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Original Article

The Antiproliferative Properties of *Uncaria tomentosa* Willd. DC. Extracts Against $Caco_2$ and HeLa Cancer Cell Lines

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ABSTRACT
Introduction: *Uncaria tomentosa* is used in Central and South American folk medicine to treat a variety of diseases. It is particularly well known for treating cancer and studies have confirmed its activity against some carcinoma cell lines. However, to date, *U. tomentosa* extracts have only been screened against a limited panel of cancer cells. **Methods:** Solvent extracts were prepared from *U. tomentosa* inner bark and their antiproliferative activities against $Caco_2$ and HeLa cancer cells were determined by an MTS based cell proliferation assay. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Methanolic and aqueous *U. tomentosa* extracts were strong inhibitors of $Caco_2$ and HeLa cell proliferation, with IC_{50} values generally below 1500 µg/mL. The methanolic extract was particularly effective, with IC_{50} values of 881 and 763 µg/mL against Caco2 and HeLa cells respectively. In contrast, the mid to lower polarity solvent extractions (ethyl acetate, chloroform and hexane) were less potent inhibitors of cell proliferation. Indeed, exposure of the $Caco_2$ cells to the chloroform and hexane extracts produced proliferation levels equivalent to those of the untreated control. Cell imaging studies detected morphological features consistent with apoptosis in $Caco_2$ cells exposed to the methanolic and aqueous *U. tomentosa* extracts, indicating that these extracts are functioning by cytotoxic mechanisms. All *U. tomentosa* extracts were nontoxic in the *Artemia franciscana* bioassay, with LC_{50} values substantially >1000 µg/mL. **Conclusions:** The antiproliferative activity of the *U. tomentosa* inner bark extracts against HeLa and $Caco_2$ cancer cell lines indicates their potential in the treatment and prevention of some cancers.

Key words: Cat's claw, Uña de gato, Rubiaceae, Anticancer activity, Antiproliferative activity, $Caco_2$, HeLa, Apoptosis.

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INTRODUCTION

Uncaria tomentosa Willd. DC. (Family Rubiaceae) is a woody vine (Figure 1a) that is restricted to tropical regions in Central and South America and is indigenous to the Amazon rainforest. It is commonly known as cat's claw (or uña de gato in Spanish) due to claw shaped thorns (Figure 1b) that allow it to climb tree trunks to heights of up to 30 metres. *U. tomentosa* has been used for hundreds (perhaps thousands) of years by various indigenous cultures and is particularly well known for its use by the Ashaninka Indians of Peru.[1] Although the Ashaninka people prepared infusions of the inner bark (Figure 1c) and used it to treat a variety of diseases, it is perhaps best known as an effective therapy for both rheumatoid arthritis and osteoarthritis. However, it is also beneficial in the treatment of asthma, allergies, bursitis, cancer, candidiasis, diabetes, gastric ulcers, gout, Crohn's disease, fibromyalgia, sexually transmitted diseases (STI's) and viral infections.[2]

Numerous studies have investigated the anti-inflammatory properties of *U. tomentosa* extracts. Exposure to *U. tomentosa* extracts inhibits cellular TNF-α production and reduces free radicals.[3] as well as activating the nuclear transcription factor NF-KB.[4] Furthermore, *U. tomentosa* extracts act as an immunostimulant by inducing the release of a lymphocyte proliferation regulating factor.[5] and prolonging lymphocyte survival.[6] Several anti-inflammatory phytochemical constituents have been identified in *U. tomentosa* extracts. Several pentacyclic oxindole alkaloids (Figure 1d) have been implicated in the anti-inflammatory activity of these extracts.[3,4,7]

U. tomentosa is also used in Peruvian folk medicine as an anticancer therapy.[7] and has recently become a popular herbal supplement in Europe and the United States of America for the prevention and treatment of cancer. Whilst much of our understanding of its anticancer properties is anecdotal, multiple studies have also reported antiproliferative activity of *U. tomentosa* extracts *in vitro*. An aqueous *U. tomentosa* extract has been reported to inhibit the proliferation of HL-60 and K562 human leukemia and Raji EBV-transformed B lymphoma cell lines.[8] Subsequent studies by the same group determined that the extract inhibited cell proliferation without inducing apoptosis.[9] Similarly, another study reported that an aqueous *U. tomentosa* extract also inhibited proliferation in normal mouse T and B lymphocytes.[6] That study reported that exposure to the extract did not induce apoptosis and instead deduced that cytostatic mechanisms were responsible for the inhibition of proliferation. In contrast, other studies have reported that *U. tomentosa* extracts induce apoptosis in lymphoblastic and breast cancer cell lines.[10,11] Similarly, apoptotic activity was reported for several *U. tomentosa* hydro-alcohol extracts against human HT-29 and SW707 (colon adenocarcinoma), KB (cervical carcinoma), MCF7 (breast carcinoma), A549 (non-small cell lung carcinoma), HL-60 (promyelocytic leukemia) cells, as well as mouse LL/2 (lung carcinoma) and B16 (melanoma) cells.[12]

