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In vitro antiproliferative activities of some Ghanaian medicinal plants

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Abstract

Background Cancer continues to pose a significant threat to human well-being due to the overwhelming rate of morbidity and mortality associated with it. Hence, the quest for newer, effective and safer anticancer agents has become more crucial. Over the years, some medicinal plants have been used to treat abnormal tissue growths (tumours) in Ghana. Even though sufficient literature points out that people found some relief in their use, there is limited scientific evidence of their antiproliferative activities.

Method Ethanolic extracts of nine medicinal plant materials from seven plant species, including the stem bark of *Terminalia superba*, *Talbotiella gentii* and *Ceiba pentandra* and the leaves of *Morinda lucida*, *Dracaena arborea*, *Dioscorea dumetorum*, *Thaumatococcus danielli*, *Ceiba pentandra* and *Talbotiella gentii*, were evaluated for antiproliferative activities against four human cancer cell lines (hepatocellular carcinoma, colorectal adenocarcinoma, cervical carcinoma, and mammary adenocarcinoma) using an MTT-based assay.

Results The extract of *C. pentandra* leaves, exhibited generally higher antiproliferative activity, which was particularly substantial against human hepatocellular carcinoma (HepG2) cells (IC_{50} = 16.3 μ g/mL) and human colorectal adenocarcinoma (RKO) cells (IC_{50} = 18.7 μ g/mL). All the other plant materials demonstrated weak (IC_{50} : 201–500 μ g/mL) to moderate (IC_{50} : 21–200 μ g/mL) antiproliferative activities against the four cancer cell lines.

Conclusion The extracts of the plant materials demonstrated varied antiproliferative activities. Extract of *C. pentandra* leaves exhibited the highest antiproliferative activity. The IC_{50} values of *C. pentandra* leaves met the benchmark to be considered effective against HepG2 and RKO cancer cell lines in particular. Therefore, there is the need to further undertake fractionation work on *C. pentandra* leaves. The antiproliferative effect of extract of *C. pentandra* leaves against other cancer cell lines and normal cell line could also be explored in the future to ascertain the anticancer potential of this plant material. Generally, findings from this work support the indigenous use of these plant materials in treating abnormal tissue growth in Ghana.

Keywords *Ceiba pentandra*, Medicinal plants, Antiproliferative activity, Cancer cells, Tumour

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Introduction

Cancer is a broad term describing a group of diseases characterised by abnormal cell growth and progression, usually beyond their natural boundaries, that can invade nearby body parts or spread to other tissues [1]. Cancer is a significant health concern and one of the leading causes of mortality. It is the second leading cause of disease and mortality globally [2, 3]. In 2022, there were an estimated 20 million new cases and nearly 10 million deaths attributed to cancers [4]. Although several drugs have been developed over the years to combat this deadly disease [5–7], the development of resistance and the reported adverse drug reactions associated with their use [8–11] have hindered treatment success, which has led to the continuous search for new agents. The frightening projection of a 57% surge in cancer-related morbidity and mortality by the year 2032 has also amplified the interest in newer effective, and safer antitumour agents [12].

Due to their diverse pharmacological potential, plants serve as rich repositories for new pharmacological agents in drug discovery [13–15]. Plants have been a source of therapeutic bioactive compounds and are the basis for developing several drugs to treat various diseases [16]. These drugs include Quinine and Artemisinin from *Cinchona* spp and *Artemisia annua*, respectively [17–19]. More importantly, the anticancer agents such as Camptothecin, Taxol and Vinblastine were first isolated from *Camptotheca acuminata*, *Taxus brevifolia* and *Catharanthus roseus*, respectively [5, 20–23].

Ghana is endowed with numerous plants of medicinal value [24, 25]. Several herbalists and traditional healers use these plants to treat or manage various diseases, including abnormal tissue growth [24, 25]. Plants such as *Morinda lucida*, *Talbotiella gentii*, *Dracaena arborea*, *Dioscorea dumetorum*, *Thaumatococcus danielli*, *Ceiba pentandra*, and *Terminalia superba* have been cited in various studies to be used in folkloric management of tumours in Ghana [26].

Previous studies on the antiproliferative properties of some of these plant species have pointed to their anticancer potential. *Morinda lucida*, for instance, has been reported by Appiah-Opong et al. [27] to induce apoptosis in human promyelocytic (leukemic) HL-60 cell line. Young et al. [28] also reported the ability of methanol extract of *Dracaena arborea* to decrease the cell viability of metastatic human breast cancer cells (MDA-MB-231). The cytotoxic potential of bark extracts of *Ceiba pentandra* has also been reported against Ehrlich ascites carcinoma (EAC), and human mammary adenocarcinoma (MCF-7) cells [29]. Despite these encouraging findings, significant gaps still exist as most of these studies have focused on one cell line. Additionally, there is a dearth of scientific work on several of these plant materials regarding their antiproliferative activities. Therefore, this

study seeks to evaluate the aforementioned plant species for their antiproliferative activities on a panel of human cancer cells. This evaluation will help to support their traditional use for treating abnormal tissue growth by traditional healers or herbalists.

Materials and methods

Chemicals and reagents

Dulbecco's modified eagle's medium (DMEM) (Gibco Life Technologies Ltd, Paisley, UK cat # 11885084), trypsin (Gibco Life Technologies Ltd, Paisley, UK cat # 11560626), phosphate buffer saline (PBS) (Gibco Life Technologies Ltd, Paisley, UK cat # 10010023), dimethylsulfoxide (DMSO) (Gletham Life Sciences, UK cat # GLS GK2245-500ML), ethanol, 5-fluorouracil (Gletham Life Sciences, UK cat # GP6168-5 g), and isopropanol.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder (cat # M5655-1G) was acquired from Sigma Aldrich (St. Louis, USA). Penicillin (Gibco Life Technologies Ltd, Paisley, UK, cat # 15140122) and streptomycin (Gibco Life Technologies Ltd, Paisley, UK, cat # 15140122), Foetal bovine serum (FBS) (ATCC 30-2020) and non-essential amino acids (NEAA) (ScienCell, Carlsbad, USA, cat # 0823).

