

Phytochemical screening and *in-vitro* assessment of the cytotoxic potential of extracts from a commercially prepared fruit skin using PC-3 prostate cancer cells

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BACKGROUND

Globally, prostate cancer (PCa) is the most frequently diagnosed cancer in men, with the fifth highest mortality rate among cancer deaths in men (Sung *et al.*, 2021).

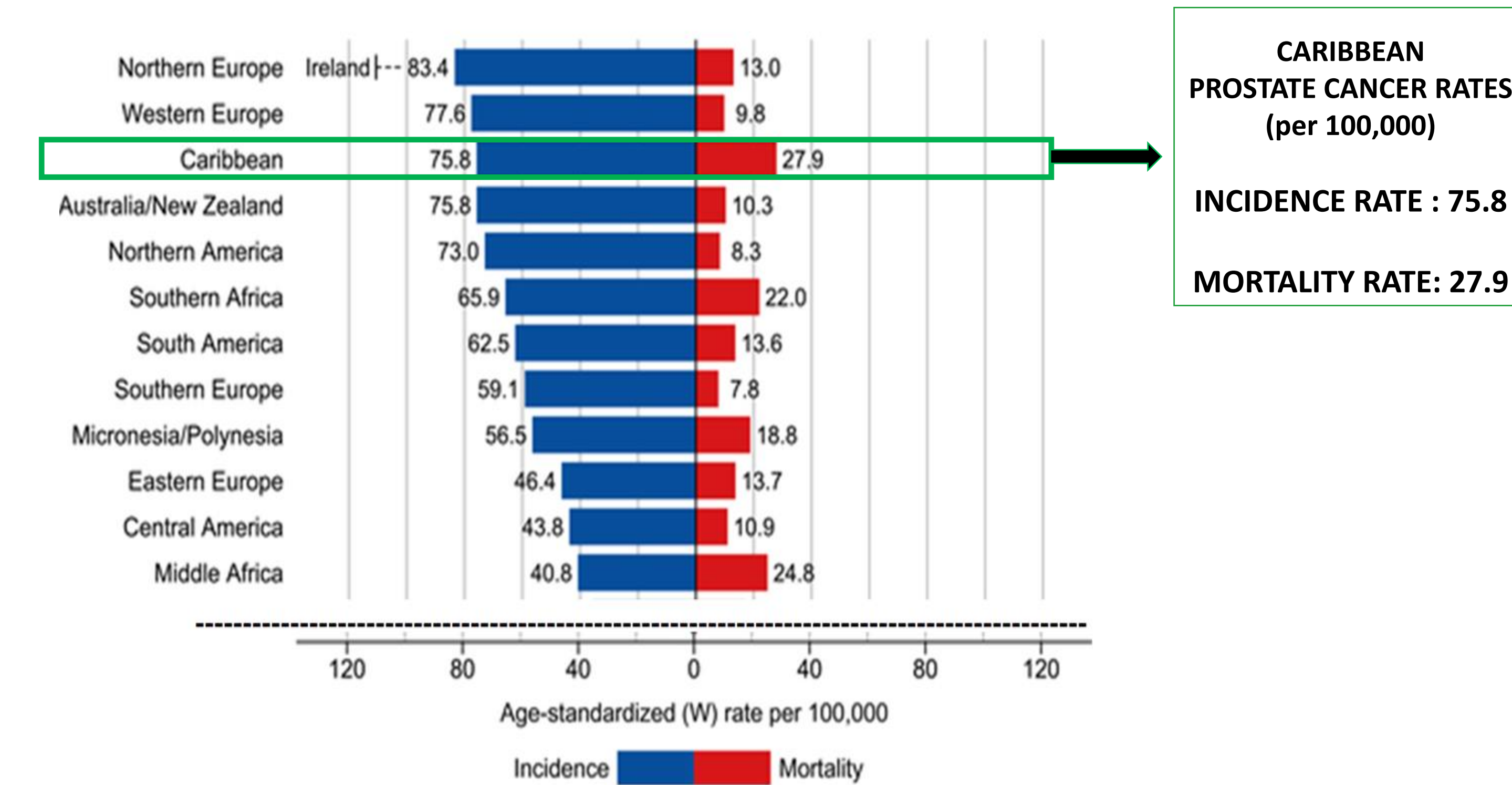


Figure 1. Worldwide Incidence and Mortality rates for Prostate Cancer (modified from Sung *et al.*, 2021)

