SUPPL.TABLES

Table S1. Chromatographic conditions used for ethyl acetate fraction

Mobile phase	**Gradient elution** Solvent **A:** 1% formic acid in water Solvent **B**: acetonitrile 0 - 5 min: (**A**:90% **B**:10%) 5 - 9 min: (**A**:80% **B**:20%) 9- 15 min: (**A**:70% **B**:30%) 15 - 25 min: (**A**:60% **B**:40%)
Column	SYKAM LC C18 (250 mm × 4.6 mm, 5 μm particle size).
Samples	Whole plant ethyl acetate fraction
Standards	Resveratrol, salicylic acid, Syringic acid, Ferulic acid, Benzoic acid, Chlorogenic acid, Apigenin, Caffeic acid, and Epicatechin
Flow rate	1 mL / min
Injection volume	50 μL
Injection concentration	1 mg /mL
Detection	UV Detector at λ 254 and 277

Table S2. Retention time of standards, sample and concentration in plant extract (ppm), of the polyphenolics in *Euphorbia peplus* ethyl acetate extract

Active constituents	**Rt of standards**	**Rt of sample**	**Concentration in plant extract mg/g**
Caffeic acid	4.19	4.12	0.4422
Syringic acid	7.62	7.69	0.1051
salicylic acid	10.25	10.22	0.4899
Ferulic acid	12.25	12.20	0.7565
Chlorogenic acid	17.38	17.32	2.060
Resveratrol	19.9	19.9	1.26694
Epicatechin	23.85	23.89	0.1269
Apigenin	24.29	24.25	0.1052
Benzoic acid	27.25	27.23	3.9761

Table S3. Cytotoxicity percentage of ethyl acetate extract from *Euphorbia peplus* at different concentrations

Concentration	1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	31.2 µg/mL
% Cytotoxicity	69.1954	57.8981	44.4991	24.4991	17.8981	6.5353

Table S4. Quantitative estimation of polyphenolic compounds in *Euphorbia peplus* ethyl acetate extract (µg/g extract)

Compound	**Concentration (µg/g)**	**Rt (min)**
Benzoic acid	25.4	5.2
Chlorogenic acid	18.7	7.3
Resveratrol	12.5	8.9
Apigenin	10.2	9.4
Epicatechin	9.6	10.1
Caffeic acid	7.8	6.5
Salicylic acid	6.3	4.7
Syringic acid	5.1	5.9
Ferulic acid	4.9	6.1