Cell lines

The cell lines included the human cancer cell lines: HepG2 (human hepatocellular carcinoma), RKO (human colorectal adenocarcinoma), Hela (human cervical carcinoma) and MCF-7 (human mammary adenocarcinoma). All the cell lines were generously donated by Prof. Mohamed Mutocheluh of the Virus Research and Molecular Biology Laboratory of the Clinical Microbiology Department of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

Cell culture

A working medium was prepared from DMEM reconstituted by adding 1% NEAA, 1% Penicillin/Streptomycin and 10% FBS. The cancer cell lines (HepG2, RKO, Hela and MCF-7) were cultured in the working medium. The cells were incubated in an incubator maintained at 37 °C and 5% CO₂ under relative humidity of 95%. The continuous logarithmic growth of the cells was ensured by subculturing the cells at least twice a week. The cells at about 80% confluence were washed with PBS, detached using trypsin and collected for centrifugation. After centrifuging at 200 x g for 5 min, the cells were resuspended in the working medium. The enumeration of cells was achieved using a haemocytometer, and the viability of cells was determined using trypan blue exclusion. Eppendorf 96-well TC-treated cell culture plates were used to seed cells at 4 × 10⁴ cells/100μL per well.

Plant materials collection and authentication

Nine plant materials (viz., *C. pentandra* bark, *C. pentandra* leaves, *D. dumetorum* leaves, *D. arborea* leaves, *M. lucida* leaves, *T. gentii* bark, *T. gentii* leaves, *T. superba* bark and *T. danielli* leaves) were collected from different parts of Ghana (Akosombo, Kwahu Bepong, and Mampong-Akwapem). Prof. Gustav Komlaga from the Department of Pharmacognosy, and Dr. George Henry Sam from the Department of Herbal Medicine, both of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, authenticated the plants. Herbarium specimens with voucher numbers (Table 1) were deposited in the Herbarium of the same Faculty.

Preparation of plant extracts

The plant materials were rinsed with tap water and air-dried at room temperature for three weeks. The dried plant materials were then ground into coarse powder. Each powdered material (2000 g) was placed in a glass jar, and 80% ethanol was added. The mixture was left for 72 h, and occasionally gently stirred. It was subsequently filtered using Whatman No. 1 filter paper. The process was performed thrice to ensure that all constituents had been extracted. The filtrate was then concentrated using rotary evaporator (R-114, Buchi, Switzerland). Drawell freeze drier (DW-10 N) was used to concentrate the sample further by removing the water. The dried extract was kept in a well-closed glass bottle and put in the refrigerator at 4°C until required. The extract of *C. pentandra* leaves which exhibited the highest antiproliferative properties was further fractionated. The extract was first suspended in water and then extracted with petroleum ether (PE). The resultant water fraction was further extracted sequentially with ethyl acetate (EtOAc) and n-butanol (n-BuOH). The four different fractions obtained (PE, EtOAc, n-BuOH, and water fraction) from the fractionation process were dried, weighed and kept in a well-closed glass bottle and put in the refrigerator at 4 °C until required.

Table 1 Medicinal plants screened for antiproliferative activities

Plant Species	Family	Part Screened Voucher #
<i>Dracaena arborea</i>	Asparagaceae	Leaves KNUST/HMI/2020/L011
<i>Talbotiella gentii</i>	Fabaceae	Leaves KNUST/HMI/2020/L013
<i>Talbotiella gentii</i>	Fabaceae	Bark KNUST/HMI/2020/SB026
<i>Ceiba pentandra</i>	Malvaceae	Bark KNUST/HMI/2020/SB027
<i>Ceiba pentandra</i>	Malvaceae	Leaves KNUST/HMI/2020/L015
<i>Morinda lucida</i>	Rubiaceae	Leaves KNUST/HMI/2020/L023
<i>Discorea dumetorum</i>	Dioscoreaceae	Leaves KNUST/HMI/2020/L012
<i>Terminalia superba</i>	Combretaceae	Bark KNUST/HMI/2020/SB028
<i>Thaumatococcus danielli</i>	Marantaceae	Leaves KNUST/HMI/2020/L014

Preparation of test solutions

Stock test solutions of crude extracts (1 mg/mL) were prepared in 1% dimethylsulfoxide (DMSO). The positive control, 5-fluorouracil (5FU) solution, was also prepared in a similar way. The negative control was prepared from 2 µl of DMSO and 1998 µl of working medium (reconstituted DMEM). Each treatment stock solution of the crude extracts was diluted with the working medium to give the different concentrations (200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 1 µg/mL). Following the confirmation of *C. pentandra* leaves as significantly bioactive, a time-dependent study was conducted on the extract by assessing its antiproliferative activities after 24 h, 48 h and 72 h of treatment at concentrations between 3.125 and 50 µg/mL. Using the working medium, the 1 mg/mL stock solution was diluted to give a concentration of 50 µg/mL. The serial dilution method was then used to prepare the other required concentrations (that is, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL). Similar approach was employed in the determinations of the antiproliferative activity of the four fractions of *C. pentandra* leaves obtained during the fractionation process. In all cases, the solutions (that is, test solutions, positive and negative control) contained ≤0.1% of DMSO which has been demonstrated to have no effect on the growth of cells [30].

Antiproliferative study

Cell viability was determined using MTT-based assay [31]. Cells seeded in 96-well plates were incubated at 37 °C and 5% CO₂ under humid conditions for 24 h for the cells to attach. The old medium was discarded, and cells were incubated in 100 mL of fresh medium containing test crude extracts at a concentration of 1 µg/mL to 200 µg/mL for all the plants' crude extracts and 3.125 µg/mL to 50 µg/mL for the *C. pentandra* fractions. All tests were performed in triplicate. The positive (5FU) and negative (DMEM) controls were also done in triplicate. This was then incubated for 72 h. To determine the time-dependent effect of extract of the active plant material (that is, *C. pentandra* leaves), the treated cells were incubated for 24 h, 48 h and 72 h. After incubation, 20 µl of MTT reagent was added to each well, and the plate was incubated for another 3 h. The supernatant was then aspirated from each well, and 120 µl of isopropanol was added, with three of the wells serving as blank and sterility control. The plate was kept in the incubator for 30 min for the formazan crystals to dissolve. The absorbance was read on iMark Microplate Reader (Bio-Rad, USA) with the measurement filter set at 595 nm. The procedure was repeated two more times.

Data analysis

The data were analysed using GraphPad Prism (version 8.0.2). Cell viability was determined by the ratio of cells cultured in the presence of extracts or fractions to cells cultured in the absence of extracts or fractions (cells were mock-treated with DMSO). Comparisons of IC_{50} values to 30 $\mu\text{g/mL}$ were done using a one-sample t-test. All other comparisons between treatments were conducted using the one-way ANOVA and Tukey's test for post-hoc analysis. In all comparisons, $p < 0.05$ was considered as statistically significant.

