

## Anticancer Activities of Some Cameroonian Medicinal Plants

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### Abstract

Natural products are well recognized as sources for drugs in several human ailments including cancers. Examples of natural pharmaceuticals from plants include vincristine, irinotecan, etoposide and paclitaxel. Despite the discovery of many drugs of natural origin, the search for new anticancer agents is still necessary, in order to increase the range available and to find less toxic and more effective drugs. It has been recommended that samples with pharmacological usage should be taken into account when selecting plants to treat cancer, as several ailments reflect disease states bearing relevance to cancer or cancer-like symptoms. Therefore, we designed the present work to investigate the cytotoxicity of six natural compounds available in our research group, with previously demonstrated pharmacological activities.

**Keywords:** Staphylococcus; Naphthoquinone; Anthraquinones; Tumour promotion; Cell cycle; Carcinoma cells

### Introduction

Compound 1 has been isolated from the roots of *Cratogeomys formosum*, the leaves of *Symphonia globulifera*, and the seeds of *Vismia laurentii*. The only reported natural source of compound 2 is *Newbouldia laevis* in which it can be isolated from the roots. Compound 3 is a key active ingredient of the ethanol extract from roots of Chinese rhubarb that has been commercialised in China for controlling powdery mildews. Compound 3 was purified from several plants including *Rumex japonicus*, *Radix Boehmeriae*, *Discocleidion rufescens*, *Senna septemtrionalis*, etc. Compounds 4 and 5 are mostly found in plants of the genus *Vismia*, whilst compound 6 was reported in *Vimia laurentii* and *Psorospermum* species. Compounds previously showed antimicrobial activities against a panel of bacteria and fungi compound exhibited antibacterial activities against *Chlorella fusa* and *Bacillus megaterium*, respectively and compound 1 showed antileishmanial and cytotoxic activities against HeLa, HT-29 and KB cell lines [1]. Compound 1 also exhibited significant antibacterial activities on *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Salmonella typhi* with minimal inhibitory concentration below 10µg/ml. In the present work, we examined at first, the cytotoxicity of compounds 1–6 against MiaPaCa-2, CCRF-CEM, and CEM/ADR5000 cell lines, then we selected compounds 1 and 2 which were tested on a panel of cancer cells. Their possible modes of action were also investigated and reported herein [2]. The six naturally occurring compounds tested included one xanthone named xanthone V1 and five quinones amongst which were three anthraquinones known as physcion; vismiaquinone and 1,8-dihydroxy-3-geranyloxy-6-methylanthraquinone, one naphthoquinone known as 2-acetylfuro-1,4-naphthoquinone and one binaphthoquinone named bisvismiaquinone. The four studied naphthoquinones have compound 3 as the basic moiety. The preliminary cytotoxicity of the six studied compounds on CCRF-CEM, CEM/ADR5000 and MiaPaca-2 is summarized in [3]. Only xanthone V1 and 2-acetylfuro-1, 4-naphthoquinone as well as doxorubicin were able to reduce the proliferation of the three cell lines by up to 50%, when tested at 20 µg/ml. Physcion was previously found to have no cytotoxic activity on some cancer cell lines such as K562, HeLa, Calu-1, Wish and Raji and was not significantly active as observed in the present work. However, the low activity of 8-dihydroxy-3-geranyloxy-6-methylanthraquinone, as well as bisvismiaquinone and vismiaquinone also bearing physcion moiety clearly highlights the low cytotoxicity of the studied anthraquinones [4]. It can be deduced that

the best cytotoxic activity of the studied compounds were obtained with the tested xanthone and naphthoquinone. Xanthone V1 and 2-acetylfuro-1,4-naphthoquinone were therefore selected and tested on several cancer cells. The IC50 values obtained are reported in and values below 20 µg/ml were recorded on 12 of the 14 tested cancer cell lines for xanthone V1 and 14/14 for 2-acetylfuro-1,4-naphthoquinone.

### Methodology

Considering the cut-off points of 4 µg/ml or 10 µM for good cytotoxic compounds, values around or below this set point were obtained by xanthone V1 on 9/14 tested cancer cell lines and 11/14 for 2-acetylfuro-1,4-naphthoquinone [5]. The most sensitive cell lines were breast MCF-7 and cervix HeLa and Caski, leukemia PF-382 and melanoma colo-38. The liver is the main organ involved in drug metabolism. Therefore AML12 hepatocyte was chosen in the present work to evaluate the cytotoxicity of the compounds on non-cancer cells. Interestingly, the two compounds were generally less toxic on AML12 cells, the IC50 being above 20 µg/ml [6]. In addition, the two compounds showed respectively, 65.8% and 59.6% inhibition of the growth of blood capillaries on the chorioallantoic membrane of quail eggs in the anti-angiogenic assay, suggesting that negative effect on tumour promotion in vivo could be expected. To the best of our knowledge, the anticancer activity of 2-acetylfuro-1, 4-naphthoquinone is being reported herein for the first time meanwhile the cytotoxicity of xanthone V1 was reported on HeLa, HT-29 and KB cell lines as shown in (Figure 1).