Our study aimed to extend these earlier studies by testing *U. tomentosa* extracts against a different panel of human carcinoma cell lines. Caco2 colorectal carcinoma cells were selected as a comparison to the HT-29 and SW707 colorectal cells examined in the Pilarski *et al*. (2010) study.[12] Caco2 cells are extremely well characterised and have been used in numerous anticancer studies, allowing for direct comparison of efficacies between these studies. HeLa (human cervical carcinoma cell line), which are perhaps the most widely used cells for scientific research and are also extremely well characterised, were included as a comparison to the KB cervical carcinoma cells screened in the Pilarski *et al*. (2010) study.[12]

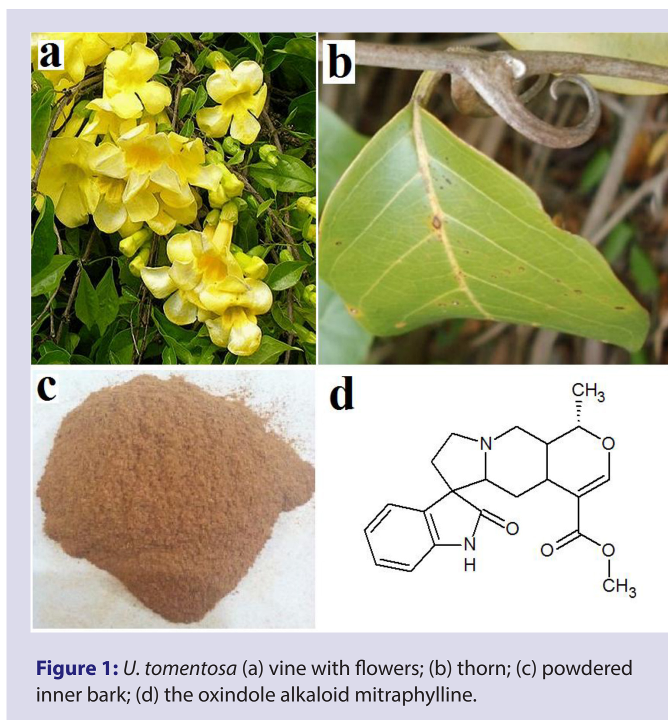

Figure 1: *U. tomentosa* (a) vine with flowers; (b) thorn; (c) powdered inner bark; (d) the oxindole alkaloid mitraphylline.

MATERIALS AND METHODS

Plant source and extraction

The *Uncaria tomentosa* Willd. DC. dried and powdered material used in this study were obtained from Noodles Emporium online herbalist, Australia. Voucher specimens are stored in the School of Natural Sciences, Griffith University, Australia. Individual 1 g quantities of the powdered *U. tomentosa* were weighed into individual tubes and 50 mL of methanol, deionised water, ethyl acetate, chloroform or hexane were added. All solvents were obtained from Ajax Chemicals, Australia and were AR grade. The ground plant materials were individually extracted in each solvent for 24 h at 4ºC with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54) under vacuum and the solvent extracts were dried by rotary evaporation. The aqueous extract was dried by freeze drying. The resultant dry extracts were weighed and redissolved in 10 mL deionised water (containing 1 % DMSO).

Qualitative phytochemical studies

Phytochemical analysis of the extracts for the presence of saponins, phenolic compounds, flavonoids, phytosterols, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids were conducted by previously described assays.[13-15]

Screen for anticancer bioactivity

Cancer cell lines

The Caco2 and HeLa carcinoma cell lines used in this study were obtained from American Type Culture Collection (Rockville, USA). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies), supplemented with 20 mM HEPES, 10 mM sodium bicarbonate, 50 μg/mL streptomycin, 50 IU/mL penicillin, 2 mM glutamine and 10 % foetal calf serum (Life Technologies). The cells were maintained as monolayers in 75 mL flasks at 37ºC, 5 % CO_2 in a humidified atmosphere until approximately 80 % confluent.