Results

Extracts from the nine plant materials used by traditional healers in Ghana to treat abnormal cell growth were evaluated for their antiproliferative activities using an MTT-based assay. The plant samples demonstrated a wide range of activities on cell viability, as shown in Fig. 1A and B. All the plant extracts inhibited cell growth in a dose-dependent manner with significant differences as shown in Fig. 1A and B and Supplementary Data S1. As summarised in Table 2, only one plant material, *C. pentandra* leaves demonstrated appreciable antiproliferative properties of IC_{50} between 16 $\mu\text{g/mL}$ and 42 $\mu\text{g/mL}$. The remaining plant samples produced moderate to weak antiproliferative activities. As shown in Fig. 1A and B, the effects of the various extracts on cell viability were concentration dependent.

Extracts from *C. pentandra* bark, *D. arborea* leaves, *M. lucida* leaves, and *T. danielli* leaves showed moderate antiproliferative properties against the cell lines with IC_{50} values ranging from 76 $\mu\text{g/mL}$ to 193 $\mu\text{g/mL}$. Extracts from *D. dumetorum* leaves, *T. gentii* leaves and bark, and *T. superba* bark, on the other hand, showed moderate antiproliferative properties against most of the cell lines. However, *T. superba* bark, *T. gentii* leaves, and *D. dumetorum* leaves displayed weak toxicity ($IC_{50} > 200 \mu\text{g/mL}$) against Hela, HepG2, and MCF-7 cell lines respectively.

Extracts of *C. pentandra* leaves demonstrated the highest activity against all the cell lines with IC_{50} values of 16.3 $\mu\text{g/mL}$, 18.7 $\mu\text{g/mL}$, 41.6 $\mu\text{g/mL}$, 30.2 $\mu\text{g/mL}$ against HepG2, RKO, Hela and MCF-7, respectively. The IC_{50} values were found to be significantly lower against HepG2 ($p < 0.001$) and RKO ($p < 0.01$) when compared to the American National Cancer Institute (NCI) threshold of $< 30 \mu\text{g/mL}$.

The cell viability of the extract of *C. pentandra* leaves was determined after 24 h, 48 h and 72 h of treatment. As shown in Fig. 2, the test materials demonstrated good inhibitory effect after 24 h, 48 h and 72 h of treatment against the HepG2 cell line. Results showed a time-dependent antiproliferative effect which was not statistically significant ($p = 0.3430$). Table 3 provides the IC_{50} values obtained for the test. The test material

demonstrated IC_{50} values ranging from 35.6 ± 1.6 to 71.4 ± 3.8 after 24 h of treatment, 21.5 ± 0.9 to 57.4 ± 4.5 after 48 h of treatment and 16.1 ± 0.6 to 40.9 ± 3.1 after 72 h of treatment.

Figure 3A and B provide the percentage inhibition of the various fractions against HepG2 and Hela cell lines. Inhibition of cell viability by the different fractions of *C. pentandra* leaves showed varied results. All four fractions inhibited cell growth in a dose-dependent fashion with significant differences as shown in Fig. 3A and B and Supplementary Data S2. Ethyl acetate fraction demonstrated the highest inhibition of cell viability against both cell lines. As shown in Table 4, ethyl acetate fraction had the lowest IC_{50} values (that is, exerted the highest inhibitory effect), with HepG2 being the most susceptible. The IC_{50} values were significantly lower against HepG2 ($p < 0.01$) and Hela ($p < 0.05$) when compared to NCI threshold of $< 30 \mu\text{g/mL}$.

Discussion

The level of antiproliferative activity has been classified as high, moderate, weak or no toxicity. This was based on protocols established by the American National Cancer Institute (NCI) and the Geran Protocol [32, 33], stated as follows: No effect = $IC_{50} \geq 501 \mu\text{g/mL}$; Weak effect = IC_{50} 201–500 $\mu\text{g/mL}$; Moderate effect = IC_{50} 21–200 $\mu\text{g/mL}$; and High effect = $IC_{50} \leq 20 \mu\text{g/mL}$. With the exception of extract of *C. pentandra* leaves, which demonstrated high antiproliferative activity ($IC_{50} \leq 20 \mu\text{g/mL}$) against two cell lines (Table 2), all other plant extracts exhibited weak or moderate antiproliferative activity against the four established cancer cell lines used.

The IC_{50} values of *M. lucida* leaves extract were within the range of 100 to 185 $\mu\text{g/mL}$ for the different cancer cells (HepG2, RKO, Hela and MCF-7), which indicated that the extract was moderately antiproliferative. The results contradicted reports on other *Morinda* species with substantial antiproliferative activities. The leaves of another species, *M. tinctoria*, have been shown to demonstrate marked activity [34]. Similarly, Noor and co-workers reported a higher antiproliferative effect (IC_{50} 39.9 $\mu\text{g/mL}$) of *M. citrifolia* leaf extract on MCF-7 [35]. Other previous studies have also demonstrated that four monoterpenes isolated from *M. morindoides* leaves exhibited marked antiproliferative activity ($< 10 \mu\text{g/mL}$) against MT-4 (human T-cell) cells [36], while damnanthal and nordamnanthal isolated from the roots of *M. elliptica* have been shown to possess marked antiproliferative activity against HL-60 and Wehi-3B (myelomonocytic leukaemia) cell lines [37]. The extract of *T. gentii* leaves produced IC_{50} values ranging from 129.9 to 201.5 $\mu\text{g/mL}$ against the cancer cells, while the bark produced IC_{50} values between 105 and 300 $\mu\text{g/mL}$. To the

