

Synopsis of cancer cell line studies

In vitro cytotoxicity of ten asterosaponins extracted from *Culcita novaeguineae* was evaluated against human leukemia K-562 and human hepatoma BEL-7402 cell lines[6-8]. Among these, three saponins (regularoside B, novaeguinoside I and II) were isolated from ethanolic extracts, while unnamed saponins (saponins 1–3) were obtained through bioassay-guided fractionation of an n-BuOH extract. Four additional asterosaponins (asterosaponin 1, thornasteroside A, marthasteroside A1, and regularoside A) were identified via further bioassay-guided fractionation of a new n-BuOH extract[6-8]. All asterosaponins exhibited moderate cytotoxicity, with asterosaponin 1 and novaeguinoside II demonstrating the highest level of bioactivity, showing inhibitory concentrations 2–4 times lower than the other saponins tested[6-8]. Later research isolated four more asterosaponins (novaeguinosides A–D) from *C. novaeguineae* and tested their ability to promote tubulin polymerisation *in vitro* as potential antitumor agents the K-562 and BEL-7402 cell lines[54]. All four asterosaponins showed cytotoxicity, with IC₅₀ values ranging from 0.7 to 9.5 μ M. Novaeguinoside C exhibited the highest potency (IC₅₀ of 1.3 and 0.7 μ M, respectively), although none of the saponins tested demonstrated the ability to polymerise tubulin[54]. The combined findings from these studies suggested that specific structural traits, such as $\Delta^9(11)$ -3 β ,6 α -dioxysteroidal aglycone with a C-3 sulfate group and an oligosaccharide moiety at C-6 as a potent configuration, are responsible for potent cytotoxic activity[54].

In one study, nine triterpenoid saponins (pacificusosides D-K and cucumarioside D) isolated from *Solaster pacificus* were tested for cytotoxicity against non-cancerous mouse epidermal cells (JB6 CI41) and a panel of human melanoma cell lines (SK-MEL-2, SK-MEL-28, and RPMI-7951) at concentrations ranging from 0.1 to 62.5 μ M for 24 h[15]. The non-cancerous JB6 CI41 served as a reference to assess cancer selectivity, with greater differences in IC₅₀ values between cancer and non-cancer cells indicating higher selectivity[15]. Among the saponins tested, pacificusosides D, F, H, K and cucumarioside D exhibited high selectivity and cytotoxicity specifically against the SK-MEL-2 cell line, with IC₅₀ values of 0.7, 0.68, 0.69, 0.75, and 0.67 μ M, respectively. In contrast, pacificusosides E, G, I, and J were less effective, displaying equal or higher IC₅₀ values compared to the JB6 cells, and overall, the RPMI-7951 cell line showed resistance to the bioactivity of these saponins[15].

Another study explored the cytotoxic properties of three asterosaponins (asteriidoside A, diplasteriosides A and B) isolated from *Diplasterias brucei* against human colon cancer

cells (HCT116), breast cancer cells (T-47D), and melanoma cancer cells (RPMI-7951)[44]. Diplasterioside A exhibited cytotoxicity across all three cell types, with IC_{50} values ranging from 60 μ M against RPMI-7951 to 153 μ M against T-47D. Diplasterioside B showed toxicity against RPMI-7951 cells (IC_{50} of 67 μ M), while asteriidoside A displayed no cytotoxicity at concentrations below 160 μ M[44].

Four asterosaponins, acanthaglycoside G, pentareguloside G, acanthaglycoside A and maculatoside, were isolated from an ethanolic extract of *Acanthaster planci* and tested for cytotoxic activity against three human cancer cell lines RPMI-7951, HT-29 (colorectal carcinoma), MDA-MB-231 (breast cancer)[42]. While acanthaglycoside G and pentareguloside G exhibited no significant inhibitory activity, acanthaglycoside A and maculatoside demonstrated effective bioactivity against both HT-29 (IC_{50} : 11 and 7 μ M, respectively) and MDA-MB-231 (IC_{50} : 11 and 8 μ M, respectively), though they were effective against RPMI-7951 cells (IC_{50} : 15 and 14 μ M, respectively). Among the tested asterosaponins, maculatoside showed the highest potential for inhibiting cell colony growth across all three cell lines[42].

The *in vitro* cytotoxicity of four other asterosaponins (lethasterioside A, thornasteroside A, anasteroside A and luidiaquinoside) isolated from *Lethasterias fusca* was assessed against three human cancer cell lines: breast T-47D, colorectal HCT-116, and melanoma RPMI-7951[41]. Thornasteroside A exhibited cytotoxicity across all three cell lines, with IC_{50} values ranging from 50 to 94 μ M. Lethasterioside A was nontoxic to the T-47D cell line but showed strong cytotoxicity against HCT-116 and RPMI-7951 cells, with IC_{50} values of 41 and 34 μ M, respectively. In contrast, anasteroside A and luidiaquinoside did not demonstrate any bioactivity at concentrations below 160 μ M for any of the tested cell lines[41].