Evaluation of cancer cell antiproliferative activity

The antiproliferative activity of the extracts was assessed as previously described.[16,17] Briefly, 1 mL of trypsin (Sigma) was added to the culture flasks and incubated at 37ºC, 5 % CO_2 for 15 min to dislodge the cancer cells. The cell suspensions were then transferred to a 10-mL centrifuge tube and sedimented by centrifugation. The supernatant was discarded, and the cells were resuspended in 9 mL of fresh media. Aliquots of the resuspended cells (70 μL, containing approximately 5000 cells) were added to the wells of a 96 well plate. A volume of 30 μL of the test extracts or cell media (for the negative control) was added to individual wells and the plates were incubated at 37ºC, 5 % CO_2 for 12 h in a humidified atmosphere. A volume of 20 µL of Cell Titre 96 Aqueous One solution (Promega) was subsequently added to each well and the plates were incubated for a further 3 h. Absorbance were recorded at 490 nm using a Molecular Devices, Spectra Max M3 plate reader. All tests were performed in at least triplicate and triplicate controls were included on each plate. The antiproliferative activity of each test was calculated as a percentage of the negative control using the following formula:

$$\text{Proliferation (\% untreated control)} = (A_{ct}/A_{cc}) \times 100$$

A_{ct} is the corrected absorbance for the test extract (calculated by subtracting the absorbance of the test extract in media without cells from the extract cell test combination) and A_{cc} is the corrected untreated control (calculated by subtracting the absorbance of the untreated control in media without cells from the untreated cell media combination).

Cell imaging studies

Cell morphological changes were measured using bright field microscopic imaging without staining. Caco2 cells were seeded into individual wells of a 96 well plate at a density of 5 x 10^3 cells/100 µL and the cells could adhere overnight. *U. tomentosa* extracts were added to the respective wells at concentrations previously determined to be the highest concentration which induced <50 % change in proliferation compared to the untreated control. Cells were incubated for 24 h at 37 °C in a humidified 5 % CO_2, 95 % air atmosphere. Each assay was performed in triplicate, and the cell morphology was qualitatively appraised after 24 h treatment by using an inverted phase microscope with camera (Olympus IX70) with an optical zoom of 20X. The images were captured via computer and analysed to scale.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 1.6 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.[18-20] Briefly, 400 µL of seawater containing approximately 43 (mean 43.2, n = 155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25 ± 1ºC under artificial light (1000 Lux). A negative control (400 µL seawater) was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was observed within 10 secs. After 24h all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water (containing 1% DMSO) and qualitative phytochemical screenings of the *U. tomentosa* extracts.

Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
M	364	36.4	+++	++	++	-	+	++	-	+	++	+++	++	-	-
W	560	56	+++	++	+++	-	+	+	+	-	-	+++	++	-	-
E	186	18.6	++	+	-	-	-	-	-	-	-	++	-	-	-
C	443	44.3	+	+	-	-	-	+	-	-	-	+	-	-	-
H	393	39.3	-	-	-	-	-	-	-	-	-	+	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay; M = methanolic *U. tomentosa* extract; W = aqueous *U. tomentosa* extract; E = ethyl acetate *U. tomentosa* extract; C = chloroform *U. tomentosa* extract; H = hexane *U. tomentosa* extract.

limits for each treatment was calculated using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments. One-way ANOVA was used to calculate statistical significance between control and treated groups with a *P* value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of the dried *U. tomentosa* powder with solvents of varying polarity yielded dried plant extracts ranging from 186 mg (ethyl acetate extract) to 560 mg (aqueous extract) (Table 1). The aqueous extract produced the highest yield of dried extracted material (560 mg). Methanol, chloroform and hexane each extracted similar yields of plant material (~400 mg), whilst ethyl acetate extracted a lower mass (186 mg). The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO) resulting in the extract concentrations shown in Table 1.

Qualitative phytochemical studies (Table 1) showed that methanol and water extracted the widest range of phytochemicals. Both showed moderate to high levels of phenolics (both water soluble and insoluble phenolics), flavonoids and tannins, as well as low to moderate levels of saponins, triterpenoids and alkaloids. The ethyl acetate extract contained only moderate levels of phenolics and flavonoids. The chloroform extract had a similar phytochemical profile, although the phenolics and flavonoids were present in lower relative abundances. Phytosterols, cardiac glycosides and anthraquinones were generally absent in all extracts.

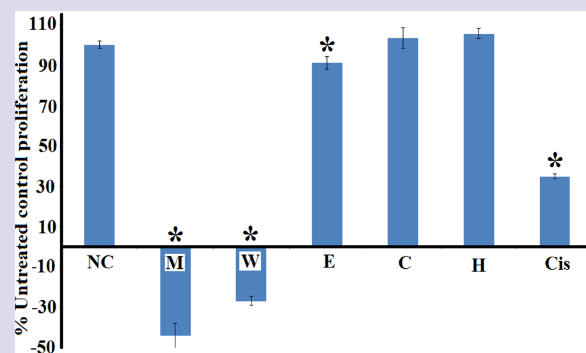

Figure 2: Anti-proliferative activity of the *U. tomentosa* extracts and untreated controls against Caco2 cancer cell lines measured as percentages of the untreated control cells. NC = untreated control; M = methanolic *U. tomentosa* extract; W = aqueous *U. tomentosa* extract; E = ethyl acetate *U. tomentosa* extract; C = chloroform *U. tomentosa* extract; H = hexane *U. tomentosa* extract; Cis = cisplatin control (50 mg/mL). Results are expressed as mean percentages ± SEM of at least triplicate determinations.* indicates results that are significantly different to the untreated control (p<0.01).”