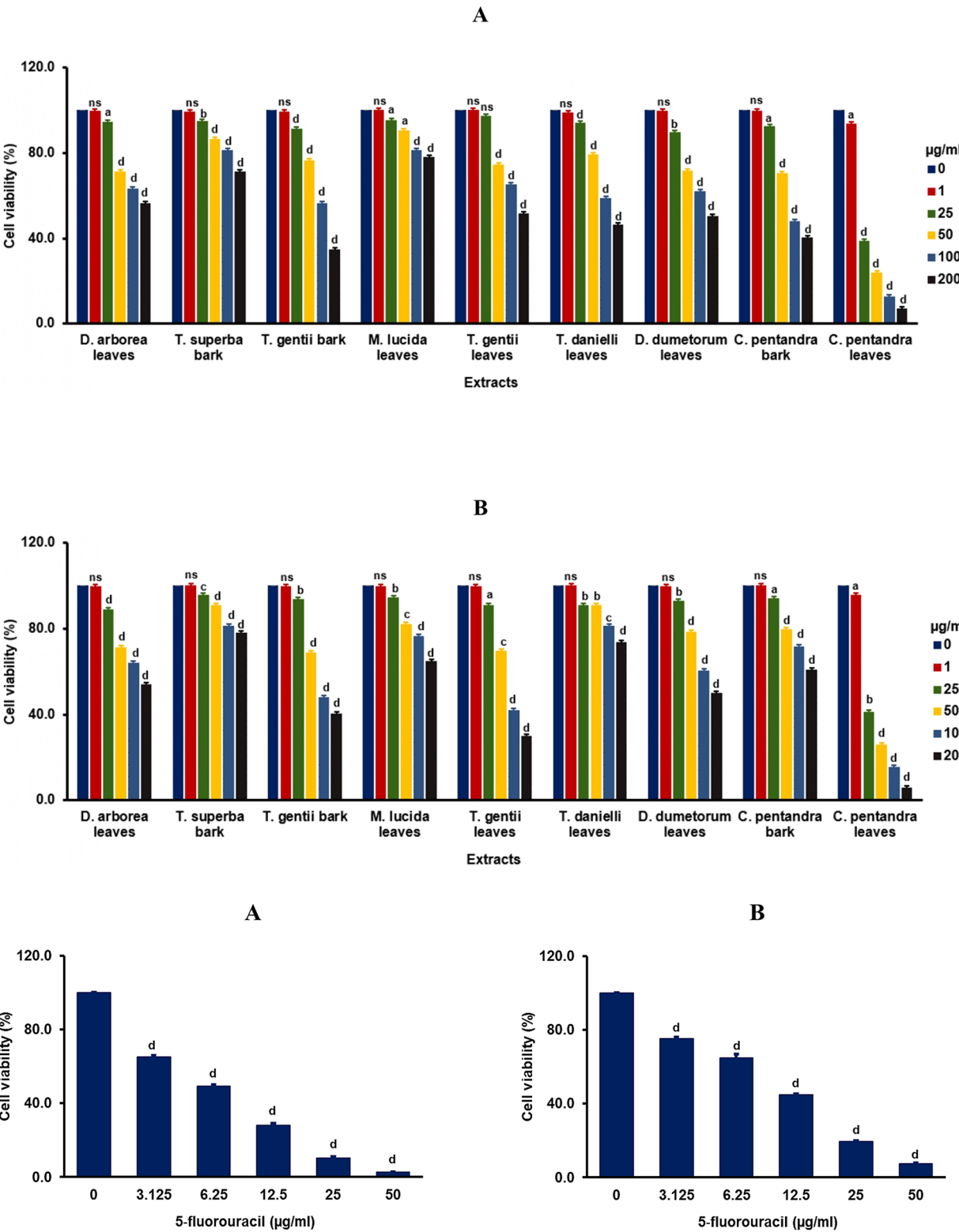


Fig. 1 A representative graph of the inhibitory effects of plant extracts on HepG2 (A) and RKO (B) cells. The cells were treated with plant extracts (1–200 µg/mL), and the cell viability was assessed at 72 h using an MTT-based assay. Data are presented as the means, with the standard deviations as error bars, from three different experiments, each conducted in triplicate. ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, and ^d $p < 0.0001$ correspond to significant difference between the untreated and treated cells as calculated by one-way ANOVA and Tukey's test, ns: difference not significant. The comparisons within the treatment are indicated in the supplementary data. Data for positive control (5-fluorouracil) are presented for comparison

Table 2 IC₅₀ values of different plant extracts on the various cancer cell lines

Plant Species/Drug	IC ₅₀ values (µg/mL ± SD) ^a			
	HepG2	RKO	Hela	MCF-7
<i>C. pentandra</i> bark	108.8 ± 1.8	121.7 ± 1.5	117.7 ± 3.1	162.9 ± 2.7
<i>C. pentandra</i> leaves	16.3 ± 0.5***	18.7 ± 1.4**	41.6 ± 2.2	30.2 ± 1.1
<i>D. dumetorum</i> leaves	92.9 ± 1.7	76.3 ± 3.9	132.5 ± 2.4	245.1 ± 1.6
<i>D. arborea</i> leaves	89.9 ± 2.2	140.1 ± 2.6	94.0 ± 1.5	163.4 ± 4.6
<i>M. lucida</i> leaves	109.7 ± 1.5	142.1 ± 2.1	181.0 ± 3.9	120.2 ± 2.6
<i>T. gentii</i> bark	298.4 ± 5.4	106.8 ± 4.8	190.2 ± 1.8	111.3 ± 1.1
<i>T. gentii</i> leaves	147.3 ± 6.8	129.9 ± 2.4	201.5 ± 1.0	132.1 ± 1.8
<i>T. superba</i> bark	176.4 ± 3.7	117.5 ± 9.3	206.4 ± 4.7	119.6 ± 2.2
<i>T. danielli</i> leaves	183.6 ± 1.6	166.2 ± 2.4	129.1 ± 2.2	193.0 ± 0.9
5- fluorouracil	5.4 ± 0.8	10.8 ± 1.2	9.6 ± 1.1	3.1 ± 0.9

^a – IC₅₀ values are presented as means and standard deviations of three independent experiments, with each experiment conducted in triplicate. Using a one-sample t-test, IC₅₀ values were compared to 30 µg/mL. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 correspond to significant differences

best of our knowledge, this is the first report on the anti-proliferative activity of this plant species (*T. gentii*).

Dracaena arborea showed moderate antiproliferative activity against all four cancer cell lines with IC₅₀ values of 89.9 to 163.4 µg/mL. Methanol extract of *D. cinnabari* has been reported to display high antiproliferative activity

Table 3 IC₅₀ values of *C. Pentandra* leaves extract on cancer cell lines at different times

Duration	IC ₅₀ values (µg/mL ± SD) ^a			
	HepG2	Hela	RKO	MCF-7
24 h	35.6 ± 1.6	62.3 ± 5.2	68.4 ± 3.6	71.4 ± 3.8
48 h	21.5 ± 0.9	49.1 ± 1.6	52.7 ± 2.4	57.4 ± 4.5
72 h	16.1 ± 0.6	40.9 ± 3.1	19.6 ± 2.2	32.4 ± 1.8

