Journal of Ethnopharmacology 341 (2025) 119363

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

A bioactive fraction from the leaves of *Ceiba pentandra* (L.) Gaertn. exhibits antiproliferative activity via cell cycle arrest at the G1/S checkpoint and initiation of apoptosis via poly [ADP-ribose] polymerase 1 (PARP1) cleavage in HeLa cells.

Bright Selorm Addy [a,b,*], Caleb Kesse Firempong [a], Gustav Komlaga [c], Patrick Addo-Fordjour [d], Seth Agyei Domfeh [a], Oluwatomisin Deborah Afolayan [a], Eric Nana Yaw Nyarko [e], Benjamin Obukowho Emikpe [f]

[a] Department of Biochemistry and Biotechnology, College of Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[b] Department of Pharmaceutical Sciences, School of Pharmacy, Central University, Accra, Ghana
[c] Department of Pharmacognosy, College of Health Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[d] Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana
[e] Department of Chemical Pathology, University of Ghana Medical School, University of Ghana, Accra, Ghana
[f] Department of Veterinary Pathology, School of Veterinary Medicine, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

ARTICLE INFO

Handling Editor: V Kuete

Keywords:
Antiproliferative activity
cell cycle arrest
mechanism of action
PARP1 cleavage
Cdk2
G1/S checkpoint
apoptosis
Ceiba pentandra

ABSTRACT

Ethnopharmacological relevance: Ceiba pentandra (L.) Gaertn. (Malvaceae) has been used in Africa traditionally to manage a variety of illnesses, including cancer. The hydroethanolic extract of the leaves of *C. pentandra* has been shown to possess antiproliferative activity. However, the fractionation of antiproliferative bioactive constituents from the leaves of *C. pentandra* and the determination of the mechanisms of action of such bioactive constituents remain unexplored.

Aim of the study: This work sought to fractionate the extract of *C. pentandra* leaves, establish the antiproliferative activity of the fractionated constituents, and determine the active constituents' possible mechanisms of action.

Material and methods: Chromatographic techniques were used to fractionate bioactive constituents from *C. pentandra* leaves. The fractionated constituents were evaluated for their antiproliferative activity against four cancer cell lines (viz hepatocellular carcinoma, colorectal adenocarcinoma, cervical carcinoma, and mammary adenocarcinoma) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT)-based assay. The possible mechanisms of action of the active constituent, Fraction A (IsoA), were also determined via western blot analysis.

Results: Six constituents were fractionated from the leaves of *C. pentandra*. Among the six constituents, IsoA stood out for its remarkable antiproliferative activity across the four cancer cell lines, with hepatocellular carcinoma (HepG2) cells being the most affected. With half-maximal inhibitory concentration (IC_{50}) values ranging from 6.4 ±1.2 μg/mL to 19.2±3.4 μg/mL, IsoA demonstrated great potential in inhibiting cancer cell proliferation. Notably, IsoA's mechanisms of action involve critical molecular targets associated with cell cycle regulation and apoptosis. It significantly increased the levels of phosphorylated cyclin-dependent kinase 2 (Cdk2 pTyr15), a key regulator of cell cycle arrest, and cleaved poly [ADP-ribose] polymerase 1 (PARP1), a hallmark of apoptosis initiation. These findings underscore the therapeutic potential of IsoA in cancer treatment.

Conclusions: IsoA demonstrated highly promising *in vitro* antiproliferative activity by effectively arresting the cell cycle at the G1/S checkpoint, halting cancer cell proliferation. Additionally, IsoA induced programmed cell death (apoptosis) through mechanisms such as PARP1 cleavage, highlighting its potential as a candidate for cancer therapy.

* Corresponding author. Central University, P.O Box 2305, Tema, Ghana.
E-mail address: brightaddy@gmail.com (B.S. Addy).

https://doi.org/10.1016/j.jep.2025.119363
Received 9 August 2024; Received in revised form 2 January 2025; Accepted 11 January 2025
Available online 13 January 2025

1. Introduction

Ceiba pentandra (L.) Gaertner, known as kapok or white cotton tree (plant name has been checked with https://www.worldfloraonline.org/), is a large tree of the Malvaceae family (Tareau et al., 2021). This plant is widely recognised among traditional African healers for managing various medical conditions (Aloke et al., 2010; Osuntokun et al., 2017; Sarfo et al., 2022).