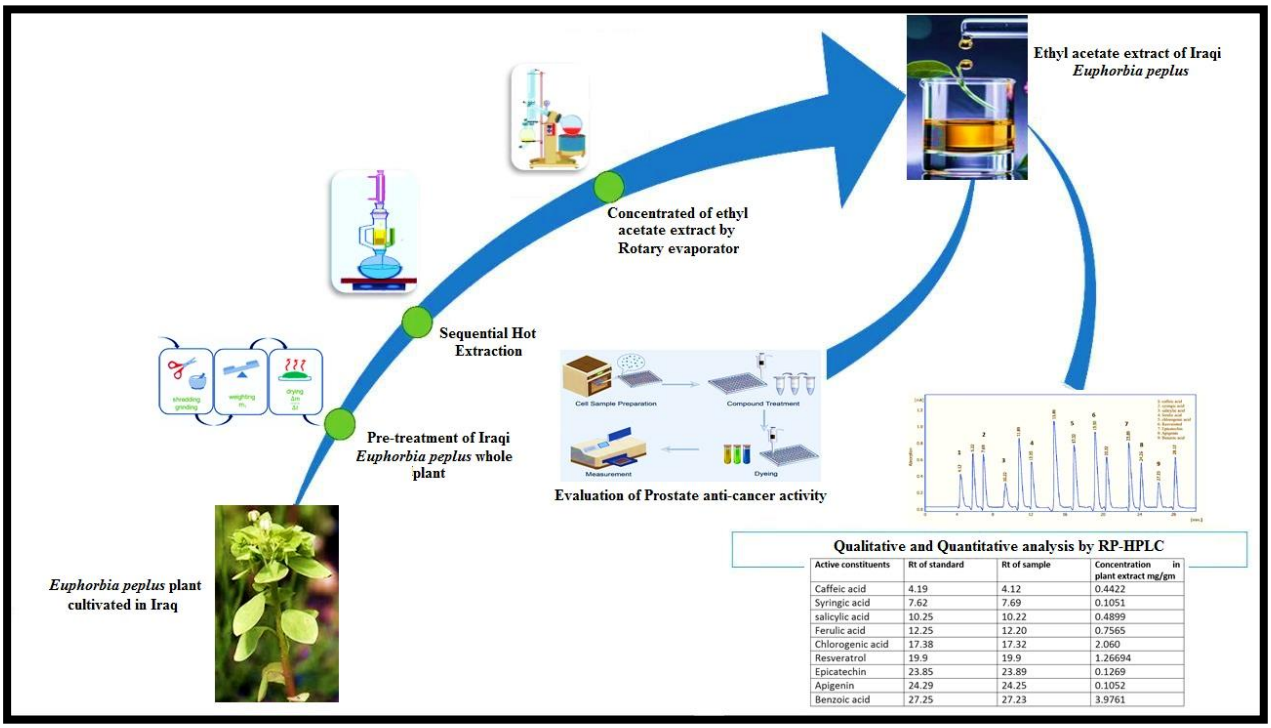

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Fig. S1. General scheme of Iraqi *Euphorbia peplus* extraction, phytochemical analysis and evaluation of prostate anticancer activity. The diagram summarizes the sequential extraction process, concentration of the ethyl acetate fraction, subsequent phytochemical profiling (HPLC) and the observed anticancer activity against PC3 prostate cancer cells.

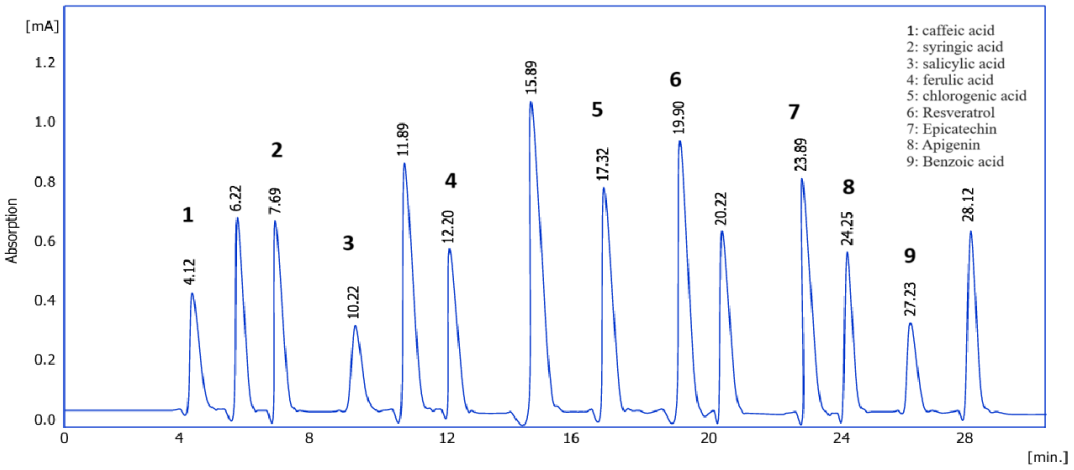


Fig. S2. HPLC chromatogram of Euphorbia peplus ethyl acetate whole plant extract showing the detection of several phenolic and flavonoid compounds. Major identified peaks include salicylic acid, caffeic acid, chlorogenic acid , syringic acid, ferulic acid, resveratrol, benzoic acid, apigenin, and epicatechin, confirming the presence of bioactive constituents with potential anticancer activity.