^a – IC₅₀ values are presented as means and standard deviations of three independent experiments, with each experiment conducted in triplicate. There was no statistical difference in IC₅₀ values between time intervals according to one-way ANOVA test

against oral squamous cell carcinoma (H103) cell lines [38]. A homoisoflavonoid isolated from another *Dracaena* species (*D. cambodiana*) has also been reported to have substantial activity (IC₅₀ = 1.4 µg/mL and 2.9 µg/mL) against K562 (chronic myelogenous leukemia) and SGC-7901 (human gastric cancer) cells, respectively [39]. The results of the effect of leave extract of *D. arborea* on HepG2 and Hela cells offered some evidence for the traditional healing practices associated with this plant material. *D. dumetorum* leaf extract also showed moderate antiproliferative activity (IC₅₀ of 76.3 to 132.5 µg/mL) against RKO, MCF-7 and HepG2 cells while displaying weak toxicity (IC₅₀ = 245.1 µg/mL) against Hela cells. The

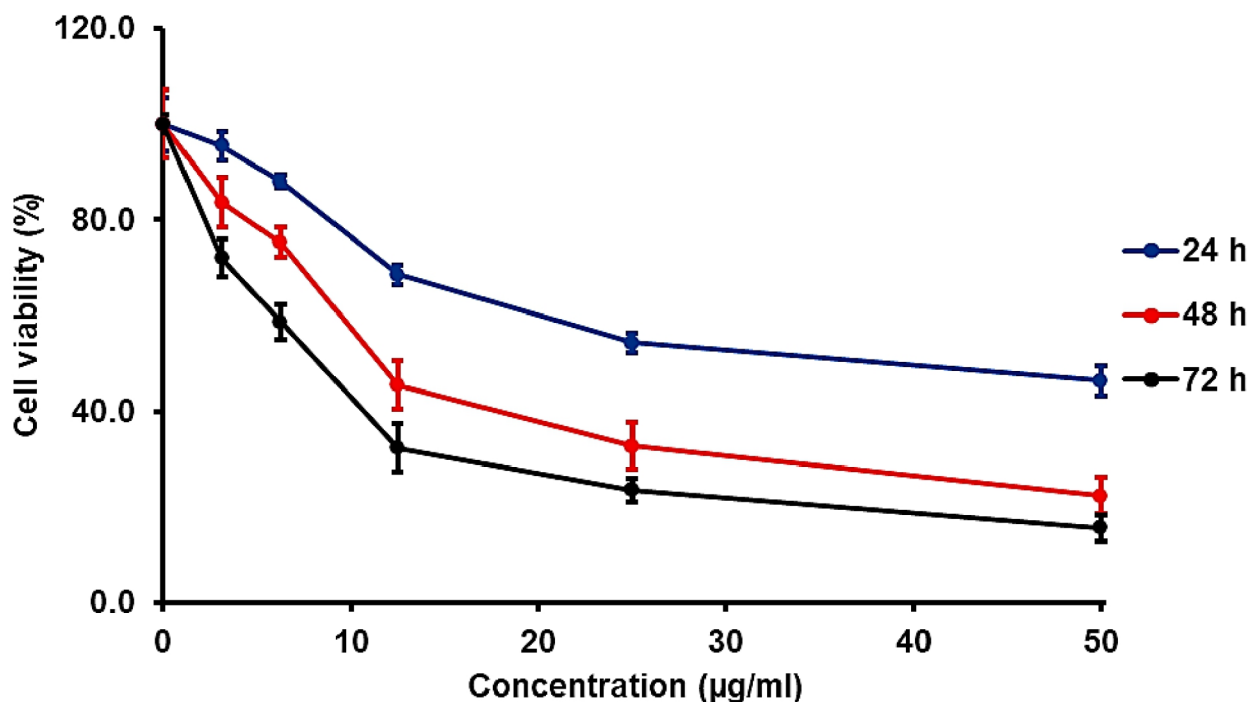


Fig. 2 A representative graph of time-dependent inhibitory effects of ethanolic extracts of *C. pentandra* leaves on HepG2 cells. The cells were treated with the ethanolic extract (3.125–50 µg/mL), and the cell viability was assessed at 24, 48 and 72 h using an MTT-based assay. Data are presented as the means, with the standard deviations as error bars, from three different experiments, each conducted in triplicate. There was no statistical difference in the viability at the various time intervals (*p* = 0.3430), as calculated by one-way ANOVA