This plant has also been used to manage cancer-related conditions such as tumours and sores. For example, the Tupuri people of Mindif in Cameroon use crushed fresh leaves for wound dressing and bandaging tumours or abscesses (Nkouam et al., 2017). However, there is a dearth of scientific data to corroborate their folkloric use for the management of cancer-like conditions. Our laboratory, therefore, recently validated the use of the leaves of *C. pentandra* in traditional medicine for the management of tumours (Addy et al., 2024), thereby heightening the interest in exploring further the antiproliferative value of the leaves of *C. pentandra.* Current studies on *C. pentandra* leaves lack comprehensive data on its fractionation, the evaluation of the antiproliferative properties of the fractions and the underlying mechanisms of action of the potential bioactive fractions. This study, therefore, addresses these gaps by employing bioassay-guided fractionation to identify bioactive fractions, investigating the antiproliferative activities of the fractions and mechanisms of action using cell viability assay and western blot, respectively.

Bioassay-guided fractionation is essential in drug discovery and development because it allows for the rational separation and identification of bioactive constituents from complex mixtures (Mani et al., 2022). This technique is crucial when dealing with natural products, which are often made up of many chemical constituents (Atanasov et al., 2021). The bioactive agents of interest can be obtained and concentrated by systematically fractionating these mixtures, making the determination of their specific biological activities easier.

Additionally, understanding the precise mechanisms by which these antiproliferative bioactive agents exhibit their activities provides valuable insights into cancer biology and could potentially lead to the development of targeted therapies (Kumar et al., 2023). Assessment of intracellular pathways offers a good way to determine the mechanism of action because intracellular signalling pathways are essential regulators of internal cellular activities, such as cell proliferation, maintenance, and cell death (Valdespino-Gómez et al., 2015). Therefore, understanding these pathways is essential for gaining insight into the mechanisms of action of antiproliferative agents, which are usually designed to promote cell death and or inhibit cell growth and division. In this regard, cell cycle arrest (Doan et al., 2019; Gao et al., 2024a; Lin et al., 2018) and initiation of apoptosis (Martí et al., 2020; Mashimo et al., 2021) are two mechanisms that have piqued the curiosity of researchers looking for new strategies to combat cancer.

Uncontrolled cell cycle progression is a crucial event in cancer development and progression. The induction of cell cycle arrest at the G1/S phase in various cancer cell lines has been demonstrated by several researchers to be a valuable process by which anti-cancer drugs function (Chang et al., 2015; Doan et al., 2019; Duronio & Xiong, 2013; Matthews et al., 2022). This is because the arrest of the cell cycle at the G1/S checkpoint leads to the suppression of cell proliferation and the prevention of tumour growth (Lee et al., 2019; Matthews et al., 2022), making it a promising therapeutic strategy for cancer treatment.

Furthermore, apoptosis, a form of programmed cell death is fundamental to tissue homeostasis and the elimination of damaged cells (Berridge, 2014; Park et al., 2023). Dysregulation of apoptosis plays a significant role in cancer development (Id & Chaudhry, 2019; Zhong Xu et al., 2016). Therefore, initiating apoptosis is an essential approach to destroying cancer cells. The cleaving or inhibition of poly [ADP-ribose] polymerase 1 (PARP1) is a critical marker of apoptosis (Chaitanya et al., 2010; Mashimo et al., 2021). Poly (ADP-ribose) polymerase 1 is an enzyme that plays a key role in DNA repair. Inhibition or cleavage of PARP1 has emerged as a promising strategy in cancer therapy, specifically in DNA damage repair deficiency (Jain & Patel, 2019). Activation of PARP1 cleavage triggers a cascade of events leading to cell death, presenting an attractive avenue for targeted cancer therapy.

Although the ethanolic extract of *C. pentandra* leaves and its ethyl acetate fraction have been shown to inhibit cell proliferation, the specific constituents responsible for these antiproliferative activities and the mechanisms of action remain unexplored. Therefore, this research aims to further fractionate the leaves of *C. pentandra*, evaluate the constituents' antiproliferative potential, and elucidate the mechanisms of action of the most active constituent.