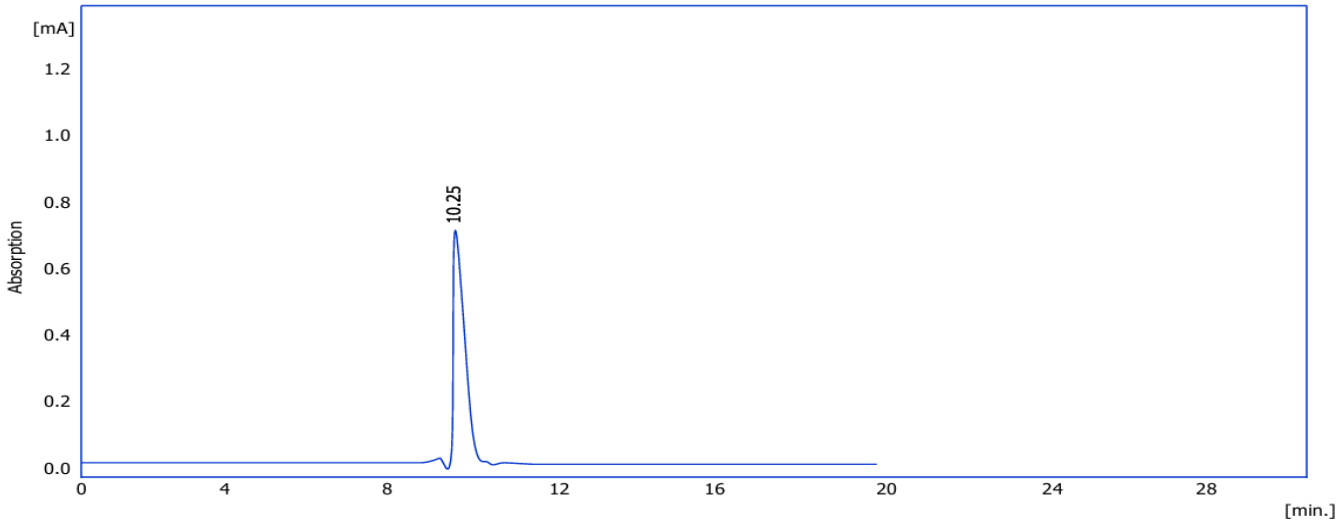


Fig. S3. HPLC chromatogram of salicylic acid standard showing a distinct peak at a retention time of 10.25 min. This reference peak was used to validate and confirm the presence of salicylic acid in the *Euphorbia peplus* ethyl acetate extract.

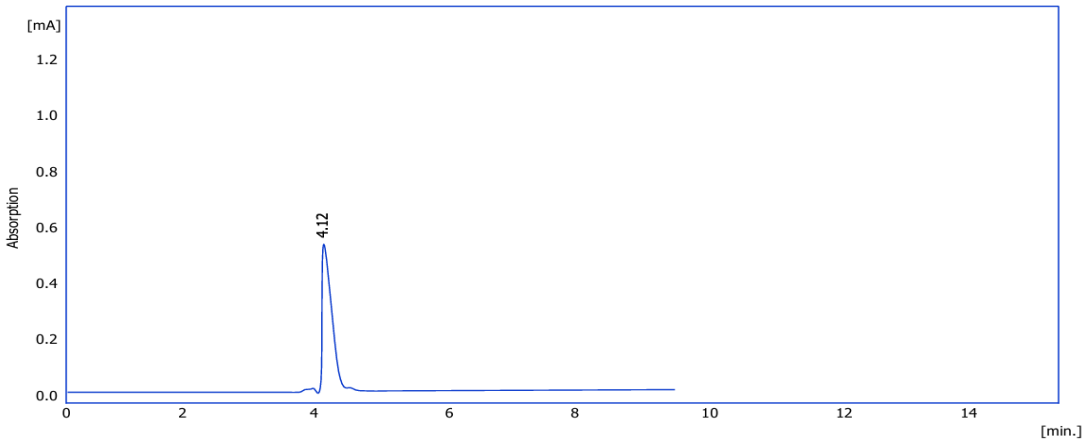


Fig. S4. HPLC chromatogram of caffeic acid standard showing a characteristic peak at a retention time of 4.12 min. This peak was used as a reference to validate and confirm the presence of caffeic acid in the *Euphorbia peplus* ethyl acetate extract.