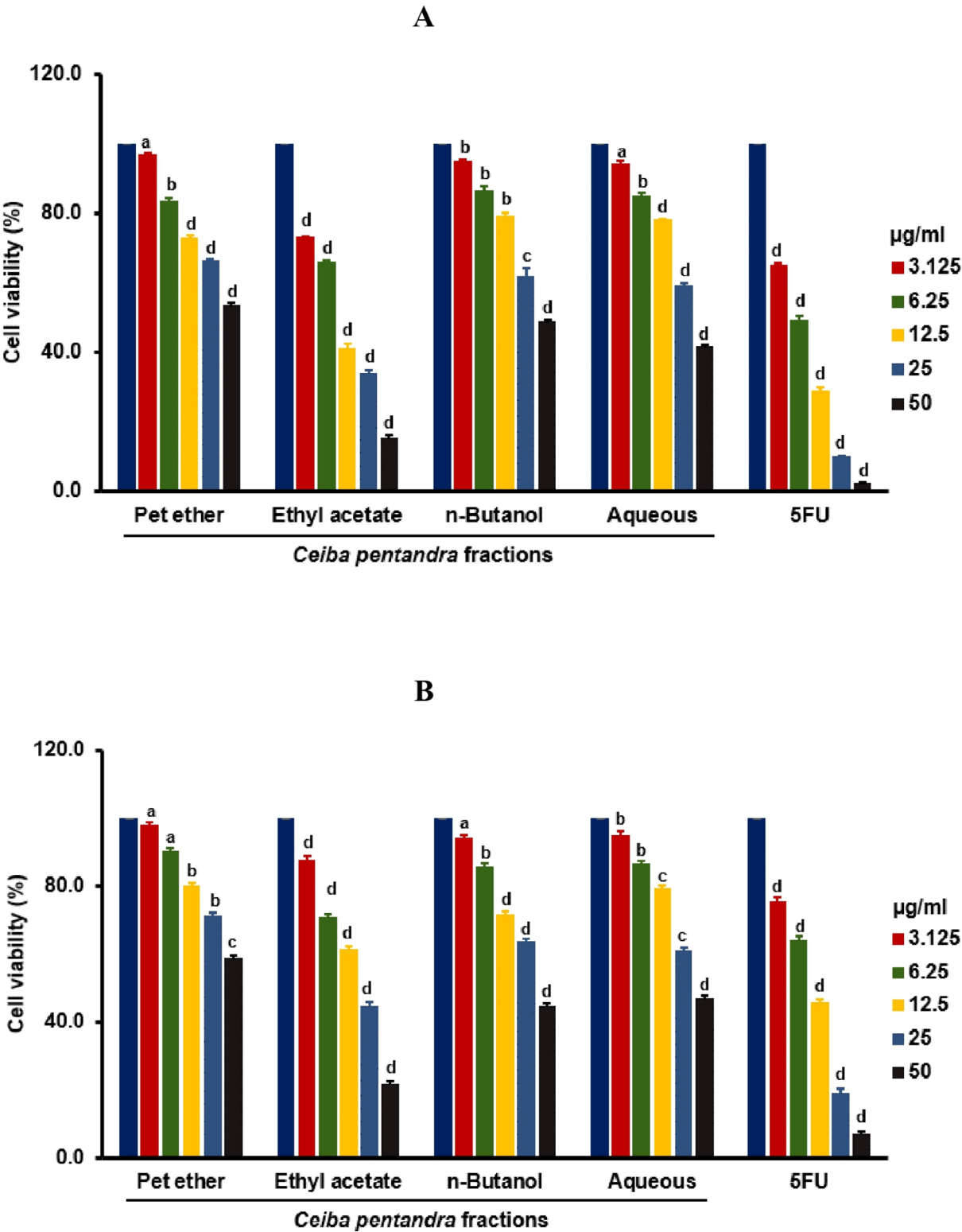


Fig. 3 A representative graph of the inhibitory effects of *C. pentandra* leaves fractions on HepG2 (A) and Hela (B) cells. The cells were treated with the fractions (3.125–50 µg/mL), and the cell viability was assessed at 72 h using an MTT-based assay. Data are presented as the means, with the standard deviations as error bars, from three different experiments, each conducted in triplicate ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, and ^d $p < 0.0001$ correspond to significant difference between the untreated and treated cells as calculated by one-way ANOVA and Tukey's test. The comparisons within the treatments are indicated in the supplementary data. **5FU**: 5-Fluorouracil

Table 4 IC₅₀ values of *C. Pentandra* leave fractions on the various cancer cell lines

Fraction	IC ₅₀ values (µg/mL ± SD) ^a			
	HepG2	Hela	RKO	MCF-7
Pet ether	> 50	> 50	> 50	> 50
Ethyl acetate	10.7 ± 1.1**	17.9 ± 2.9*	> 50	> 50
n-Butanol	44.1 ± 3.6	40.8 ± 5.2	> 50	> 50
Aqueous	36.4 ± 4.2	43.6 ± 3.4	> 50	> 50
5- fluorouracil	5.4 ± 0.6	10.7 ± 1.4	9.4 ± 1.0	3.2 ± 0.4

^a – IC₅₀ values are presented as means and standard deviations of three independent experiments, with each experiment conducted in triplicate. Using a one-sample t-test, IC₅₀ values were compared to 30 µg/mL. **p* < 0.05 and ***p* < 0.01 correspond to significant differences

results were, however, at variance with similar studies on other *Dioscorea* species, which reported high antiproliferative activity (IC₅₀ = 19.77 µg/mL) for *D. bulbifera* against CCRF-CEM (acute lymphoblastic leukemia) cell line [40].

Thaumatococcus danielli leaves exhibited moderate antiproliferative activities (IC₅₀ of 129.1 to 193.0 µg/mL) against the selected panel of cancer cell lines. Although significant antioxidant activity has been reported for the seeds [41] and the leaves [42, 43] of *T. danielli*, there is a dearth of published work on the antiproliferative activity of any part of this plant. Similarly, published reports on the other species of *Thaumatococcus* (*T. flavus*) concerning its antiproliferative properties are scanty. *T. superba* stem bark was another medicinal plant material evaluated in the present study that demonstrated weak to moderate antiproliferative effects (IC₅₀ between 117.5 µg/mL and 206.4 µg/mL) against the cancer cells. Our finding is similar to other researchers, such as Fyhrquist et al., who reported a wide range of antiproliferative effect (IC₅₀ range of 18.0 µg/mL to 250.0 µg/mL) for other species of *Terminalia* [44]. The findings of Spiegler and co-workers also revealed that the extract of *T. superba* stem bark had either no activity or weak activity [45].

Although the bark of *C. pentandra* demonstrated moderate antiproliferative activity (IC₅₀ = 108.8 ± 1.8 to 162.9 ± 2.7), its leaves exhibited the highest antiproliferative activities (IC₅₀ of 16.3 to 41.6 µg/mL) against the four established cancer cell lines with HepG2 (IC₅₀ = 16.3 µg/mL) and RKO (IC₅₀ = 18.7 µg/mL) cells being the most susceptible. These findings were consistent with earlier studies in which the stem bark of *C. pentandra* revealed antiproliferation potential against EAC (Ehrlich ascites carcinoma), MCF-7 and B16F10 (murine melanoma) cells [29]. The aerial part of *C. pentandra* had also been reported to show substantial antiproliferative activity against HepG2 and MCF-7 cell lines with IC₅₀ of 14.895 µg/mL and 18.859 µg/mL, respectively [46]. The antiproliferative potency of the extract of *C. pentandra* leaves is further demonstrated by its ability to substantially inhibit cell growth after 48 h and 24 h of treatment.

The results of *C. pentandra* leaves extract were within the criteria established by the American National Cancer Institute, which states that, for a crude extract to be considered promising for further work, it must show IC₅₀ < 30 µg/mL. Therefore, the *C. pentandra* leaves could be considered as a good candidate for further work since it demonstrated significantly lower bioactivity against HepG2 (IC₅₀ = 16.3 µg/mL, *p* < 0.001) and RKO (IC₅₀ = 18.7 µg/mL, *p* < 0.01) when compared to 30 µg/mL. The findings also give credence to its use by traditional healers and herbalists for the treatment of cancer-like diseases.