As seen in Figure 1, The Caribbean boasts the highest PCa mortality rate in the world with one of the highest incidence rates. In Trinidad & Tobago, PCa continues to be the leading cause of cancer-related mortality in men with an incidence rate on the rise (Warner *et al.*, 2018). After therapy, PCa may relapse to become castration-resistant prostate cancer (CRPC) which is difficult to treat. This latter situation has fuelled the search for novel treatment options inclusive of plant-derived chemotherapeutic agents (Lichota and Gwozdinski, 2018).

METHODOLOGY

Preparation of extracts was carried out by repeated cold maceration of the fruit skin, followed by filtration, drying and liquid-liquid extraction with solvents (Figure 2). The effect of extracts E1- E5 on PC-3 cells viability was assessed *in-vitro* using the MTT assay and the extract with the highest cytotoxic activity E3, was selected for further testing. The IC₅₀ value of E3 was calculated from dose-response curves and Selectivity Index determined using PNT1A cells (Figure 3 and Table 1). Abiraterone Acetate (AA) a drug in current clinical use for CRPC, was employed as a positive control (Figure 4). Qualitative effects of E3 on PC-3 and PNT1A cells morphology were captured over 120h exposure (Figure 5). Phytochemical screening of E3 using chemical visualization agents was performed (Figure 6 and Table 2). Subsequently, the extract was subjected to further separation using preparative thin layer chromatography (PTLC) to yield S1 – S5 (results not shown). MTT assay was executed at every step to guide the purification process, with S1 and S2 revealed as the most cytotoxic sub-fractions (Figures 7 & 8 and Table 3).