Antiproliferative activity

Aliquots of each extract were tested for the ability to block the proliferation of Caco2 (Figure 2) and HeLa (Figure 3) cell lines. Three of the *U. tomentosa* extracts displayed significant (p<0.01) antiproliferative effects against Caco2 cells (Figure 2). The methanolic and aqueous extracts were particularly potent. Indeed, negative Caco2 proliferation was noted for both extracts, suggesting that the antiproliferative activity may be due to apoptosis rather than cytostatic mechanisms. The ethyl acetate extract also inhibited Caco2 cell proliferation, although only by 8.9 % inhibition compared to the negative control cell proliferation. Neither the chloro-

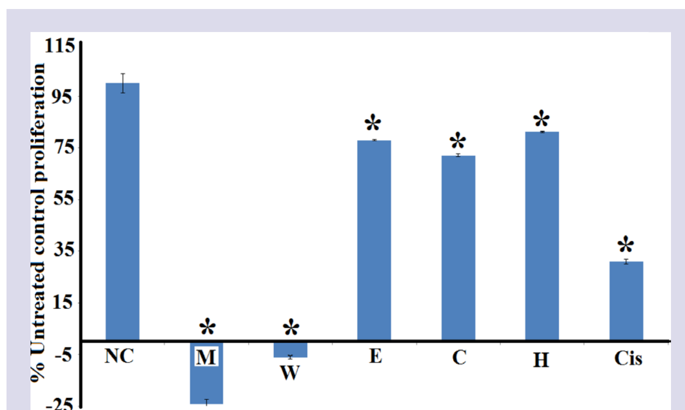

Figure 3: Anti-proliferative activity of the *U. tomentosa* extracts and untreated controls against HeLa cancer cell lines measured as percentages of the untreated control cells. NC = untreated control; M = methanolic *U. tomentosa* extract; W = aqueous *U. tomentosa* extract; E = ethyl acetate *U. tomentosa* extract; C = chloroform *U. tomentosa* extract; H = hexane *U. tomentosa* extract; Cis = cisplatin control (50 mg/mL). Results are expressed as mean percentages ± SEM of at least triplicate determinations. * indicates results that are significantly different to the untreated control (p<0.01).

form nor the hexane extract significantly affected Caco2 cell proliferation. Control cells.

The extracts were similarly effective at inhibiting HeLa cancer cell proliferation, although HeLa cell proliferation was inhibited by a broader range of extracts. Indeed, all *U. tomentosa* extracts displayed significant (p<0.01) antiproliferative effects against the HeLa cell line. Interestingly, negative proliferation was noted for the methanolic and aqueous *U. tomentosa* extracts (Figure 3), indicating that the antiproliferative activity was not only cytostatic in nature, but may be cytotoxic. The ethyl acetate, chloroform and hexane extract also inhibited HeLa cell proliferation, albeit by a lesser amount (by 22, 28 and 19 % compared to the negative control cell proliferation respectively).

The antiproliferative efficacy was further quantified by determining the IC_{50} values for each extract which inhibited cell proliferation against each cell line (Table 2). The methanolic extract was particularly effective at inhibiting cancer cell proliferation at low to moderate concentrations, with IC_{50} values against Caco2 and HeLa lines of 881 and 763 µg/mL respectively. The aqueous extract was also a strong inhibitor of Caco2 and HeLa proliferation, with IC_{50} values of 1645 and 1444 µg/mL respectively. Whilst several other extracts had antiproliferative activity, we were unable to determine IC_{50} values as the inhibition of proliferation did not exceed 50 % at any concentration tested.

Cell imaging studies

The antiproliferative studies presented in this manuscript demonstrate that the methanolic and aqueous *U. tomentosa* extracts have profound effects on cellular proliferation. Indeed, Figures 2 and 3 that not only was cell growth blocked, but negative cellular proliferation was recorded. This decrease in relative cell proliferation compared to the untreated control cells indicated that these extracts may not just inhibit growth, but may act via cytotoxic/apoptotic mechanisms. However, whilst this is indicated by these results, further studies are required to test this hypothesis and cell imaging studies were undertaken to confirm this finding. The Caco2 cell line was chosen for cell imaging studies as it is a commonly used cell line in similar studies allowing for comparative studies, has well defined cell morphology, has a reasonable doubling time compared with the other cell lines and was responsive to the *U. tomentosa* extracts.