The extract of *C. pentandra* leaves was further fractionated, yielding four fractions which were assessed for their antiproliferative activity. The PE fraction demonstrated the least effectiveness against all four cell lines with IC₅₀ values greater than 50 µg/mL. The most active fraction was the ethyl acetate fraction with appreciable effectiveness (IC₅₀ between 10.7 ± 1.1 µg/mL and 68.62 µg/mL). This is consistent with the work of Orabi and his co-workers, who demonstrated the effectiveness of ethyl acetate extract of *C. pentandra* against diethylnitrosamine-induced hepatocellular carcinoma in rats [47]. One of the most important biological events in the development of malignancies is oxidative stress [48, 49]. It has been shown that the ethyl acetate fraction of *C. pentandra* possesses a very high level of antioxidant activity and can significantly reduce oxidative stress [50]. Therefore, the tremendous antiproliferative effect of the ethyl acetate fraction found in this study may have resulted from its capacity to decrease oxidative stress.

Conclusion

The study concluded that *C. pentandra* leaf extract exhibit high antiproliferative effect with HepG2 and RKO cells being the most susceptible. Extracts of *M. lucida*, *C. pentandra* bark, *T. danielli* leaves and *D. arborea* leaves demonstrated moderate antiproliferative activity while the other plant materials displayed varied antiproliferative activities. The findings of the present study therefore support the indigenous use of these medicinal plants in treating abnormal tumour growth in the various communities in Ghana. Furthermore, the ethyl acetate fraction of *C. pentandra* leaves was found to demonstrate a high antiproliferative effect against HepG2 and Hela cancer cells. It is therefore essential to undertake isolation work on the ethyl acetate fraction in order to further identify the bioactive compounds that are responsible for the antiproliferative activity observed in *C. pentandra* leaves. Identification of bioactive compounds and the determination of their mechanisms of action will help to better understand the therapeutic potential of *C. pentandra* leaves.

Abbreviations

DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
FBS	Foetal bovine serum
IC ₅₀	Half-maximal inhibitory concentration
MTT	Methyl blue thiazole tetrazolium bromide
NEAA	Non-essential amino acids
PBS	Phosphate buffered saline
SD	Standard deviation

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40816-024-00383-w>.

Supplementary Material 1

Supplementary Material 2

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Author contributions

This project was undertaken in partnership by all authors. Authors CKF and GK designed and supervised the entire project. BSA performed the in vitro experiments, analysed the data and wrote the original draft of the manuscript. Author OA prepared the extracts and assisted with the conduct of the in vitro experiments. PAF co-supervised the work and revised the manuscript. SAD designed the in vitro assay protocol, interpreted the data, and assisted with the drafting of the original manuscript. BOE coordinated the project and revised the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

No ethical approval and consent to participate was sought as it is not applicable.

Competing interests

The authors declare no competing interests.

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References

- Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of Cancer. *Mol Cancer Res*. 2023;21(11):1142–7.
- Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, et al. The global burden of cancer 2013. *JAMA Oncol*. 2015;1(4):505–27.
- Ritchie H, Roser M. Causes of Death. Our World in Data. 2019. <https://our-worldindata.org/causes-of-death>
- Chhikara BS, Parang K. Global Cancer statistics 2022: the trends projection analysis. *Chem Biol Lett*. 2023;10(1):1–16.
- Pan L, Chai H-B, Kinghorn AD. Discovery of new anticancer agents from higher plants. *Front Biosci*. 2013;4:142–56. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf>
- Falzone L, Salomone S, Libra M. Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front Pharmacol*. 2018;9(NOV).
- Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis*. 2023;10(4):1367–401.
- Lau PM, Stewart K, Dooley M. The ten most common adverse drug reactions (ADRs) in oncology patients: do they matter to you? *Support care cancer*. 2004;12(9):626–33.
- Singh S, Dhasmana DC, Bisht M, Singh PK. Pattern of Adverse Drug Reactions to Anticancer Drugs: A Quantitative and Qualitative Analysis. *Indian J Med Paediatr Oncol*. 2017;38(2):140–5. <https://pubmed.ncbi.nlm.nih.gov/28900321>
- Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist*. 2019;2(2):141–60.
- Yan H, Wang P, Yang F, Cheng W, Chen C, Zhai B, et al. Anticancer therapy-induced adverse drug reactions in children and preventive and control measures. *Front Pharmacol*. 2024;15(February):1–14.
- Jacques F, Isabelle S, Rajesh D, Sultan E, Colin M, Marise R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 2014.
- Gurnani N, Mehta D, Gupta M, Mehta BK, Natural, Products. Source of Potential Drugs Natural Products Lab, School of Studies in Chemistry & Biochemistry. *Afr J Basic Appl Sci*. 2014;6(6):171–86.
- Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2016;21(5).
- Douglas KA, Li P, Fletcher JN, Chai H. The relevance of higher plants in lead compound Discovery Programs. *J Nat Prod*. 2012;74(6):1539–55.
- Atanasov AG, Waltenberger B, Pferschy-wenzig E. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv*. 2016;33(8):1582–614.
- Gachelin G, Garner P, Ferroni E, Tröhler U, Chalmers I. Evaluating Cinchona bark and quinine for treating and preventing malaria. *J R Soc Med*. 2017;110(1):31–40.
- Bryson A. The prophylactic influence of quinine. *Med Times Gaz*. 1854;8:6–7.
- Tu Y. From Artemisia annua L. to Artemisinins: The Discovery and Development of Artemisinins and Antimalarial agents. Academic; 2017.
- Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA. Plant Antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from camptotheca acuminata 1, 2. *J Am Chem Soc*. 1966;88(16):3888–90.
- Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant Antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. *J Am Chem Soc*. 1971;93(9):2325–7.
- Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J Nat Prod*. 2012;75(3):311–35.
- Sarker SD, Nahar L, Miron A, Guo M. Chapter Two - Anticancer natural products. In: Sarker SD, Nahar LBT-AR in MC, editors. *Medicinal Natural Products: A Disease-Focused Approach*. Academic Press; 2020. pp. 45–75. <http://www.sciencedirect.com/science/article/pii/S0065774320300014>
- Irvine FR. Woody plants of Ghana. Woody plants of Ghana. Oxford University Press; 1961.
- Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi MRA, Ekper JA, et al. Traditional medicine and pharmacopoeia: contribution to the revision of ethnobotanical and floristic studies in Ghana. Organization of African Unity/Scientific, Technical & Research Commission; 2000.
- Agyare C, Spiegler V, Asase A, Scholz M, Hempel G, Hensel A. An ethnopharmacological survey of medicinal plants traditionally used for cancer treatment in the Ashanti region, Ghana. *J Ethnopharmacol*. 2018;212(October):137–52. <https://doi.org/10.1016/j.jep.2017.10.019>
- Appiah-Opang, Regina, Tuifour I, Annor Kwakye, George, Blankson-Darku, Anna Doris, Cramer, Precious, Kissi-Twum, Abena Adomah, Uto, Takuhiro, Ocloo A. Antiproliferative, antioxidant activities and apoptosis induction by morinda lucida and taraxacum of *J Glob Biosci*. 2016;5(November):4281–91
- Ko YS, et al. Anticancer effect of Dracaena arborea leaf extract through down-regulation of VCAM-1 and EMT proteins via suppressing Akt/PKC pathway. *Yakhak Hoeji*. 2018;62(4):203–12.