RESULTS

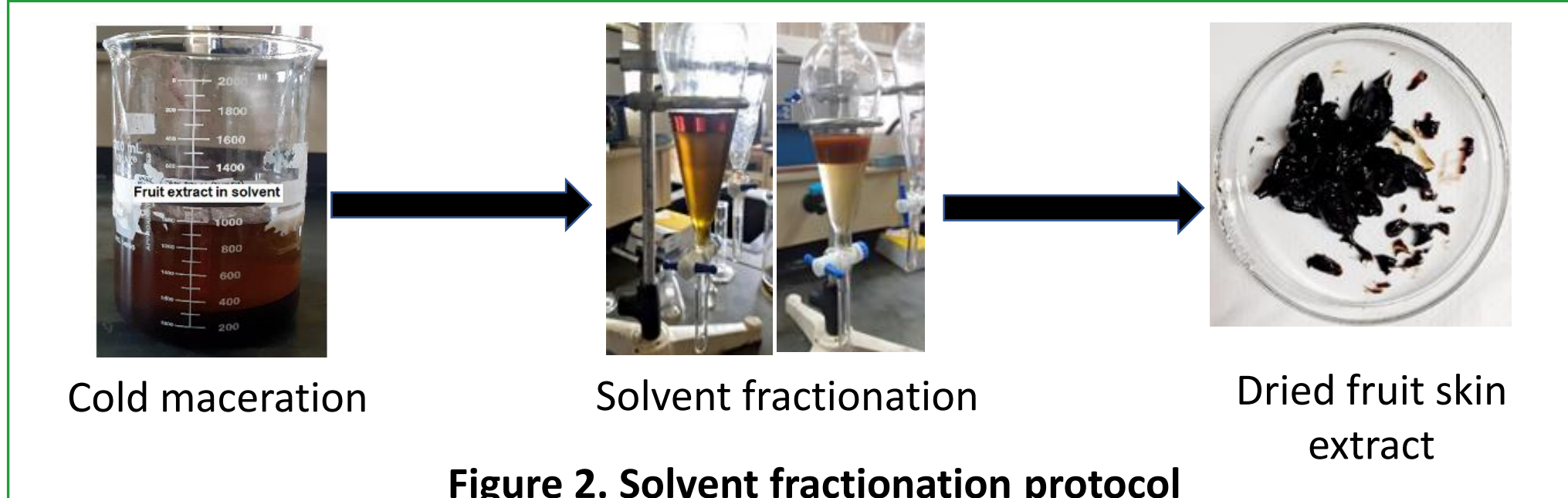


Figure 2. Solvent fractionation protocol

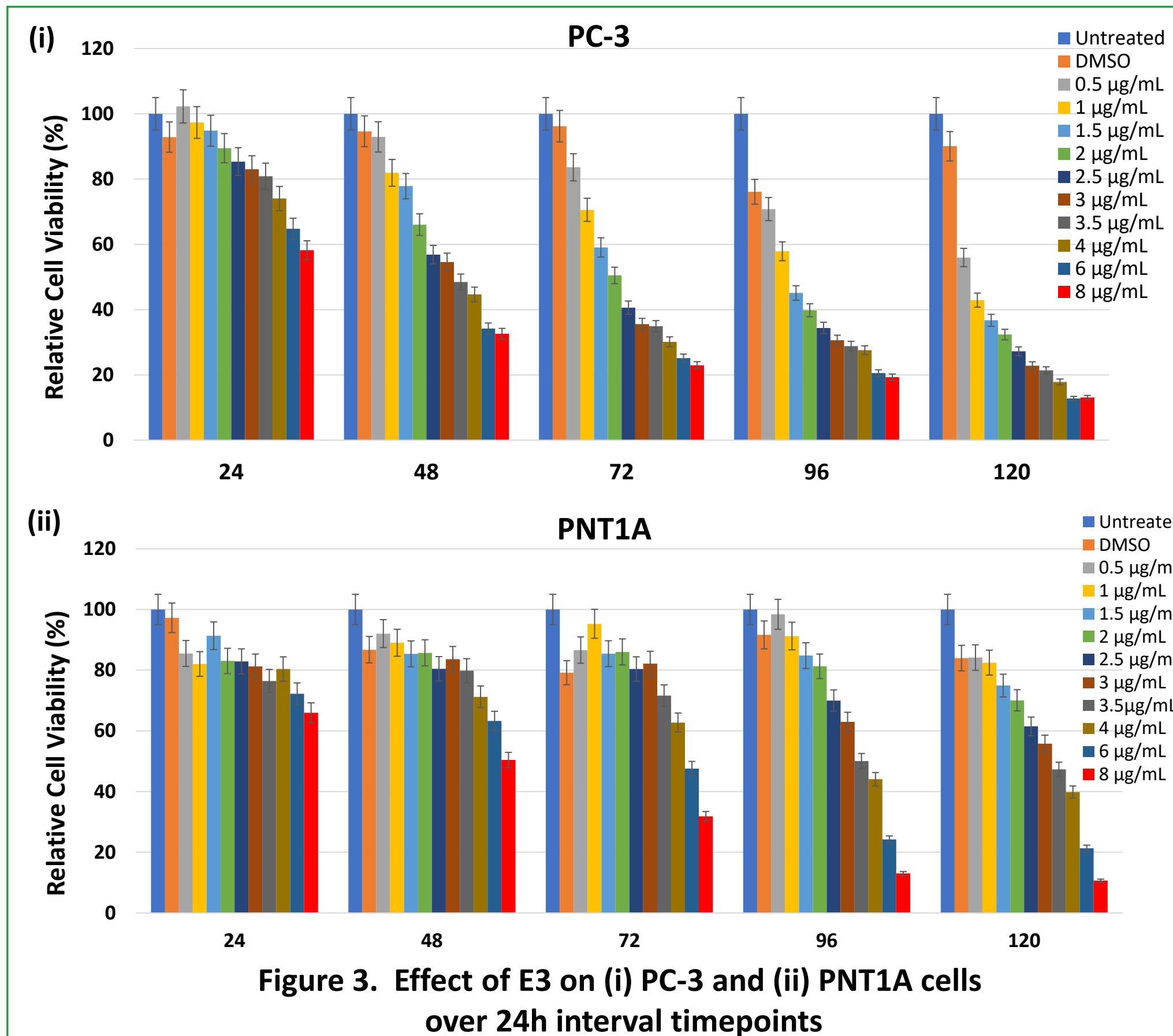


Figure 3. Effect of E3 on (i) PC-3 and (ii) PNT1A cells over 24h interval timepoints