2. Materials and methods

2.1. Materials and reagents

Cell cycle and apoptosis western blot cocktail (cat # ab139417) was purchased from Abcam, UK. Products obtained from ThermoFisher Scientific, Massachusetts, USA include Radioimmunoprecipitation assay (RIPA) buffer (cat # 89900), Protease and phosphatase inhibitor cocktail (cat # 78440), ethylenediaminetetraacetic acid (cat # 17892) and Pierce ECL western blotting substrate with cat # 32109. Also utilised were 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) with cat # M5655-1G and 2-β-mercaptoethanol (cat # 1610710) obtained from Bio-Rad, California, USA and Sigma Aldrich, St. Louis, USA, respectively. Penicillin (cat # 15140122), streptomycin (cat # 15140122) and Dulbecco's modified eagle medium (DMEM) (cat # 11885084) were purchased from Gibco Life Technologies Ltd., Paisley, UK. Nonessential amino acids (NEAA) (cat # 0823) and dimethylsulfoxide (DMSO) (GK2245-500ML) were obtained from ScienCell, Carlsbad, USA and Gletham Life Sciences, UK, respectively while foetal bovine serum (# 30-2020) was obtained from ATCC, USA. The manufacturers' guidelines were followed for preparing all chemicals and reagents.

2.2. Plant collection and extraction

The collection and extraction of the leaves of *C. pentandra* have been described in our previous study (Addy et al., 2024). Briefly, the plant material was collected from Kwahu Bepong in the Eastern Region of Ghana, authenticated, and a specimen with voucher number (KNUST/HMI/2020/L015) was deposited. The plant material was rinsed with tap water and air-dried at room temperature for three weeks. The dried plant material was then extracted with 80 % ethanol. The filtrate was then concentrated using a rotary evaporator (R-114, Buchi, Switzerland). Drawell freeze drier (DW-10 N) was used to concentrate the sample further by removing the water. The extract was then fractionated using the modified Kupchan method. Firstly, the extract was suspended in water and then extracted with petroleum ether. The resultant water fraction was further extracted sequentially with ethyl acetate (EtOAc) and n-butanol (n-BuOH). The EtOAc fraction previously established to possess the highest antiproliferative activity was further fractionated using column chromatography.

2.3. Fractionation using column chromatography

A chromatographic glass column (2 × 50 cm) preloaded with a slurry of Si-60 silica gel (40–63 μm, Merck) and petroleum ether as the stationary phase was loaded with the EtOAc fraction (260 g) and gradient eluted with pet ether, EtOAc and MeOH in increasing order of polarity. Beginning with 100% pet ether, a solvent ratio of pet ether to EtOAc was varied (9:1, 8:2, 7:3, 6:4–1:9, v/v) as the columns were progressively run until 100% EtOAc and then a mixture of EtOAc and MeOH (99:1–90:10) was used for the final elution. For each solvent ratio, the column was run until the eluate was clear. Volumes of 50mL eluates were collected in test tubes. Based on the thin layer chromatography profile of the

fractions, they were bulked together to obtain six fractions labelled fractions A (0.98 mg), B (1.6 mg), C (0.83 mg), D (1.1 mg), E (1.7 mg), and F (1.3 mg). The fractions were concentrated to dryness using rotary evaporation under vacuum. The resulting residues were dried and stored at −4 °C until use.

2.4. Cell culture

All cell lines used, including human mammary adenocarcinoma (MCF-7), hepatocellular carcinoma (HepG2), colorectal adenocarcinoma (RKO), human cervical carcinoma (HeLa) cells, were kindly 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. A working cell culture medium was prepared by supplementing DMEM with 10% heat-inactivated foetal bovine serum (FBS), 1% NEAA, and 1% Penicillin-Streptomycin. The cells were grown in the DMEM working medium in a humidified incubator with 5% carbon dioxide (CO_2) set at 37 °C.

2.5. Antiproliferative study

For each cell line, cells were seeded in 96-well plates at 4×10^4 cells per well and incubated at 37 °C and 5% CO_2 under humid conditions for 24 h to allow for attachment. Stock test solutions (1 mg/mL) of the fractionated constituents and the positive control, 5-fluorouracil (5FU), were prepared in 1% DMSO. A 20 μg/mL concentration of the test solution was subsequently prepared by diluting the stock solution with the working medium. The serial dilution method was used to prepare the other concentrations (from 10 μg/mL to 1.25 μg/mL) using the working medium. The positive control, 5-fluorouracil (5FU) solution, was prepared using a similar approach. The negative control was prepared by adding 2 μl of DMSO to 1998 μl of DMEM to make a 0.1% DMSO solution, which has been established to produce no effect on the growth of cells (Moskot et al., 2019). After removing the old media, the cells were treated by incubation in freshly prepared test substances (Fractions A, B, C, D, E, and F) and the positive control at triplicate concentrations. Following a 72-hour incubation period, each well received 20 μl of MTT reagent and then incubated for another 3 h. The supernatant was then aspirated from each well, and 120 μl of absolute isopropanol was added, with three of the wells serving as blanks. The plate was then incubated for 30 min for the formazan crystals to dissolve. The absorbance was read on a Bio-Rad iMark Microplate Reader with the measurement filter set at 595 nm. The procedure was repeated two more times. Percentage cell viability values of test substances and 5FU were computed from the absorbance values using the following formula:

$$\textit{Percentage cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100$$

The % cell viability values were then used to determine the half-maximal inhibitory concentration (IC_{50}) values of test substances and 5FU. Fractions with IC_{50} values ≤ 10 μg/mL were considered for further probing.

2.6. Western blot analysis

To understand the possible mechanisms of action of IsoA, the western blotting technique was used to assess the concentrations of cleaved-PARP1 and Cdk2 pTyr15 proteins. Firstly, HeLa cells were cultured, prepared, and treated as previously described with slight modification. Briefly, a 6-well plate was used for seeding cells at 5 x 10^5 cells per well and incubated for 24 h to allow for attachment. The negative control, different concentrations (5.2, 10.4 and 15.6 μg/mL) of IsoA, and the positive control were prepared using reconstituted DMEM media. The old medium on the cells was discarded, and 2 mL of each treatment was added to the appropriate well in triplicate. After treating the cells for 24 h, proteins were extracted as instructed by the manufacturer. Total protein was extracted with an ice-cold RIPA buffer containing ethylenediaminetetraacetic acid (EDTA), phosphatase, and protease inhibitor cocktail. The sample buffer containing 2-beta mercaptoethanol was used to denature the extracted proteins. The denatured proteins were resolved using a 4 - 20% gradient polyacrylamide gel and then transferred to a polyvinylidene difluoride (PVDF) membrane. Non-fat milk (5%) in 1× Tris-buffered saline with Tween-20 (TBST) was used to block the PVDF membrane. A cocktail of specific primary antibodies prepared (1:250) in 3% non-fat dry milk in 1X TBST was used to investigate the target proteins (beta-actin, phosphorylated Cdk2, phosphorylated histone, and cleaved-PARP1). The primary antibodies were allowed to bind to the PVDF membrane for eighteen hours at 4 °C. Afterward, the PVDF membrane was probed by incubating at room temperature for 60 min using a secondary antibody cocktail of HRP conjugated antibodies (anti-mouse and anti-rabbit) (1:2500). Using Pierce ECL western blotting substrate, enhanced chemiluminescence was employed to illuminate the bands. A blot scanner (C-DIGIT) by Li-COR Bioscience, USA, was used to acquire the images. ImageJ software (National Institute of Health) was then employed to analyse the data.

2.7. Statistical 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 fractions to cells cultured in the absence of fractions (cells were mock-treated with a working culture medium). Analysis of variance (ANOVA) was employed to analyse the data where applicable. Tukey's multiple comparison test was used to perform a post hoc test where there was a difference. Differences in the results were deemed statistically significant when $p < 0.05$.

3. Results

3.1. Fractionation of bioactive constituents

Previous results from the evaluation of medicinal plants as antiproliferative agents *in vitro* led us to establish the antiproliferative activity of the ethanolic extract and ethyl acetate fraction of *C. pentandra* leaves, necessitating further fractionation and determination of the antiproliferative activity of the fractionated constituents. The fractionation process produced six constituents labelled as Fractions A – F, which were evaluated for their antiproliferative activities.

3.2. Antiproliferative activity

The study investigated the antiproliferative effects of six fractionated constituents from *C. pentandra* leaves. The inhibition of cell growth by the six fractions is shown in Figure 1 below as representative graphs. Fraction A (IsoA) demonstrated the highest efficacy among the tested fractions, showing potent antiproliferative activity across four distinct cell lines, with cell viability reduced to below 10%. Conversely, Fractions B, D, E, and F exhibited limited growth inhibition, with cell viability levels remaining above 70% even at the highest treatment concentration of 20 μg/mL, indicating weak inhibitory effects. Fraction C showed moderate antiproliferative effects at the same concentration, reducing cell viability to below 50%. These results suggest that IsoA possesses significant antiproliferative capabilities, outperforming the other fractions and effectively inhibiting cell proliferation. In contrast, Fractions B, D, E, and F show minimal efficacy, with Fraction C providing only moderate inhibitory effects, underscoring the potential of IsoA as a good candidate for further antiproliferative research.