Additionally, ten steroidal saponins (certonardosides B2, B3, H2-4, I2, J2, J3, O1, and P1) isolated from *Certonardoa semiregularis* were tested for cytotoxicity against a small panel of human solid tumour cell lines (A549, SK-OV-3, SK-MEL-2, XF498, and HCT15). All but one saponin demonstrated moderate to significant cytotoxicity[49, 51]. Certonardosides P1 and J3 were shown to be the most potent, with ED_{50} values ranging from 0.35 to 2.17 μ g/mL and 0.45 to 2.76 μ g/mL, respectively. Certonardoside H3 showed considerable cytotoxicity, with ED_{50} values between of 0.52 and 7.20 μ g/mL. Notably, the HCT15 cell line demonstrated greater resilience, compared to the other four tumour cell lines, recording the highest effective dosage requirement (8.13, 6.14, 5.04, 7.20, >30, 8.32, 8.20, 2.76, 2.28, and 2.17 μ g/mL, respectively) in all but one case[49, 51].

Four asterosaponins—aphelasteroside A, an asterone analogue of thornasteroside A, thornasteroside A, and versicoside A—isolated from *Asterias microdiscus* were tested for cytotoxic effects against four human cancer cell lines: HT-29, MDA-MB-231, THP-1 (monocytic leukemia), and Raji (Burkitt's lymphoma), as well as a normal mouse epidermal cell line (JB6 Cl41), at concentrations up to 100 μM [40]. Aphelasteroside A and the asterone analogue demonstrated cytotoxicity against THP-1 and Raji cells, with IC_{50} values below 100 μM , while the other three cell lines consistently required concentrations greater than 100 μM to achieve similar effects. THP-1 cells were particularly sensitive, with versicoside A achieving the lowest tested IC_{50} value of 23 μM . Thornasteroside A exhibited the least variation in inhibitory concentration across between the tested cell lines, ranging from 28 to 48 μM . The study highlighted that thornasteroside A and versicoside A to be promising candidates for future investigation into their anticancer mechanisms, particularly for breast cancer[40].

Six new asterosaponins, leptasteriosides A–F, were isolated from the alcoholic extract of the Far Eastern starfish *Leptasterias ochotensis* and cytotoxic activities tested against human breast cancer T-47D and melanoma RPMI-7951 cell lines[48]. Leptasteriosides D, E, and F exhibited slight cytotoxic effects against T-47D and against RPMI-7951 cell lines with IC_{50} values of 68 to 143 μM , and 91 to 151 μM , respectively. In contrast, leptasteriosides A, B, and C displayed significant inhibition of both cell lines, with IC_{50} values of 2, 10, and 23 μM against T-47D, and 27, 17 and 30 μM against RPMI-7951, respectively[48].

Eleven polyhydroxysteroidal glycosides (anthenosides A–K) were isolated from an ethanol extract of *Anthena chinensis* and evaluated for cytotoxicity against human leukemia K-562, hepatoma BEL-7402, and glioblastoma U87 cells[55, 56]. Among the isolated compounds, anthenoside A showed the highest cytotoxicity, with IC_{50} values of 1.62, 3.09, and 2.79 μM across the three cell lines. The greatest bioactivity was observed in a mixture of anthenosides J and K (ratio unknown), which achieved an IC_{50} of 0.60 μM against K-562 cells. The U87 cell line was generally less sensitive, requiring higher IC_{50} value ($> 25 \mu\text{M}$) for some anthenosides compared to the K-562 and BEL-7402 cell lines, where IC_{50} values ranged between 2.8 to 8.3 μM . The other anthenosides did not exhibit significant cytotoxic effects[55, 56].

The cytotoxic activities of three polyhydroxysteroidal saponins (hesperuside A, B, and C) isolated from the ethanol extract of *Craspidaster Hesperus*, were tested against human tumour cells hepatoma BEL-7402, leukemia MOLT-4, and lung cancer A-549 *in vitro*[53].

Hesperuside B demonstrated the highest cytotoxicity across all tested tumour cells, with IC₅₀ values of 2.67, 0.68 and 1.84 µM, respectively. Hesperusides A and C were deemed less potent, with IC₅₀ values ranging from 2.12 to 5.72 µM. The MOLT-4 leukemia cells were the most susceptible to hesperuside exposure, requiring the lowest IC₅₀ concentrations[53].