This study thus confirms the cytotoxic potency of xanthone V1 on a large number of cancer cell lines [7]. The effects on xanthone V1 and 2-acetylfuro-1, 4-naphthoquinone on the cell cycle distribution, apoptosis induction and caspase 3/7 activity were investigated in CCRF-CEM cell line. Xanthone V1 was able to induce cell cycle arrest at

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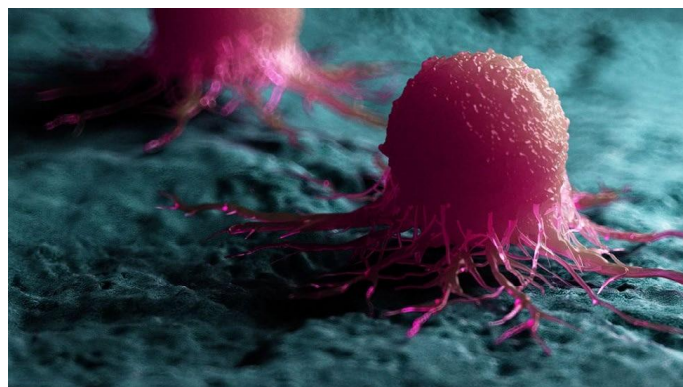
**Figure 1:** Cell lines.

higher concentration. At  $2 \times \text{IC}_{50}$ , the cell number in S-phase gradually increased with time upon treatment with xanthone V1, and the highest amount was observed after 72 h, suggesting a cycle arrest at this phase [8]. Up to 8.51% and 10.53% apoptotic cells were observed after 72 h upon treatment of CCRF-CEM cells with xanthone V1 at  $\text{IC}_{50}$  and  $2 \times \text{IC}_{50}$ , respectively. This result is in accordance with the activation of caspases 3/7, though the effect at  $2 \times \text{IC}_{50}$  was less than that obtained at a concentration corresponding to  $\text{IC}_{50}$  value [9]. This is obviously due to the fact that, despite the activation of caspase induced by this compound, the ratio of cell number-activity might still be better at  $\text{IC}_{50}$  than at  $2 \times \text{IC}_{50}$ . 2-acetylfuro-1,4-naphthoquinone induced cell cycle arrest in S-phase when tested at  $2 \times \text{IC}_{50}$  and  $\text{IC}_{50}$  values in a time-dependant manner. This compound induced apoptosis, even though without caspase 3/7 activation. This suggests that the activation of caspase might not be the main pathway for apoptosis induction by 2-acetylfuro-1,4-naphthoquinone. The overall results of the present work highlight the anticancer potency of xanthone V1 and 2-acetylfuro-1,4-naphthoquinone, and clearly justify the fact that all compounds with any pharmacological activity should also be evaluated for its cytotoxicity. The most sensitive cancer cell lines to xanthone V1 and 2-acetylfuro-1,4-naphthoquinone was Colo-38, HeLa and Caski with  $\text{IC}_{50}$  values being closer or lower those obtained with doxorubicin. In addition, MCF-7 and PF-382 also showed high sensitivity to xanthone V1 and 2-acetylfuro-1,4-naphthoquinone respectively [10]. The cytotoxicity of acetylfuro-1,4-naphthoquinone is being reported for the first time. Regarding the medical impact of these cancers, the activities of these two molecules could be considered as very important. In fact, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among females worldwide, accounting for 23% of the total cancer cases and 14% of the cancer deaths, meanwhile cervix and colon cancers are also amongst the most common cancer in economically developing and developed world respectively. Cell lines were obtained from different sources; Prof. Axel Sauerbrey, University of Jena, Jena, Germany: CCRF-CEM, CEM/ADR5000, HL-60; German Collection for Microorganisms and Cell Culture (DSMZ), Braunschweig, Germany: PF-382; Dr. Jörg Hoheisel, DKFZ, Heidelberg, Germany: MiaPaCa-2, Capan-1 pancreatic adenocarcinoma, MCF-7 breast adenocarcinoma, SW-680 colon carcinoma cells; Tumor Bank, German Cancer Research Center, Heidelberg, Germany: 786-0 renal carcinoma cells, U87MG glioblastoma-astrocytoma cells, A549 lung adenocarcinoma, Caski and HeLa cervical carcinoma cells, Colo-38 skin melanoma cells; ATCC, USA: AML12 hepatocytes [11].