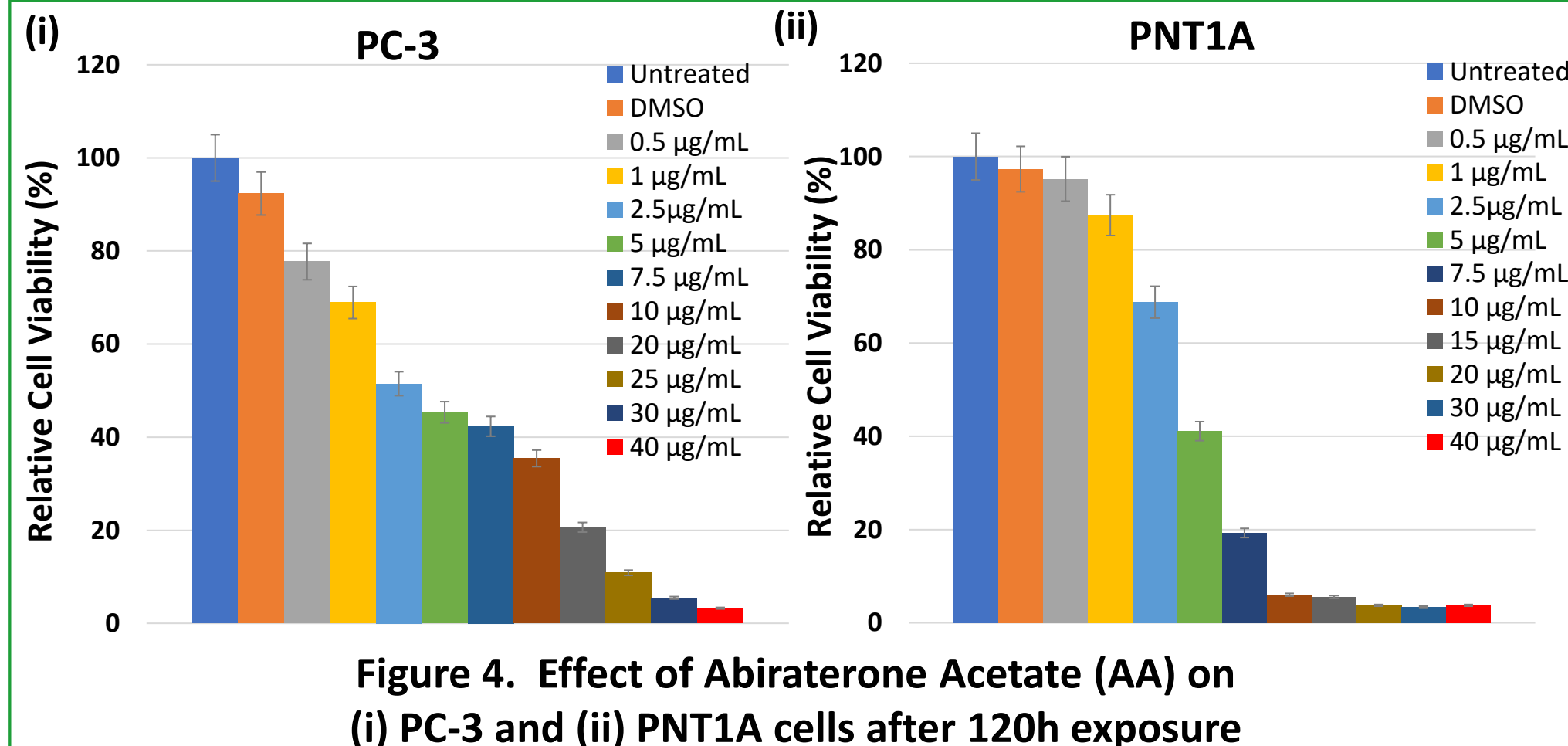


Figure 4. Effect of Abiraterone Acetate (AA) on (i) PC-3 and (ii) PNT1A cells after 120h exposure

EXTRACTS/ +VE CONTROL	PC-3	PNT1A	SI
E1	4.55	9.52	2.09
E2	6.54	11.12	1.70
E3	0.714	3.10	4.34
E4	38.96	23.55	0.60
E5	>400	405.90	≥ 1.00
AA	3.193	3.698	1.16

Table 1. IC₅₀ (µg/mL) and SI values of E1 – E5 and Abiraterone Acetate (AA) after 120h exposure