Eight steroidal saponins isolated from a methanol extract of *Culcita novaeguineae* were tested for cytotoxic activity against five human cancer cell lines: hepatoma Hep-G2, epidermoid carcinoma KB, prostate LNCaP, breast MCF7, and melanoma SK-Mel2[37]. Halitylosides D demonstrated the strongest cytotoxicity, achieving the lowest IC₅₀ values across all five cell lines (75.01, 32.66, 31.80, 33.96, and 32.99 µM, respectively). Halityloside B also showed moderate activity, with IC₅₀ values ranging from 39.68 to 80.22 µM. Halityloside A and culcitoside C5 exhibited weaker bioactivity, with IC₅₀ values between 48.59 to 92.04 µM for the KB, LNCaP, MCF7, and SK-Mel2 cell lines. Neither compound achieved an IC₅₀ below 100 µM for the Hep-G2 cell line. The remaining four saponins did not exhibit significant cytotoxic effects below 100 µM in any of the tested cell lines. Overall, Hep-G2 cells were the most resistant, requiring the highest IC₅₀ doses for all tested saponins[37].

The anti-cancer potential of three asterosaponins (regularoside A, archasterosides A, and B) isolated from *Archaster typicus*, was tested against human HeLa and mouse epidermal JB6 Cl41 cells[57]. Among the tested compounds, archasteroside B exhibited the highest potency, with IC₅₀ values of 14 and 18 µM against HeLa and JB6 Cl41 cells, respectively. Archasteroside A showed moderate activity, with IC₅₀ values of 24 and 37 µM. In contrast, regularoside A displayed only weak bioactivity against HeLa cells, requiring 110 µM to achieve an IC₅₀, and was deemed inactive to JB6 cells at concentrations below 50 µM[57].

In a separate study, a bioassay guided fractionation of a methanolic extract from *Certonardoa semiregularis* led to the isolation of two steroidal saponins, certonardosides P2 and I3. These compounds were tested for cytotoxicity against five human cancer cell lines: A549 (lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (glioblastoma), and HCT15[50]. Certonardoside P2 demonstrated strong cytotoxic activity against the SK-MEL-2 cell line, with an IC₅₀ value of 2.67 µM. The other cell lines were less sensitive, with IC₅₀ values of 19.5, 26.5, and 18.2 µM for SK-OV-3, XF498, and HCT15, respectively. Certonardoside P2 was not active against A549 cells at concentrations below 30 µg/mL. Certonardoside I3 showed no bioactivity against any of the tested cell lines at concentrations under 30 µg/mL[50].

Moreover, fifteen steroidal saponins (certonardosides A-N and culcitoside C6), were isolated from a methanol extract of *Certonardoa semiregularis*, guided active in a brine shrimp assay[36]. These saponins were evaluated for cytotoxicity against a panel of five human solid tumour cell lines, including A549 lung, SK-OV-3 ovary, SK-MEL-3 melanoma, XF498 Glioblastoma and HCT15 colorectal. Certonardosides L and N demonstrated considerable cytotoxicity against all five cell lines, with effective dosages (ED_{50}) between 3.9 to 8.4 $\mu\text{g/mL}$. Certonardoside C showed the highest bioactivity, with an ED_{50} value of 3.8 $\mu\text{g/mL}$ against SK-MEL-2, although it displayed only moderate cytotoxicity against the other four cell lines, with ED_{50} values ranging from 15.8 to 25.9 $\mu\text{g/mL}$ ED_{50} . Furthermore, certonardosides A, H, K, M, and culcitoside C6 demonstrated moderate to strong bioactivity against the SK-MEL-2 cell line, with ED_{50} values between 6.7 to 16.3 $\mu\text{g/mL}$. Their effects on the other four cell lines, however, were limited to non-existent. Certonardosides B, D-G, I, and J showed no significant bioactivity for any cell line tested below 30 $\mu\text{g/mL}$. Overall, SK-MEL-2 appeared distinctly more susceptible to saponin exposure compared to the other tested cell lines[36].

Eight steroidal saponins (certonardosides A, C, D, F, H-J, and halityloside D), isolated from the brine shrimp active fraction of a methanolic extract of *Certonardoa semiregularis*, were evaluated for their potential antiviral activity and cytotoxicity using three host cell lines: African green monkey (*Chlorocebus sabaeus*) Vero cells, human leukemia MT-4, and human cervical HeLa cell lines[38]. Although the saponins exhibited only weak antiviral activity, the cytotoxicity tests revealed notable anti-cancer potential. Certonardosides A and C were particularly cytotoxic against the MT-4 cell line, with IC_{50} values of 6.8 and 1.4 μM , respectively. However, these compounds had limited effects on the other cell lines, with only Certonardoside C showing weak cytotoxicity against the Vero cells, achieving an IC_{50} of 97.5 μM . The remaining six saponins demonstrated moderate cytotoxicity against the tested cell lines, with IC_{50} values ranging from 39.7 to 79.9 μM . Each of these six saponins was selective for one or two cell lines, while exhibiting no effect on the third[38].