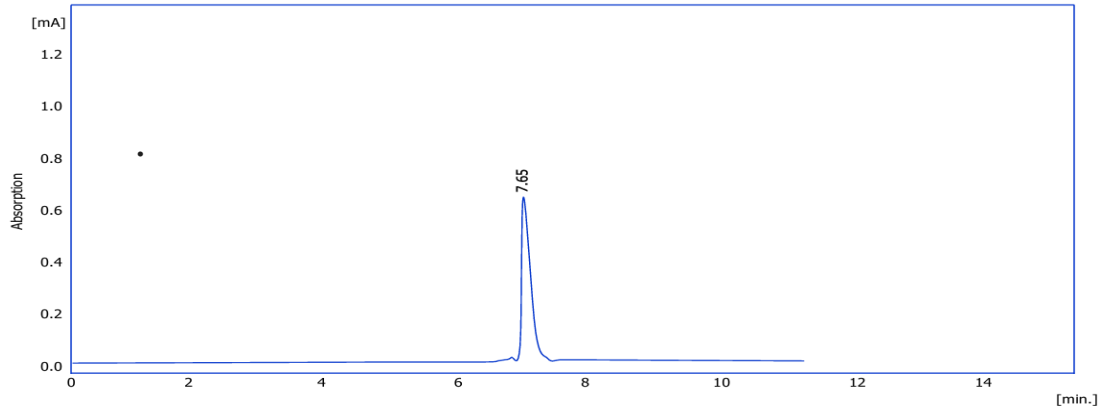


Fig. S5. HPLC chromatogram of syringeic acid standard showing a distinct peak at a retention time of 7.65 min. This reference peak was used to confirm the presence of syringeic acid in the *Euphorbia peplus* ethyl acetate extract**.**

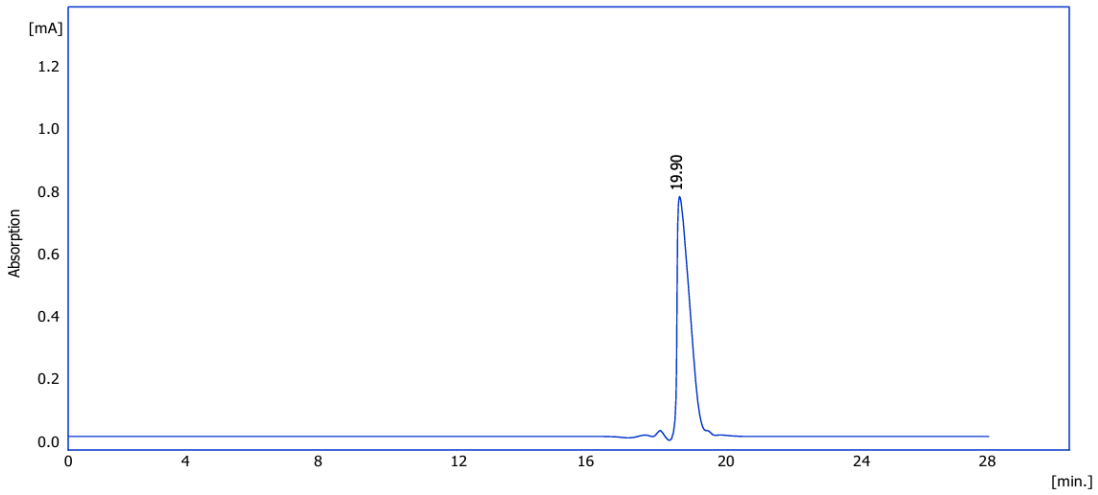


Fig. S6. HPLC chromatogram of resveratrol standard showing a distinct peak at a retention time of 19.90 min. This reference peak was used to validate and confirm the presence of resveratrol in the *Euphorbia peplus* ethyl acetate extract.