The key organelles for comparison between treated and untreated cells in these studies were the nucleus, cytoplasm, vacuoles and cell membrane, all of which were clearly visible at 20X optical magnification of the cell in the untreated control cell image (Figure 4a). The outline of the cell is clear, distinct and compact without abnormalities in size and density. Stress granules were not visible at this magnification. These are normally indicators of cellular stress, if present. Typical apoptotic phenomena were noted in the morphology of all cells tested against the methanolic (Figure 4b) and aqueous (Figure 4c) extracts when tested at sub-lethal concentrations. These images are consistent with the findings of the cell proliferative assays: both the methanolic and aqueous *U. tomentosa* extracts inhibited Caco2 proliferation to below the level at the onset of the experiment. This correlates with the morphological phenomena noted. Apoptosis is generally characterised by the presence of an intact membrane, marginalisation of cytoplasm and chromatin condensation.[21] These characteristics were clearly visible in the Caco2 cells treated with methanolic and aqueous *U. tomentosa* extracts (Figure 4b and Figure 4c respectively). Marginalisation of the cytoplasm of cells undergoing apoptosis also produces an enormously large centrally placed vacuole which is clearly visible in both Figures. The presence of stress granules in the visible fields (Figures 4a and 4b) indicates that the cell is under stress. 'Blebbing' is the appearance of 'spherical bubbles' on the surface of the membrane and is a typical characteristic generally apparent in apoptotic cells. 'Blebbing' is also seen in cells tested against the methanolic and aqueous *U. tomentosa* extracts (Figures 4a and 4b respectively). These cells also displayed a loss in membrane integrity with no clear outline of the cell visible, extensive chromatin condensation and the presence of numerous vacuoles. Also, visible for both treatments were the 'ghosts' of cell where cellular contents are no longer present within cell. The

Table 2: The IC_{50} of the *U. tomentosa* extracts against the Caco2 and HeLa cancer cell lines and detection of toxicity and quantification of LC_{50} values (µg/mL).

Extract	Antiproliferative activity (IC_{50}: µg/mL)		24 h Artemia nauplii toxicity
	Caco2	HeLa	LC_{50} (µg/mL)
M	881	763	1150
W	1645	1444	1392
E	CND	CND	-
C	DNI	CND	-
H	DNI	CND	-

Numbers indicate the mean IC_{50} or LC_{50} values of triplicate determinations. CND indicates that IC_{50} values could not be obtained as the inhibition of proliferation did not exceed 50 % at any dose tested. DNI = did not inhibit. - indicates that the extract was not toxic at any concentration tested.

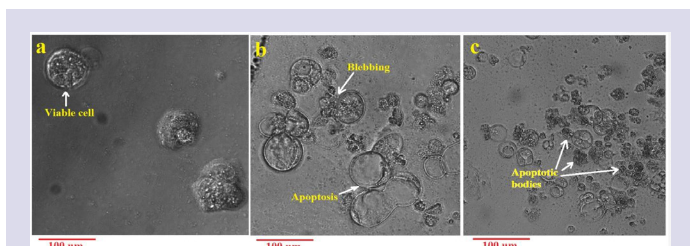

Figure 4: Effects of *U. tomentosa* extracts on the morphological characteristics of Caco2 cells: (a) viable Caco2 cells without exposure to extracts; (b) Caco2 cells exposed to the methanolic *U. tomentosa* extract; (c) Caco2 cells exposed to the aqueous *U. tomentosa* extract.

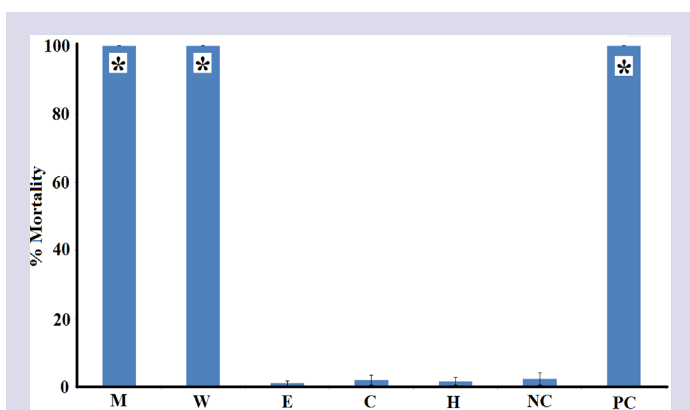

Figure 5: The lethality of the *U. tomentosa* extracts (2000 µg/mL) and the potassium dichromate control (1000 µg/mL) towards *Artemia* nauplii following 24 h exposure. M = methanolic *U. tomentosa* extract; W = aqueous *U. tomentosa* extract; E = ethyl acetate *U. tomentosa* extract; C = chloroform *U. tomentosa* extract; H = hexane *U. tomentosa* extract; NC = negative control; PC = positive control (1000 µg/ml potassium dichromate). All tests were performed in at least triplicate and the results are expressed as mean ± SEM. * indicates results that are significantly different to the negative control (p<0.01).

morphological changes observed with both treatments are indicative of the induction of apoptosis.