The calculated IC_{50} values are presented in Table 1 below. Four constituents (fractions B, D, E and F) demonstrated varied low activities. Their half-maximal inhibitory concentration (IC_{50}) values ranged between 32.1±1.0 and 94.6±8.8. In contrast, Fraction C reduced the

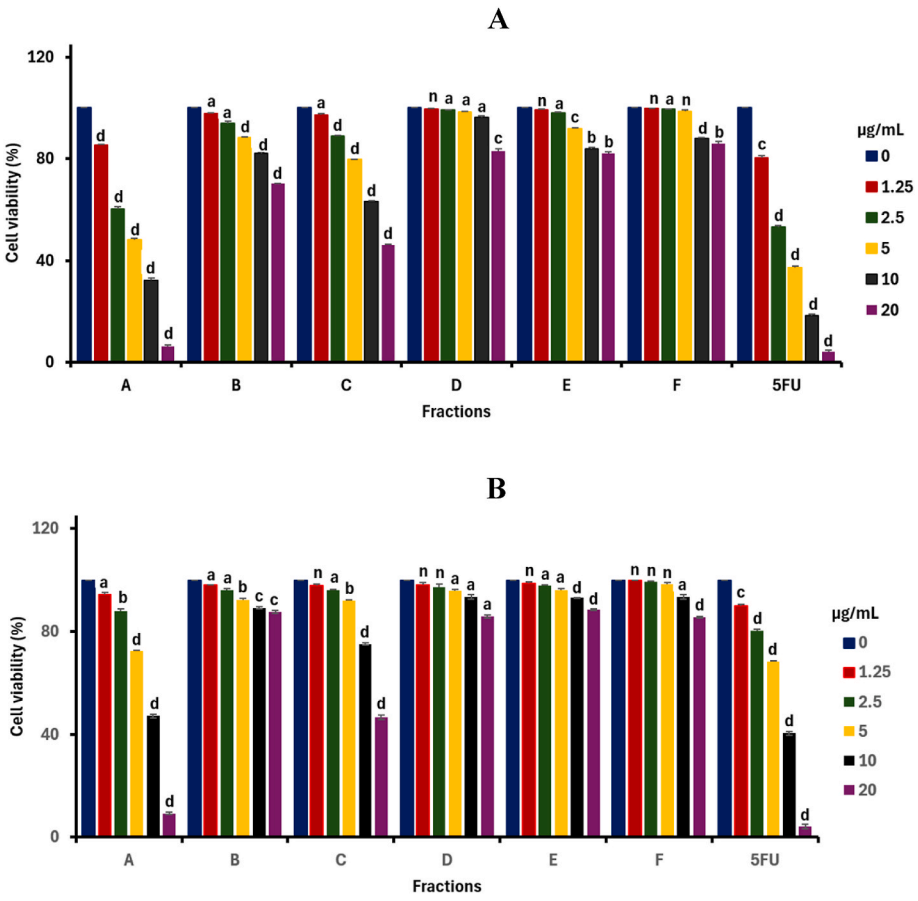

Figure 1. Inhibitory effects of *C. pentandra* fractions on HepG2 (A) and HeLa (B) cells. The cells were treated with fractions (1.25 – 20 µg/mL), and the cell viability was assessed at 72 h using an MTT-based assay. Data are presented as the means, with the standard deviations as error bars, from three experiments conducted in triplicate. [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$, and [d]$p < 0.0001$ correspond to significant differences between the untreated and treated cells as calculated by one-way ANOVA and Tukey's test, n: difference not significant. The comparisons within the treatments are indicated in the supplementary data S2. **5FU:** 5-Fluorourail.