29. Kumar R, Kumar N, Ramalingayya GV. Evaluation of *Ceiba pentandra* (L.) Gaertner bark extracts for in vitro cytotoxicity on cancer cells and in vivo antitumor activity in solid and liquid tumor models. *Cytotechnology*. 2016.
30. Moskot M, Jakóbkiewicz-Banecka J, Kloska A, Piotrowska E, Narajczyk M, Gabig-Cimińska M. The role of dimethyl sulfoxide (DMSO) in gene expression modulation and glycosaminoglycan metabolism in lysosomal storage disorders on an example of mucopolysaccharidosis. *Int J Mol Sci*. 2019;20(2):1–18. <https://doi.org/10.3390/ijms20020304>
31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1–2):55–63.
32. Suffness M, Pezzuto JM. Methods in plant biochemistry: assays for bioactivity. London: In: Academic; 1990. pp. 71–133.
33. Geran RI, Li G, NH G, MM M. AM S. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. 1972.
34. Deepti K, Amperayani KR, Yarla NS, Parimi UD. In vitro cytotoxic and genotoxic evaluation of *Morinda Tinctoria* Roxb. Leaf Extracts *Pharm Chem J*. 2017;51(4):295–300.
35. Noor A, Gunasekaran S, Vijayalakshmi MA. Article in *Pharmacognosy Research* - October 2017. *Pharmacognosy Res*. 2018;10(October):24–30.
36. Cimanga RK, Kambu K, Tona L, Hermans N, Apers S, Totte J, et al. Cytotoxicity and in vitro susceptibility of *Entamoeba histolytica* to *Morinda morindoides* leaf extracts and its isolated constituents. *J Ethnopharmacol*. 2006;107(1):83–90.
37. Alitheen NB, Manaf AA, Yeap SK, Shuhaimi M, Nordin L, Mashitoh AR. Immunomodulatory effects of *damnacanthal* isolated from roots of *Morinda Elliptica*. *Pharm Biol*. 2010;48(4):446–52.
38. Al-Afifi NA, Alabsi AM, Shaghayegh G, Ramanathan A, Ali R, Alkoshab M, et al. The in vitro and in vivo antitumor effects of *Dracaena cinnabari* resin extract on oral cancer. *Arch Oral Biol*. 2019;104(June):77–89.
39. Liu J, Mei W-L, Wu J, Zhao Y-X, Peng M, Dai H-F. A new cytotoxic homoisoflavonoid from *Dracaena cambodiana*. *J Asian Nat Prod Res*. 2009;11(2):192–5.
40. Kuete V, Djeussi DE, Mbaveng AT, Zeino M, Efferth T. Cytotoxicity of 15 Cameroonian medicinal plants against drug sensitive and multi-drug resistant cancer cells. *Complement Altern Med*. 2013;186:196–204.
41. Chinedu SN, Iheagwam FN, Anichebem CJ, Ogunnaike GB, Emiloju OC. Antioxidant and biochemical evaluation of *Thaumatococcus daniellii* seeds in rat. *J Biol Sci*. 2017;17:8.
42. Iwueke AV, Ejekwumadu NJ, Chukwu EC, Nwodu JA, Akalonu C. Nutritional composition and GC-MS phytochemical analysis of *Thaumatococcus daniellii* leaves. *Eur J Nutr Food Saf*. 2020;81–6.
43. Chinedu SN, Iheagwam FN, Makinde BT, Thorpe BO, Emiloju OC. Data on in vivo antioxidant, hypolipidemic and hepatoprotective potential of *Thaumatococcus daniellii* (Benn.) Benth leaves. *Data Br*. 2018;20:364–70.
44. Fyhrquist P, Mwasumbi L, Vuorela P, Vuorela H, Hiltunen R, Murphy C, et al. Preliminary antiproliferative effects of some species of *Terminalia*, *Combretum* and *Pteleopsis* collected in Tanzania on some human cancer cell lines. *Fitoterapia*. 2006;77(5):358–66.
45. Spiegler V, Greiffer L, Jacobtorweihen J, Asase A, Lanvers-Kaminsky C, Hempel G et al. In vitro screening of plant extracts traditionally used as cancer remedies in Ghana – 15-Hydroxyangustilobine A as the active principle in *Alstonia boonei* leaves. *J Ethnopharmacol*. 2021;265(September):113359. <https://doi.org/10.1016/j.jep.2020.113359>
46. Abouelela ME, Orabi MAA, Abdelhamid RA, Abdelkader MSA. Chemical and Cytotoxic Investigation of Non-polar Extract from *Ceiba Pentandra* (L.) Gaertn : a study supported by computer based screening. *J Appl Pharm Sci*. 2018;8(07):57–64.
47. Orabi MAA, Abouelela ME, Darwish FMM, Abdelkader MSA, Elsadek BEM, Al Awadh AA, et al. *Ceiba pentandra* ethyl acetate extract improves doxorubicin antitumor outcomes against chemically induced liver cancer in rat model: a study supported by UHPLC-Q-TOF-MS/MS identification of the bioactive phytomolecules. *Front Pharmacol*. 2024;15(February):1–15.
48. Jiang H, Zuo J, Li B, Chen R, Luo K, Xiang X et al. Drug-induced oxidative stress in cancer treatments: Angel or devil? *Redox Biol*. 2023;63:102754. <https://www.sciencedirect.com/science/article/pii/S2213231723001556>
49. Arfin S, Jha NK, Jha SK, Kesari KK, Ruokolainen J, Roychoudhury S, et al. Oxidative stress in cancer cell metabolism. *Antioxidants*. 2021;10(5):1–28.
50. Abouelela ME, Orabi MAA, Abdelhamid RA, Abdelkader MS, Madkor HR, Darwish FMM et al. Ethyl acetate extract of *Ceiba pentandra* (L.) Gaertn. reduces methotrexate-induced renal damage in rats via antioxidant, anti-inflammatory, and antiapoptotic actions. *J Tradit Complement Med*. 2020;10(5):478–86. <https://www.sciencedirect.com/science/article/pii/S2225411019300598>

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