Quantification of toxicity

All extracts were initially screened at 2000 µg/mL in the assay (Figure 5). For comparison, the reference toxin potassium dichromate (1000 µg/mL) was also tested in the bioassay. Figure 5 shows the % mortality induced by each extract and by the control toxin following 24 h exposure. The potassium dichromate reference toxin was rapid in its onset of mortality, inducing mortality within the first 3 h of exposure and 100 % mortality was evident following 4-5 h (unpublished results). Similarly, the methanolic and aqueous *U. tomentosa* extracts displayed 100 % mortality rates at 24h and were thus deemed to be toxic. All other extracts induced only low levels of mortality which were not significantly different to the mortality determined for the negative control. These extracts were therefore deemed to be nontoxic.

To further quantify the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay. Table 2 shows the LC_{50} values of the extracts towards *A. franciscana*. No LC_{50} values are reported for the ethyl acetate, chloroform or hexane *U. tomentosa* extracts as less than 50 % mortality was seen for all concentrations tested. Extracts with LC_{50} values >1000 µg/mL towards *Artemia* nauplii have been defined as being nontoxic.[22] As LC_{50} values >1000 µg/mL were determined for the methanolic and aqueous extracts, they were also deemed to be nontoxic.

DISCUSSION

The antiproliferative efficacy of *U. tomentosa* inner bark extracts was examined against two human cancer cell lines *in vitro*; Caco2 (colorectal) and HeLa (cervical). The methanolic and aqueous extract were determined to have the greatest antiproliferative actitity, although all extracts displayed inhibitory activity. The methanolic extract was particularly effective, with IC_{50} values of 881 and 763 µg/mL against Caco2 and HeLa cells respectively. This antiproliferative activity is consistent with earlier studies screening against different cancer cell lines. A study by Pilarski *et al*. reported potent antiproliferative activity for *U. tomentosa* hydro-alcohol extractions against HT-29 (IC_{50} values generally 500-800 µg/mL) and SW707 colon adenocarcinoma cells (IC_{50} values generally 50-400 µg/mL).[12] The authors of that study determined that the use of higher alcohol content solvents greatly increased the potency of the extract. This is consistent with the findings of our study in which the methanolic extract was a substantially more potent inhibitor of cell proliferation than the aqueous extract.

The antiproliferative efficacies for the higher alcohol content extracts against colorectal cancer cells (HT-29 and SW707) reported in the Pilarski *et alet al* study was substantially higher than those determined in our study against the Caco2 cells (881 µg/mL, compared to 50 and 500 µg/mL for the SW707 and HT-29 cells respectively).[12] These differences may be due to the different cell lines tested between the two studies. The SW707 cells were particularly susceptible to the *U. tomentoa* extracts, with IC_{50} values approximately 10 % of those determined for the HT-29 cells. It is also possible that the different extraction protocols used in the two studies may also contribute to the different efficacies. In our study, the plant material was extracted over night with room temperature solvent. In comparison, the Pilarski *et al* study extracted for 8 h in boiling solvent.[12]

Similar trends were evident when the *U. tomentoa* extracts were tested against the different cervical cancer cell lines used in the two studies. An IC_{50} value of 763 µg/mL was determined for the methanolic extract against the HeLa cells in our study, compared to approximately 35 µg/mL against the KB cells tested in the Pilarski *et al* study.[12] Interestingly, KB cells are indistinguishable from HeLa cells by STR PCR DNA profiling.[23] Therefore, the KB cell line is derived from HeLa cells. Thus, it is likely that the different IC_{50} values between the two studies is due to the different extraction protocols used in these studies rather than being due to the choice of carcinoma cells studied. Several other studies have also reported antiproliferative activity for *U. tomentosa* extracts against other cell lines including HL-60 and K562 human leukemia and Raji EBV-transformed B lymphoma cell lines.[8,9] and normal mouse T and B lymphocytes.[6] Unfortunately, these studies screened the extracts at fixed concentrations and thus did not report an IC_{50}, making a comparison of potency between these studies impossible.

An examination of the phytochemistry of the *U. tomentosa* extracts was beyond the scope of our study. However, previous studies have identified several compounds which inhibit cell cycle progression and/or induce apoptosis. Several pentacyclic oxindole alkaloids have been highlighted for their antiproliferative activity. Of these, mitraphylline has been perhaps the best studied. This compound inhibited proliferation in GAMG glioma cells and SKN-BE (2) neuroblastoma cells.[24] human lymphoblastic leukemia T cells.[10] MHH-ES-1 Ewings sarcoma and MT-3 breast cancer cells.[7] HT-29 and SW707 colon carcinoma cells, KB cervical cancer cells, MCF7 breast cancer cell, A549 lung carcinoma cells and

HL-60 promyelocytic leukemia cells.[12] Furthermore, mitraphylline was a potent inhibitor of proliferation, with an IC_{50} as low as 12 μg/mL.[24] Several other similarly potent antiproliferative oxindole alkaloids including pteropodine, isopteropodine, speciophylline, isomitraphylline and uncarine F have also been identified in *U. tomentosa* extracts.[7,10,12]