Table 1
IC_{50} values of chromatographic fractions of *C. pentandra* leaves on a panel of cancer cell lines

Treatment	IC_{50} values (µg/mL ± SD)[a]			
	HepG2	Hela	RKO	MCF-7
Fraction A	6.8±1.2	10.4±0.6	13.4±0.3	19.2±3.4
Fraction B	32.1±1.0	56.8±1.2	76.8±7.7	66.7±7.4
Fraction C	16.4±3.1	19.2±0.7	24.5±1.2	21.3±2.9
Fraction D	53.7±2.6	76.0±5.6	78.1±4.2	92.5±10.4
Fraction E	41.4±7.2	89.7±7.5	46.9±3.2	94.6±8.8
Fraction F	37.4±5.3	56.7±18.7	87.3±8.1	49.9±6.2
5FU	5.1±0.4	9.8±0.5	11.5±1.3	3.4±0.2

[a] IC_{50} values are presented as means and standard deviations of three independent experiments, with each experiment conducted in triplicate.

proliferation (IC_{50} = 16.4±3.1, 19.2±0.7, 24.5±1.2 and 21.3±2.9) for HepG2, Hela, RKO and MCF-7, respectively. The highest bioactivity was displayed by IsoA in all four cell lines with IC_{50} = 6.8±1.2, 10.4±0.6, 13.4±0.3 and 19.2±3.4 for HepG2, Hela, RKO and MCF-7 respectively. Due to its remarkable antiproliferative activity, IsoA has subsequently been partially characterised using NMR data showing that IsoA has a tetraterpenoid nucleus (Supplementary Data S1). Characterising the structure was essential because the structural features of natural bioactive agents are closely linked to their biological activities (Barba-Ostria et al., 2022; Gao et al., 2024b).

3.3. Mechanisms of action

The protein concentrations of cleaved-PARP1, Cdk2 pTyr15, and H3 pSer10 in HeLa cells were determined, with beta-actin (ACTB) as the loading control. As indicated in Figure 2A, IsoA and the positive control caused an observable increase in the concentration of cleaved-PARP1, while no band was observed for the negative control. Similarly, as depicted in Figure 2B, there were elevated levels of Cdk2 pTyr15 in the cells that received 5.2 µg/mL, 10.4 µg/mL and 15.6 µg/mL of IsoA compared to the untreated cells. However, no band was observed for Histone H3 pSer10 for any of the samples (treated and untreated).

Figure 3 illustrates the proposed mechanism of action of IsoA. The elevated levels of phosphorylated Cdk2 (Cdk2 pTyr15) and cleaved PARP1 led to the arrest of the cell cycle and the initiation of apoptosis, respectively. IsoA produced an inhibitory effect on PARP1 by fragmenting it, resulting in irreversible commitment of cells to undergo apoptosis. Additionally, the elevation of Cdk2 pTyr15 caused the dissociation of cyclin E from Cdk2, rendering Cdk2 inactive. The inactivation of Cdk2 leaves retinoblastoma (Rb) unphosphorylated and, hence, free to bind E2F. This prevents E2F from migrating into the nucleus to transcribe protein factors required for the cell cycle progression. The absence of these factors causes the arrest of the cell cycle.

4. Discussion

As medicinal plants continue to emerge as good sources of bioactive agents against many ailments, including cancer, the fractionation of