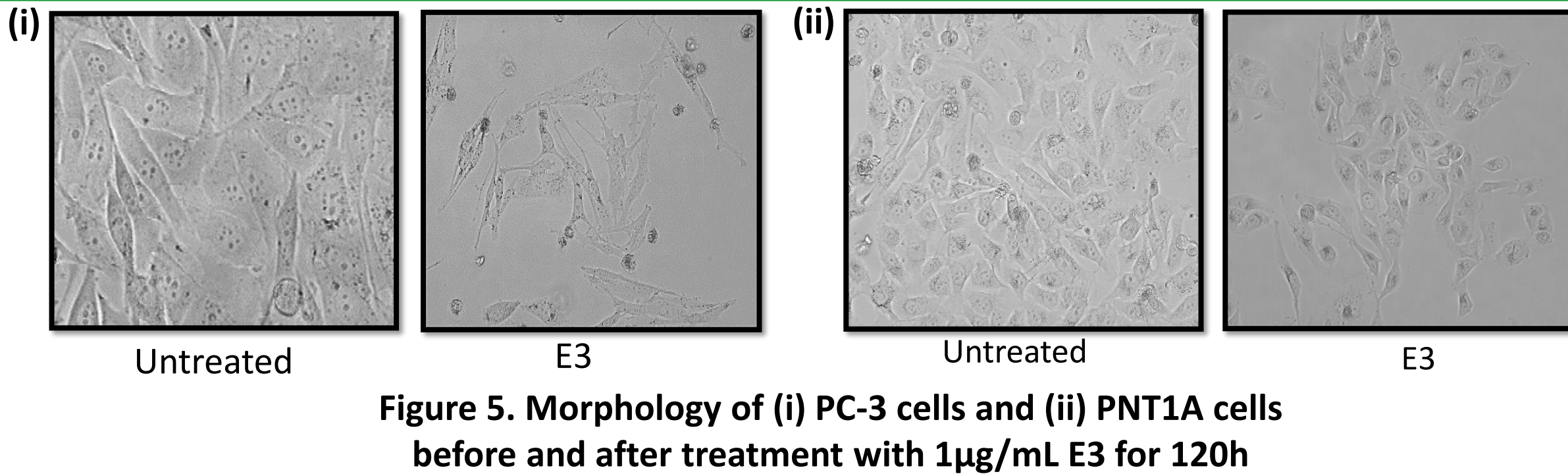


Figure 5. Morphology of (i) PC-3 cells and (ii) PNT1A cells before and after treatment with 1µg/mL E3 for 120h

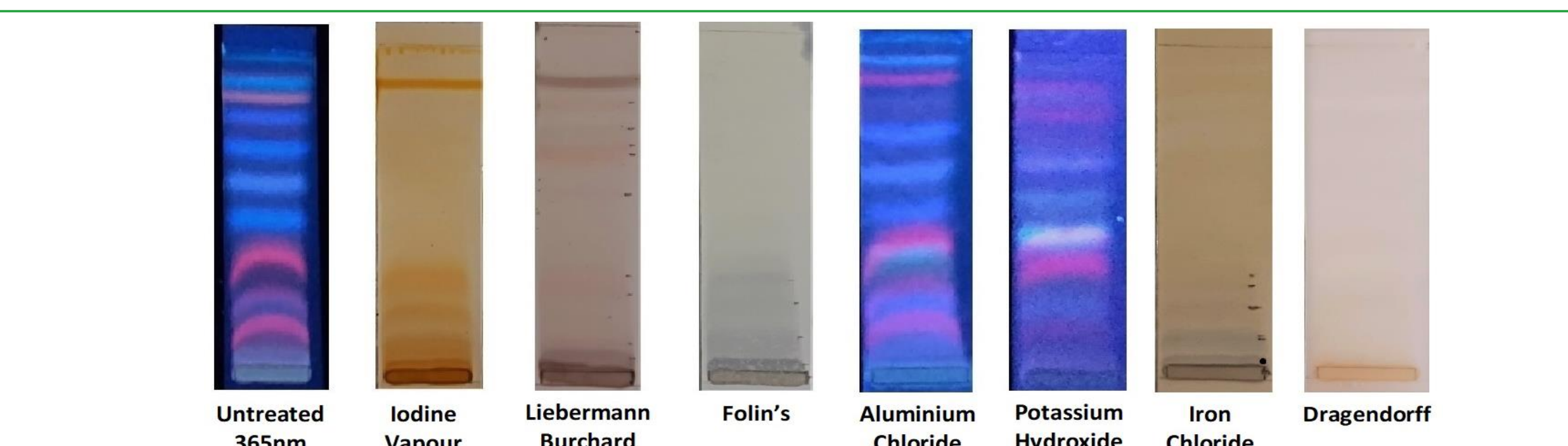


Figure 6. Analytical TLC of E3 before and after application of visualization sprays