Our understanding of the antiproliferative mechanism(s) of the *U. tomentosa* extracts and the isolated compounds is currently limited. However, it appears that multiple mechanisms may be involved and that these mechanisms may be dose dependent. Aqueous *U. tomentosa* extracts inhibit the proliferation of HL-60 and K562 human leukemia, as well as Raji EBV-transformed B lymphoma cell lines via cytostatic mechanisms, without inducing apoptosis.[9] Another study also reported that a similar aqueous *U. tomentosa* extract also inhibited proliferation in normal mouse T and B lymphocytes by blocking cell cycle progression.[6] That study reported that apoptosis was not induced by the extract. Interestingly, both Sheng *et al* (2005).[9] and Akesson *et al* (2003).[6] studies reported that the cytostatic activity was due to quinic acid and quinic acid glycosides rather than oxindole alkaloids. In contrast, other studies have reported that *U. tomentosa* extracts induce apoptosis in lymphoblastic and breast cancer cell lines.[10,11] human HT-29 and SW707 (colon adenocarcinoma), KB (cervical carcinoma), MCF7 (breast carcinoma), A549 (non-small cell lung carcinoma) and HL-60 (promyelocytic leukemia) cells.[12] Those studies reported that oxindole alkaloids were responsible for the induction of apoptosis. Thus, two distinct mechanisms may be responsible for the antiproliferative activity of the *U. tomentosa* extracts, with quinic acid glycosides having cytostatic effects, whilst the oxindole alkaloids induce apoptosis.

The findings reported here also demonstrate that all *U. tomentosa* extracts were nontoxic towards *A. franciscana* nauplii. Extracts with LC_{50} values >1000 μg/mL towards *Artemia* nauplii have been defined as being nontoxic.[22] However, further studies using normal human cell lines are required to verify the safety of these extracts for therapeutic use. Furthermore, bioactivity driven separation studies are required to isolate the active components and determine their mechanism of action.

CONCLUSION

The potent antiproliferative activity and lack of toxicity of the methanolic and aqueous *U. tomentosa* extracts indicates their potential in the treatment and prevention of some cancers. Purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents.

ACKNOWLEDGEMENTS

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ABBREVAITIONS USED

DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50% mortality; **IC_{50}:** The concentration required to achieve 50% effect.