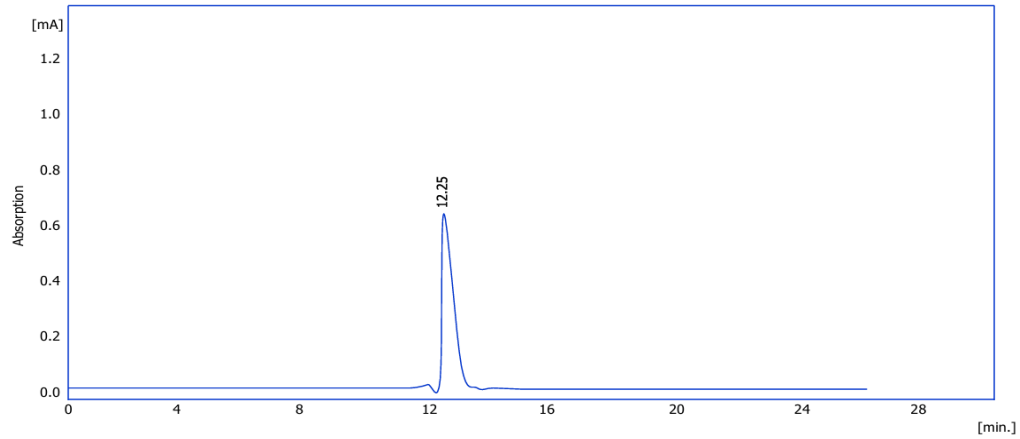


Fig. S7. HPLC chromatogram of ferulic acid standard showing a distinct peak at a retention time of 12.25 min. This reference peak was used to validate and confirm the presence of ferulic acid in the *Euphorbia peplus* ethyl acetate extract.

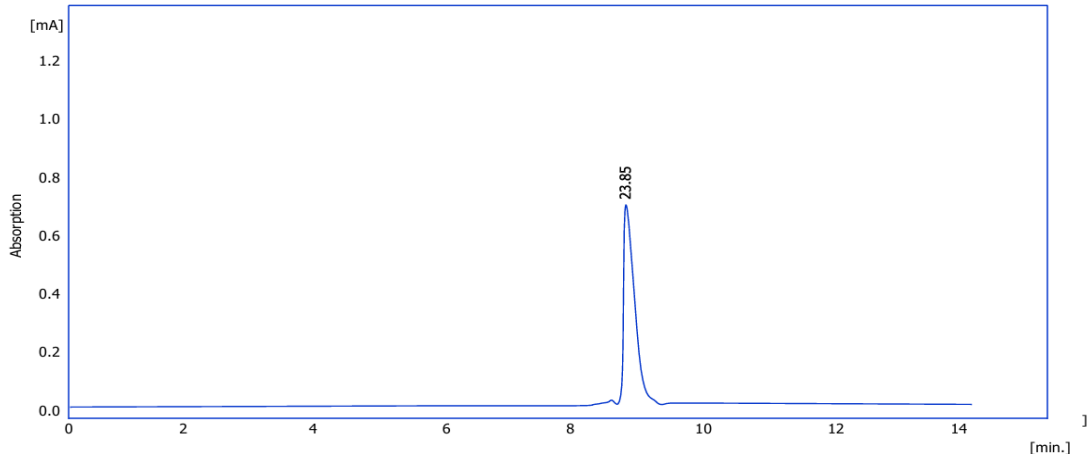


Fig. S8. HPLC chromatogram of epicatechin standard showing a distinct peak at a retention time of 23.85 min. This reference peak was used to validate and confirm the presence of Epicatechin in the *Euphorbia peplus* ethyl acetate extract.