Visualization Reagents	Active Constituents	Result
Iodine Vapour	Organic Compounds	+
Liebermann Burchard	Steroids/Triterpenes	+
Folin's	Phenols	+
Aluminium Chloride	Flavonoids	+
Potassium Hydroxide	Anthraquinones/ Coumarins	+
Iron Chloride	Tannins	+
Dragendorff	Alkaloids	-

Table 2. Phytochemical Screening of E3

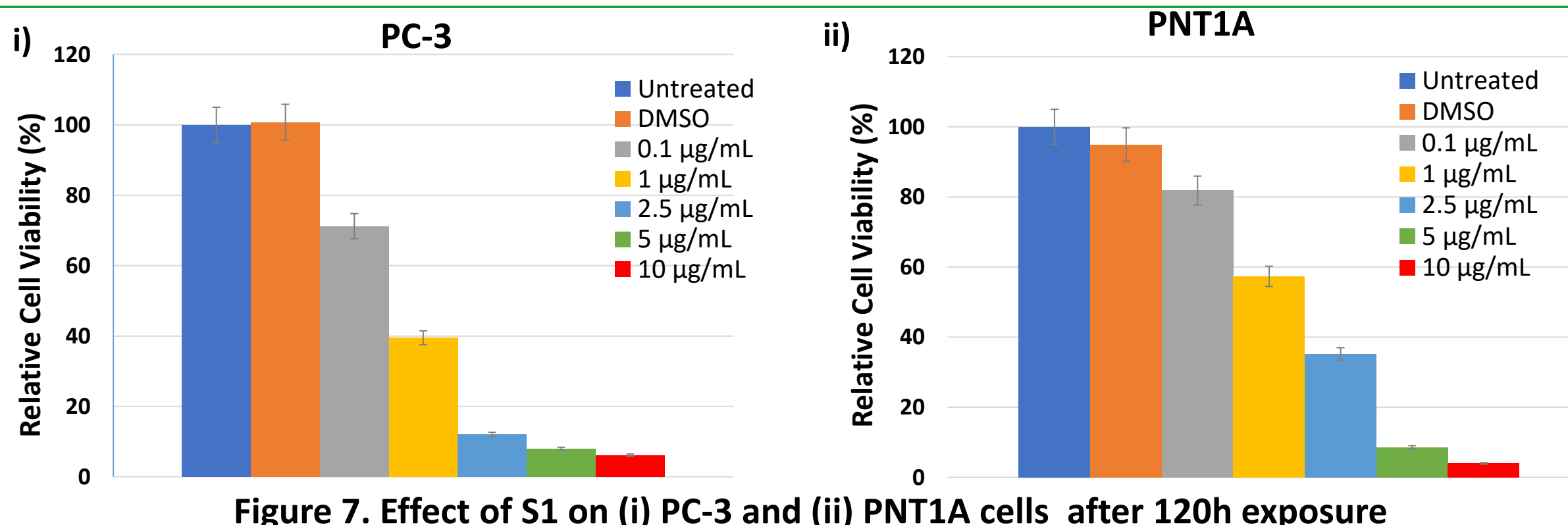


Figure 7. Effect of S1 on (i) PC-3 and (ii) PNT1A cells after 120h exposure

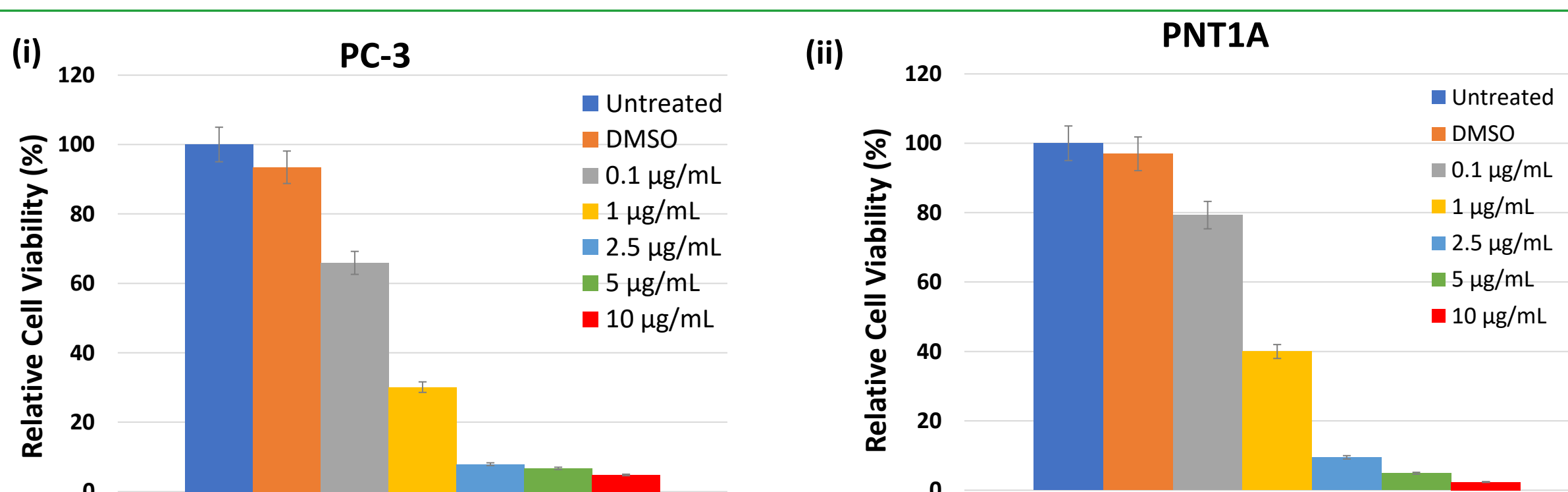


Figure 8. Effect of S2 on (i) PC-3 and (ii) PNT1A cells after 120h exposure

PTLC Sections	PC-3	PNT1A	Selectivity Index
S1	0.345	1.116	3.23
S2	0.240	0.483	2.01

Table 3. IC₅₀ (µg/mL) and SI values of S1 and S2 after 120h exposure

RESULTS CONTINUED

Five (5) extracts E1 – E5 were isolated, with E3 as the most cytotoxic against PC-3 cells; IC₅₀ = 0.714µg/mL and SI = 4.34 after 120h exposure (Figure 3 and Table 1). Treatment of PC-3 cells with 1µg/mL E3 for 120h, induced morphological changes including membrane/nucleus disruption, shrinkage of cells and loss of adhesion properties (Figure 5). Additionally observed was a drastic decrease in cell population. Images of PNT1A cells after the same treatment showed a decrease in cell population but no obvious visible changes to the cell morphology. Phytochemical analysis of E3 confirmed the presence of seven (7) families of organic