Six steroidal saponins isolated from a methanolic extract of *pentaceraster gracilis*, were evaluated for cytotoxicity against five human cancer cell lines including Hep-G2, KB, LNCaP, MCF7, and SK-MEL-2[43]. Maculatoside showed significant cytotoxic effect against the Hep-G2 and SK-Mel2 cell lines, with IC_{50} values of 16.75 and 19.44 μM , respectively. Moderate effect was observed on KB, LNCaP and MCF7 with 36.53, 39.75 and 47.34 μM . The other five saponins showed no cytotoxicity at concentrations below 100 μM against any of the tested cell lines[43].

Three steroidal saponins (polyacanthoside A, marthasteroside B, and psilasteroside) isolated from the methanolic extract of *Astropecten polyacanthus* were tested for cytotoxic activity against five human cancer cell lines: HepG2, KB, LNCaP, MCF7, and SK-Mel2[52]. Marthasteroside B and psilasteroside exhibited moderate cytotoxic effects across all five cell lines, with IC₅₀ values ranging from 28.71 to 49.03 μ M. However, polyacanthoside A showed no bioactivity at concentrations below 100 μ M[52].

The cytotoxicity of six triterpenoid saponins (pacificusosides A-C and cucumariosides C1, C2, and A10) isolated from an alcoholic extract of *Solaster pacificus* was tested against human embryonic kidney HEK 293, colorectal carcinoma HT-29, melanoma RPMI-7951, and breast MDA-MB-231 cell lines[28]. All three cucumarioside saponins exhibited strong cytotoxicity against all tested cell lines, with IC₅₀ values ranging from 3.6 to 7.9 μ M. Pacificusoside C was similarly cytotoxic to three of the four cell lines, with IC₅₀ values between 6.2 and 6.7 μ M, though it was less effective against RPMI-7951, with an IC₅₀ value of 23.7 μ M. Pacificusoside B demonstrated moderate cytotoxicity to all cell lines, with IC₅₀ values between 20.4 and 28.6 μ M, whereas pacificusoside A showed no cytotoxicity below 40 μ M[28].

Seven steroidal saponins (cariniferoside A and F; halityloside A, B, and D; halityloside A 6-O-sulfate, and 4-O-methyl halityloside A 6-O-sulfate), isolated from *Asteropsis carinifera*, were tested for *in vitro* cytotoxicity against human cancer cell lines HCT-116, T-47D, and RPMI-7951[39]. Of these, cariniferoside A demonstrated moderate cytotoxicity against all tested cell lines, with IC₅₀ values of 32, 37, and 66 μ M, respectively. Halityloside A and B showed weak bioactivity, with halityloside A active only against HCT-116 (IC₅₀ of 150 μ M) and halityloside B active only against T-47D and RPMI-7951 cells, with IC₅₀ values of 154 and 128 μ M, respectively[39].

The cytotoxic activities of three steroidal saponins (anthenosides A, A1, and A2) isolated from *Anthenea aspera* were evaluated against human melanoma RPMI-7951 and breast cancer T-47D cells[47]. All three anthenoside saponins demonstrated weak inhibition of T-47D cell viability, with IC₅₀ values of 133, 139, and 158 μ M, respectively. However, none of the saponins displayed cytotoxic effects against RPMI-7951 cells at concentrations below 150 μ M[47].

Four steroidal saponins (echinasteroside B, granulatoside A, linkoside K and forbeside L) isolated from *Mithrodia clavigera* were tested for cytotoxic activity against human skin melanoma cells SK-MEL-28, SK-MEL-5, and RPMI-7951[46]. Forbeside L demonstrated the

most significant cytotoxicity among the tested compounds, with moderate effects at IC₅₀ values of 75, 79, and 84 µM against the three cell lines, respectively. Granuloside A and linckoside K exhibited weak cytotoxic activity, with IC₅₀ values ranging from 83 to 164 µM. In contrast, echinasteroside B showed no cytotoxic activity at concentrations below 200 µM[46].

Three steroidal saponins (plancisides A–C) isolated from the ethanolic extract of *Acanthaster planci* were tested for *in vitro* cytotoxicity against human colon cancer HCT-116, breast cancer T-47D and melanoma RPMI-7951 cells[45]. Planciside A exhibited moderate cytotoxicity against HCT-116 and RPMI-7951 cells, with IC₅₀ values of 36 and 58 µM, respectively, but showed no toxicity below 120 µM against the T-47D cell line. Planciside B and C showed no cytotoxic activity at any concentration tested with these three cell lines[45].