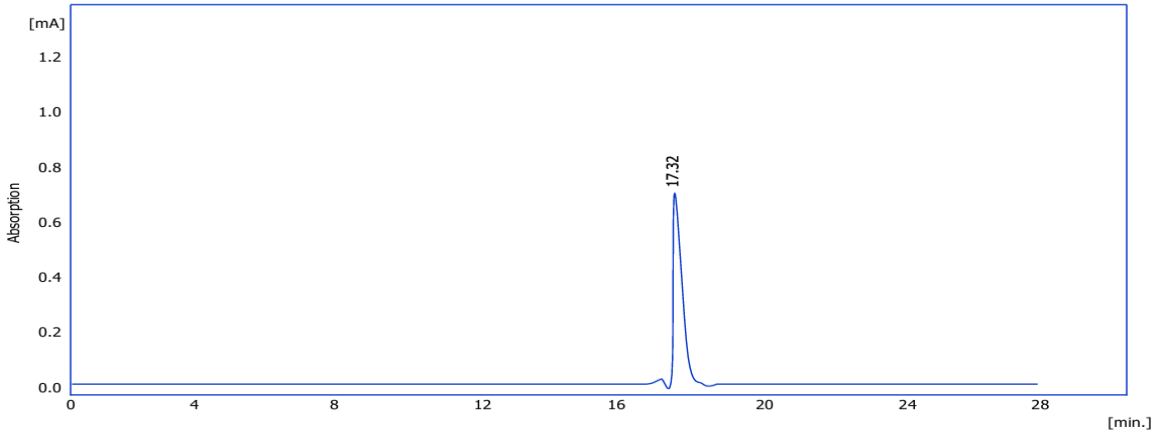


Fig. S9. HPLC chromatogram of chlorogenic acid standard showing a distinct peak at a retention time 17.32 min. This reference peak was used to validate and confirm the presence of cholinergic acid the *Euphorbia peplus* ethyl acetate extract.

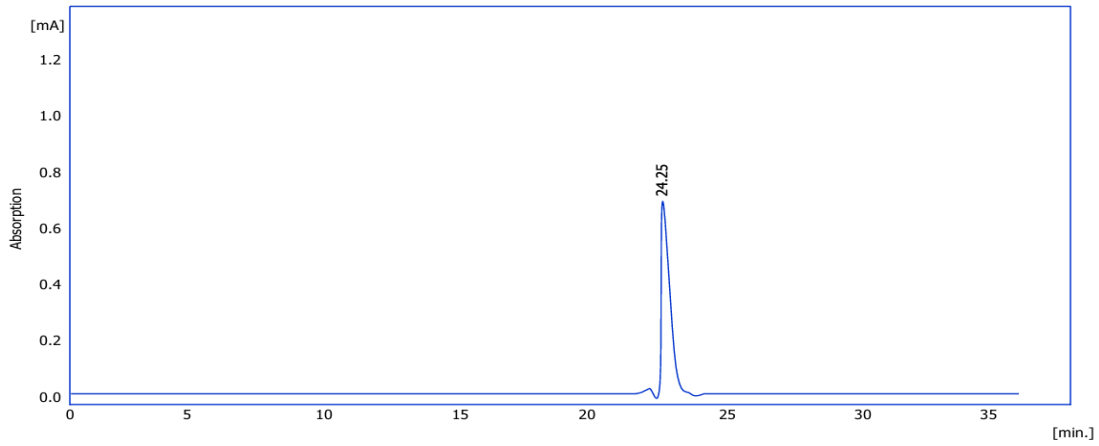


Fig. S10. HPLC chromatogram of Apigenin standard showing a distinct peak at a 24.25 min. This reference peak was used to validate and confirm the retention time of presence of Apigenin in the *Euphorbia peplus* ethyl acetate extract.