compounds (Figure 6 and Table 2). Five sub-fractions S1 - S5, were isolated from E3 after performing PTLC. From these, the most cytotoxic against the PC-3 cells were S1 and S2 with IC₅₀ values of 0.345µg/mL and 0.240µg/mL respectively (Figures 7 & 8); the corresponding SI values were 3.23 and 2.01 (Table 3). S1 and S2 were found to be 9 to 13-fold more cytotoxic for the PC-3 cells than abiraterone acetate (AA) and about two to three times more selective (Tables 1 & 3).

DISCUSSION

Phytochemical screening of E3 using chemical visualization agents revealed the presence of specific families of organic compounds: steroids/ triterpenes, phenols, tannins, flavonoids and anthraquinones/ coumarins. This spectrum of bioactive compounds has been found to display anticancer properties e.g., a semisynthetic taxane (diterpene) derived from extracts of the European Yew tree, used clinically as Docetaxel for the treatment of PCa (Lichota and Gwozdinski, 2018).

CONCLUSION

The present results suggest that S1 and S2 extracts/natural products may be promising candidates for further development into pharmaceutical agents or botanical supplements. By seeking to unearth novel, more efficacious cancer treatments, this work is contributing to the UN SDG #3, which focuses on good health and well-being.

ACKNOWLEDGEMENTS

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