## Discussion

A panel of fourteen cancer cell lines including human CCRF-CEM leukemia cells and their multidrug-resistant subline, CEM/ADR5000, PF-382 leukemia T-cells, and HL-60 promyelocytic leukemia, MiaPaCa-2 and Capan-1 pancreatic adenocarcinoma, MCF-7 breast adenocarcinoma, SW-680 colon carcinoma cells, 786-0 renal carcinoma cells, U87MG glioblastoma-astrocytoma cells, A549 lung adenocarcinoma, Caski and HeLa cervical carcinoma cells, Colo-38 skin melanoma cells, as well as AML12 hepatocytes, were used. CCRF-CEM, CEM/ADR5000 and MiaPaCa-2 cells were used for the preliminary assay and the most active compounds were tested on other cell lines. Leukemia cells were maintained in RPMI 1640 containing 100 units/ml penicillin and 100  $\mu\text{g}/\text{ml}$  streptomycin and supplemented with heat-inactivated 10% fetal bovine serum. All cultured cells were maintained in a humidified environment at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$ . Doxorubicin was used as a positive control [12]. The concentration of DMSO was not greater than 0.1% in all experiments. Alamar Blue or Resazurin reduction assay was used to assess the cytotoxicity of the studied samples. The assay tests cellular viability and mitochondrial function. Briefly, adherent cells were grown in tissue culture flasks, and then harvested by treating the flasks with 0.025% trypsin and 0.25 mM EDTA for 5 min. Once detached, cells were washed, counted and an aliquot was placed in each well of a 96-well cell culture plate in a total volume of 100  $\mu\text{l}$ . Cells were allowed to attach overnight and then treated with samples. The final concentration of samples ranged from 20-0.16  $\mu\text{g}/\text{ml}$ . After 48 h, 20  $\mu\text{l}$  resazurin 0.01% w/v solution was added to each well and the plates were incubated at  $37^{\circ}\text{C}$  for 1–2 h. Fluorescence was measured on an automated 96-well Infinite M2000 Pro™ plate reader using an excitation wavelength of 544 nm and an emission wavelength of 590 nm. For leukemia cells, aliquot of  $5 \times 10^4$  cells/ml were seeded in 96-well plates, and extracts were added immediately. After 24 h incubation, plates were treated with resazurin solution as above mentioned. Doxorubicin was used as positive control [13]. Each assay was done at least three times, with two replicates each. The viability was compared based on a comparison with untreated cells as shown in (Figure 2).

Natural products are well recognized as sources of drugs in several human ailments. In the present work, we carried out a preliminary screening of six natural compounds, xanthone V1; 2-acetylfuro-1,4-naphthoquinone; physcion; bisvismiaquinone; vismiaquinone; 1,8-dihydroxy-3-geranyloxy-6-ethylanthraquinone against MiaPaCa-2 pancreatic and CCRF-CEM leukemia cells and their multidrug-resistant subline, CEM/ADR5000. Compounds 1 and 2 were then tested in several other cancer cells and their possible modes of action



**Figure 2:** Cancer cells.

were investigated. The tested compounds were previously isolated from the Cameroonian medicinal plants *Vismia laurentii* and *Newbouldia laevis*. The preliminary cytotoxicity results allowed the selection of xanthone V1 and 2-acetylfuro-1,4-naphthoquinone, which were then tested on a panel of cancer cell lines [14]. The study was also extended to the analysis of cell cycle distribution, apoptosis induction, caspase 3/7 activation and the anti-angiogenic properties of xanthone V1 and 2-acetylfuro-1,4-naphthoquinone. IC50 values around or below 4 µg/ml were obtained on 64.29% and 78.57% of the tested cancer cell lines for xanthone V1 and 2-acetylfuro-1, 4-naphthoquinone, respectively. The most sensitive cell lines were breast MCF-7, cervix HeLa and Caski, leukemia PF-382 and melanoma colo-38. The two compounds showed respectively, 65.8% and 59.6% inhibition of the growth of blood capillaries on the chorioallantoic membrane of quail eggs in the anti-angiogenic assay. Upon treatment with two fold IC50 and after 72 h, the two compounds induced cell cycle arrest in S-phase, and also significant apoptosis in CCRF-CEM leukemia cells. Imaging of the vascularized quail eggs was performed using a digital camera with 3×-magnification objective. For illumination, a mercury-arc-lamp was used which provided a high fraction of blue and UV-light to obtain good contrast values between yolk and vessels. The pictured image section had a size of 5×5 mm. Following image acquisition, quantitative analysis was performed using a software routine which was written in the Image J-macro language, and the total small vessels number was then determined by the system. The percentage inhibition of vascularization was calculated as previously described.

## Conclusion

The overall results of the present study provided evidence for the cytotoxicity of compounds xanthone V<sub>1</sub> and 2-acetylfuro-1, 4-naphthoquinone, and bring supportive data for future investigations that will lead to their use in cancer therapy.

## Acknowledgement

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## Conflict of Interest

None

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