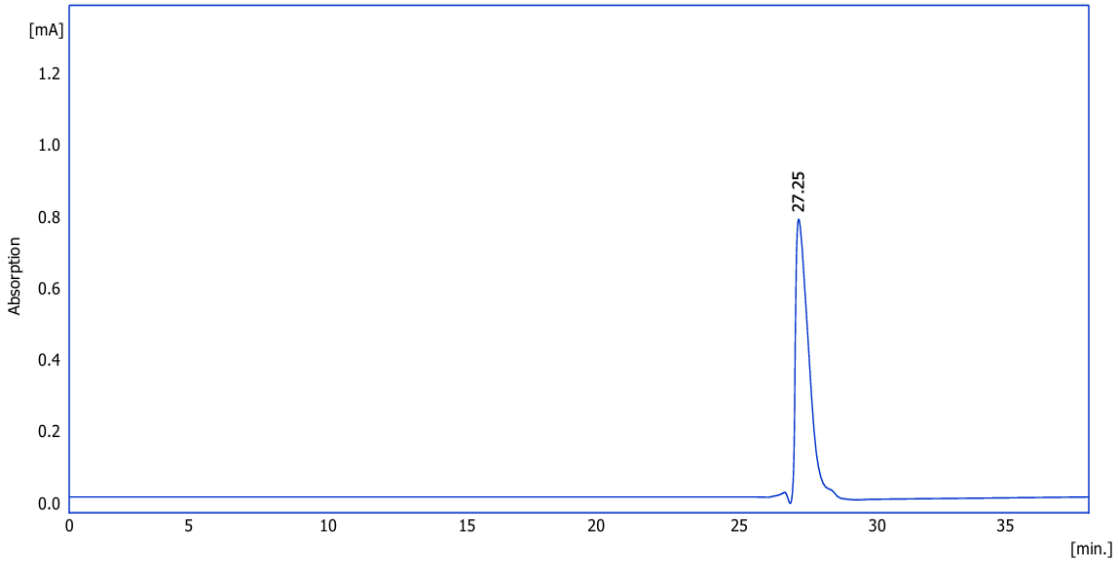


Fig. S11. HPLC chromatogram of Benzoic acid standard showing a distinct peak at a 27.25 min. This reference peak was used to validate and confirm the retention time of presence of Benzoic acid in the *Euphorbia peplus* ethyl acetate extract.

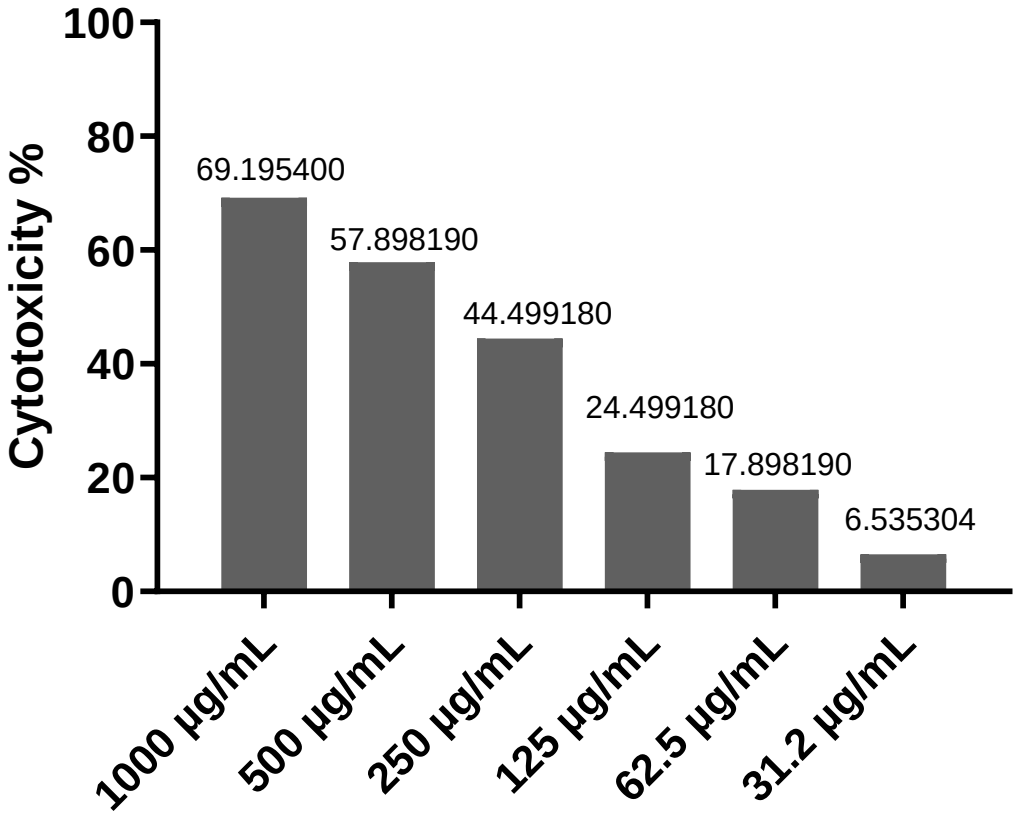


Fig. S12. Inhibitory curve showing the effect of *Euphorbia peplus* ethyl acetate extract on cell viability. The x- axis represents the concentration of the ethyl acetate (in µg) and the represents the percentage of cell death (Cytotoxicity).

