenolic constituents.

### Cytotoxicity against PC-3 cells

The MTT assay demonstrated significant, dose-dependent cytotoxicity. The IC<sub>50</sub> was 162.5 µg/mL, indicating moderate potency (Fig. S12 & S13). The optical density (OD) curve over time for treated cells and the viability of PC-3 cells treated with the IC<sub>50</sub>

concentration compared to untreated controls are shown in Fig. S14 & S15. At 1000 µg/mL, cell viability decreased sharply, with morphological changes including shrinkage and detachment. Quantitative analysis revealed that benzoic acid, chlorogenic acid and resveratrol were among the most abundant compounds, potentially linked to cytotoxicity.

## Discussion

### Rationale for using whole plant extraction

The use of the whole plant likely contributed to the observed cytotoxic effects on PC-3 cells, as bioactive compounds are unevenly distributed among different plant parts. Employing the entire plant increases the diversity of secondary metabolites, such as phenolic acids and flavonoids, which may act synergistically to enhance anticancer activity. This approach ensures that compounds present in lower concentrations in individual plant parts are also included, providing a comprehensive evaluation of the plant's therapeutic potential (23, 24).

### Phytochemical composition and novelty

The presence of resveratrol, apigenin and epicatechin in Iraqi *E. peplus* represents a novel finding compared with earlier reports that focused mainly on terpenoids, sterols, saponins and other flavonoids (25-27). Environmental factors such as soil mineral content, prolonged sunlight (> 3000 hr/year) and abiotic stresses (drought, salinity) may enhance biosynthesis of secondary metabolites (28). Local ecotypes may also express unique biosynthetic pathways due to geographic isolation (29).

### Interpretation of cytotoxicity results

Plant extracts usually exhibit IC<sub>50</sub> values of 100-500 µg/mL against PC-3 cells, whereas standard anticancer drugs such as docetaxel show IC<sub>50</sub> in the low micromolar range (30, 31). Although less potent than clinical drugs, the extract falls within the effective natural product range. Benzoic acid, the most abundant compound, has been reported to show anticancer effects against PC-3 and other human cancer cell lines with IC<sub>50</sub> ranging from 85.5-670 µg/mL (32).

The phytochemical analysis indicated that phenolic compounds, known for antioxidant and free radical scavenging properties, are major contributors to the cytotoxic activity (33). These substances affect key cellular processes including apoptosis, metastasis, angiogenesis and cell proliferation (15, 21). The synergistic action of these compounds likely contributes to the overall cytotoxic effect observed.

### Comparison with previous *Euphorbia* studies

These findings align with earlier studies on other *Euphorbia* species. For instance, *E. helioscopia* hydro-methanolic and aqueous extracts exhibited higher total phenolic content than flavonoid levels (27). Similarly, other extracts from different sections of *E. peplus* were shown to suppress the growth of multiple cancer cell types (24).

### Role of key polyphenols in anticancer activity

- **Resveratrol:** induces apoptosis via caspase-3 activation and Bcl-2 downregulation (34).
- **Caffeic acid:** inhibits proliferation and induces cell cycle arrest (34).

- **Apigenin:** inhibits PI3K/Akt signaling and promotes apoptosis (34).
- **Ferulic acid:** exerts antioxidant activity and sensitizes cancer cells to apoptosis (34).
- **Epicatechin:** suppresses angiogenesis and metastasis by downregulating VEGF and MMPs (35).

### Proposed mechanism and future work

The MTT assay does not distinguish between necrosis and apoptosis. Studies on Euphorbiaceae indicate apoptosis may occur via intrinsic and extrinsic pathways, involving ROS generation, Bcl-2/Bax modulation, caspase activation and p53 regulation (26, 27).

Future studies will employ Annexin V/PI staining and Western blotting for apoptotic markers (caspase-3, Bax, Bcl-2, p53) to confirm the mechanism, along with *in vivo* validation (36).

### Conclusion

The present study provides valuable insight into the phytochemical composition and anticancer potential of the *E. peplus* ethyl acetate extract collected from Iraq. Qualitative and quantitative analyses revealed the presence of nine polyphenolic compounds, among which several were reported for the first time in Iraqi samples of this species. These compounds—particularly benzoic acid and chlorogenic acid—are believed to play a major role in the cytotoxic activity of the extract. The ethyl acetate fraction exhibited a significant inhibitory effect on prostate cancer (PC-3) cell viability, with an IC<sub>50</sub> value of 162.5 µg/mL, indicating moderate but promising cytotoxic potential.

Overall, the findings suggest that *E. peplus* could serve as a valuable source of bioactive phytochemicals with potential therapeutic application in prostate cancer management. This study contributes to the growing body of evidence supporting the pharmacological importance of the *Euphorbia* genus.

### Future perspectives

Further investigations are recommended to isolate and characterize the active constituents responsible for the observed anticancer effects and to evaluate their precise mechanisms of action. *In vivo* studies and molecular-level analyses are also necessary to confirm the safety, efficacy and possible synergistic interactions of these compounds, paving the way for their potential development into novel anticancer agents derived from natural sources.

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### Authors' contributions

TZAJ designed the study and MMH conducted the study. The manuscript was co-written by both authors. All authors have read and approved the final manuscript.

### Compliance with ethical standards

**Conflict of interest:** The authors do not have any conflict of interest to declare.

**Ethical issues:** None

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