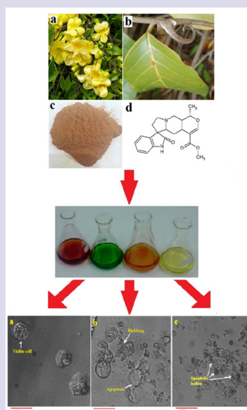
CONFLICT OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Reinhard KH. *Uncaria tomentosa* (Willd.) DC. Cat's claw, una de gato, or savéntaro. The Journal of Alternative and Complementary Medicine. 1998;5(2):143-51.
2. Ferlow K. Cat's claw. Healing vine of Peru. Herbal Medicine 2016:231. Available at: http://www.positivehealth.com/article/herbal-medicine/cat-s-claw-healing-vine-of-peru . 2017.
3. Sandoval M, Charbonnet RM, Okuhama NN, *et al*. Cat's claw inhibits TNF [alpha] production and scavenges free radicals: role in cytoprotection. Free Radical Biology and Medicine. 2000;29(1):71-8.
4. Sandoval-Chacon M, Thompson JH, Zhang XJ, *et al*. Antiinflammatory actions of cat's claw: the role of NF-kB. Alimentary Pharmacology and Therapeutics. 1998; 12(12):1279-90.
5. Wurm M, Kacani L, Laus G, *et al*. Pentacyclic oxindole alkaloids from *Uncaria tomentosa* induce human endothelial cells to release a lymphocyte-proliferation regulating factor. Planta Medica. 1998;64(8):701-4.
6. Akesson C, Pero RW, Ivars F. CMed 100, a hot water extract of *Uncaria tomentosa*, prolongs lymphocyte survival *in vivo in vivo*. Phytomedicine. 2003;10(1):23-33.
7. Giménez DG, Prado EG, Rodriguez TS, *et al*. Cytotoxic effect of the pentacyclic oxindole alkaloid mitraphylline isolated from *Uncaria tomentosa* bark on human Ewing's sarcoma and breast cancer cell lines. Planta Medica. 2009;76(2):133-6.
8. Sheng Y, Pero RW, Amiri A, *et al*. Induction of apoptosis and inhibition of proliferation in human tumor cells treated with extracts of *Uncaria tomentosa*. Anticancer Research. 1998;18(5A):3363-8.
9. Sheng Y, Åkesson C, Holmgren K, *et al*. An active ingredient of Cat's Claw water extracts: Identification and efficacy of quinic acid. Journal of Ethnopharmacology. 2005;96(3):577-84.
10. Bacher N, Tiefenthaler M, Sturm S, *et al*. Oxindole alkaloids from *Uncaria tomentosa* induce apoptosis in proliferating, G0/G1-arrested and bcl-2-expressing acute lymphoblastic leukaemia cells. British Journal of Haematology. 2006;132(5):615-22.
11. Riva L, Coradini D, Di Fronzo G, *et al*. The antiproliferative effects of *Uncaria tomentosa* extracts and fractions on the growth of breast cancer cell line. Anticancer Research. 2001;21(4A):2457-61.
12. Pilarski R, Filip B, Wietrzyk J, *et al*. Anticancer activity of the *Uncaria tomentosa* (Willd.) DC. preparations with different oxindole alkaloid composition. Phytomedicine. 2010;17(14):1133-9.
13. Sirdaarta J, Matthews B, White A, *et al*. GC-MS and LC-MS analysis of Kakadu plum fruit extracts displaying inhibitory activity against microbial triggers of multiple sclerosis. Pharmacognosy Communications. 2015;5(2):100-15. DOI: 10.5530/pc.2015.2.2.
14. Kalt FR, Cock IE. GC-MS analysis of bioactive *Petalostigma* extracts: Toxicity, antibacterial and antiviral activities. Pharmacognosy Magazine. 2014;10(37 I): S37-S48. DOI: 10.4103/0973-1296.127338.
15. Vesoul J, Cock IE. An examination of the medicinal potential of *Pittosporum phylloraeoides*: Toxicity, antibacterial and antifungal activities. Pharmacognosy Communications. 2011;1(2):8-17. DOI: 10.5530/pc.2011.2.1.
16. Arkhipov A, Sirdaarta J, Rayan P, *et al*. An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. Pharmacognosy Communications. 2014;4(3):62-76. DOI: 10.5530/pc.2014.3.7.
17. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacognosy Communications. 2014;4(4):71-82. DOI: 10.5530/pc.2014.4.8.
18. Sirdaarta J, Matthews B, Cock IE. Inhibitory activity of Kakadu plum fruit extracts against microbial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. Journal of Functional Foods. 2015;17:610-20. http://dx.doi.org/10.1016/j.jff.2015.06.019.
19. Hübsch Z, van Zyl RL, Cock IE, van Vuuren SF. 2014. Interactive antimicrobial and toxicity profiles of conventional antimicrobials with Southern African medicinal plants. South African Journal of Botany. 2014;93:185-97. http://dx.doi.org/10.1016/j.sajb.2014.04.005.
20. Cock IE, Winnett V, Sirdaarta J, *et al*. The potential of selected Australian medicinal plants with anti-Proteus activity for the treatment and prevention of rheumatoid arthritis. Pharmacognosy Magazine. 2015;11(1):S190-S208. DOI: 10.4103/0973-1296.157734.
21. Ziegler U, Groscurth P. Morphological features of cell death. Physiology. 2004;19(3):124-8.
22. Ruebhart DR, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. Journal of Toxicology and Environmental Health Part A. 2009;72(24):1567-75.
23. Azari S, Ahmadi N, Tehrani MJ, *et al*. Profiling and authentication of human cell lines using short tandem repeat (STR) loci: Report from the National Cell Bank of Iran. Biologicals. 2007;35(3):195-202.
24. Prado EG, Giménez DG, De la Puerta Vázquez R, *et al*. Antiproliferative effects of mitraphylline, a pentacyclic oxindole alkaloid of *Uncaria tomentosa* on human glioma and neuroblastoma cell lines. Phytomedicine. 2007;14(5):280-4.

PICTORIAL ABSTRACT



SUMMARY

- *Uncaria tomentosa* methanolic and aqueous inner bark extracts were good inhibitors of Caco2 and HeLa cancer cell proliferation (IC_{50} values <1500 µg/mL).
- The methanolic *U. tomentosa* extract was particularly effective, with IC_{50} values of 881 and 763 µg/mL against Caco2 and HeLa cells respectively.
- The aqueous extract also displayed good, antiproliferative activity against Caco2 and HeLa cells, with an IC_{50} of 1645 and 1444 µg/mL respectively.
- Cell imaging studies indicated that the antiproliferative activity was due to apoptosis.
- The *U. tomentosa* extracts were nontoxic in the Artemia franciscana bioassay.

ABOUT AUTHORS

Dr Ian Edwin Cock leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including Aloe vera, South Asian and South American tropical fruits, as well as Australia plants including Scaevola spinescens, Pittosporum phylliraeoides, Terminalia ferdinandiana (Kakadu plum), Australian Acacias, Syzygiums, Petalostigmas and Xanthorrhoea johnsonii (grass trees). This range of projects has resulted in nearly 200 scientific publications in a variety of peer reviewed journals.