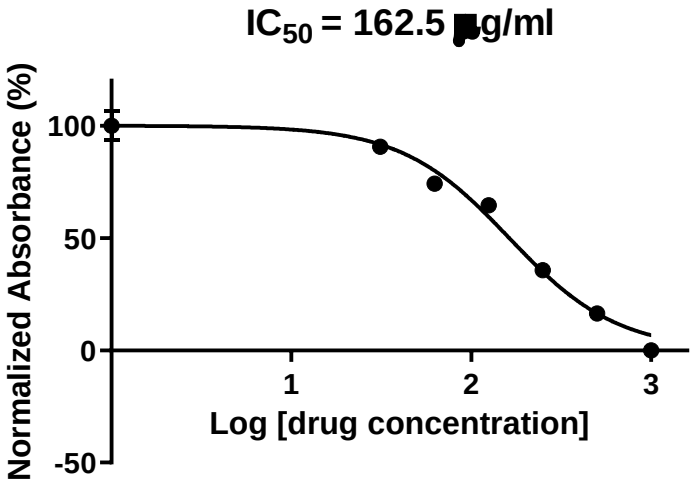


Fig. S13. Dose-response curve used to determine the IC50 value of *Euphorbia peplus* ethyl acetate extract on ells. The x-axis represents the concentration of the drug; the y axis is the percentage of normal absorbance.

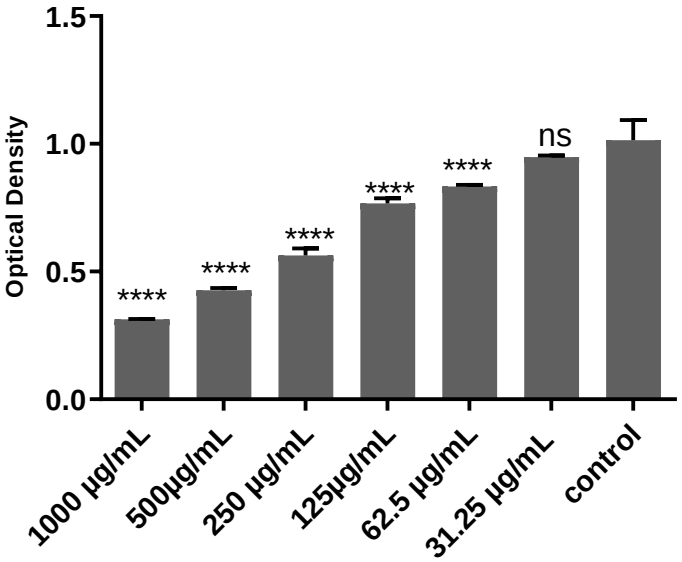
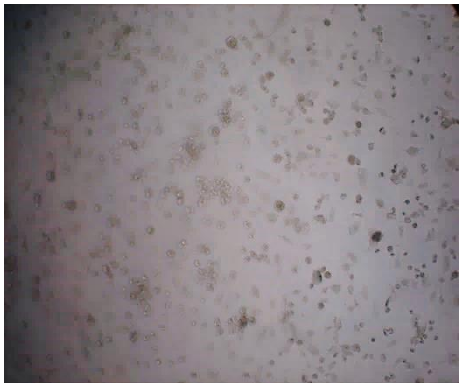


Fig. S14. Optical density (OD) reading curve over time for cells treated with *E. peplus* ethyl acetate extract. The x-axis represents concentrations in ug/mL and the y-axis represents the OD value at 492 nm. Each data point represents the mean ± standard deviation of triplicates.

PC3(un-treated)



PC3 (treated)

Fig. S15. The viability of PC-3 cell treated with IC50 of the ethyl acetate fraction of the Iraqi *Euphorbia peplus* whole plant (right) and untreated Cells (left).