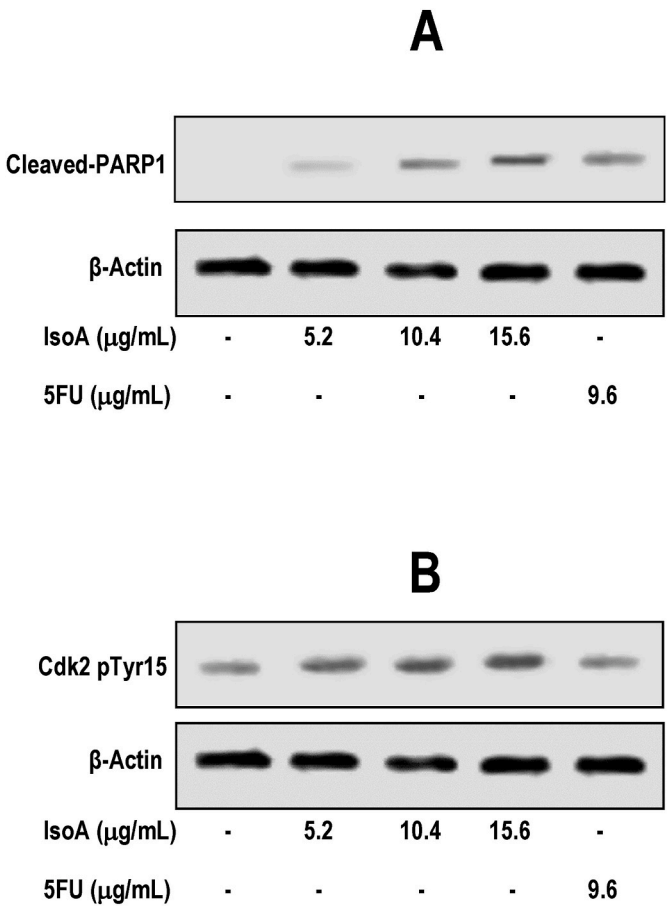

Figure 2. Cleaved-PARP1 and Cdk2 pTyr15 levels in the presence of IsoA in HeLa cells. The cells were cultured in the presence of IsoA and 5-fluorouracil (5FU) as an experimental control. The proteins were assessed after 24 h by Western blot using the Cell Cycle and Apoptosis Western Blot Cocktail (Abcam). The blotted PVDF membrane was developed using the chemiluminescence western blotting reagents. The protein bands were acquired using the C-DIGIT blot scanner.

bioactive candidates, determination of the activities of the fractionated constituents and the possible mechanisms of action are needed.

The current study reports findings on the antiproliferative activity of constituents fractionated from *C. pentandra* leaves. The six fractions demonstrated varied activities against the panel of cancer cells used. The most significant activity was demonstrated by IsoA against the panel of cancer cell lines, highlighting its potential as a therapeutic agent. The IC_{50} values of IsoA, which ranged from 6.4±1.2 μg/mL to 19.2±3.4 μg/mL, points to its bioactivity, particularly against HepG2 and HeLa cancer cells. The IC_{50} values demonstrated by IsoA suggest a potent antiproliferative effect across various cancer cell lines. Particularly, the lowest IC_{50} value of 6.4±1.2 μg/mL and 10.4 μg/mL against HepG2 and HeLa cells, respectively, signifies a high sensitivity, therefore positioning IsoA as a promising candidate for further investigation in hepatocellular carcinoma and cervical cancer therapy.

IsoA demonstrates lower IC_{50} values than many terpenoids with antiproliferative activity (Ramteke et al., 2021). For example, terpenoids such as linalool and limonene have been shown to produce IC_{50} values in the range of 20-50 μg/mL against different cancer cell lines (Alipanah et al., 2021). Similarly, terpenoids such Actein (IC_{50} = 27 μg/mL), Astragaloside IV (IC_{50} = 150-200 μg/mL), Escin (IC_{50} = 10-60 μg/mL), and Betulinic acid ($IC_{50} \leq 50$ μg/mL), etc. have been shown to demonstrate higher IC_{50} values (Einbond et al., 2009; Miao et al., 2008; Qi et al., 2010; Rabi & Bishayee, 2009; Zhou et al., 2009) compared to IsoA. This comparative advantage of IsoA highlights its potential as an effective antiproliferative agent within the terpenoid class. Furthermore, since the structural diversity of terpenoids can influence their bioactivity (Ghosh, 2020; Li & Tao, 2024), the unique structure of IsoA may contribute to its enhanced antiproliferative properties. Unlike monoterpenoids (e.g., linalool, limonene) and triterpenoids (e.g., Actein, Astragaloside IV, Escin, Betulinic acid), IsoA's classification as a tetraterpenoid suggests a unique molecular configuration, including extended conjugated functional groups that may enhance interactions with cancer-specific targets. These structural features likely contribute to IsoA's ability to efficiently disrupt cancer cell proliferation through different mechanisms (Câmara et al., 2024; Koklesova et al., 2020), making it a promising candidate for anticancer therapy. The mechanistic pathways through which IsoA exerts its effects could, therefore, also differ from other terpenoids, offering potential synergistic effects when combined with other chemotherapeutic agents since studies have shown that combining different terpenoids can lead to enhanced anti-cancer effects through complementary mechanisms of action (Castañeda et al., 2022; Kłos & Chlubek, 2022). This could consequently serve as a launchpad for developing novel and more effective cancer treatment strategies.

Figure 2A shows no band for cleaved-PARP1 in the untreated cells that received neither IsoA nor 5FU, indicating the absence of cleaved-PARP1 in the untreated cells. In contrast, cleaved-PARP1 was present in the cells exposed to the various concentrations of IsoA and 9.6 μg/mL

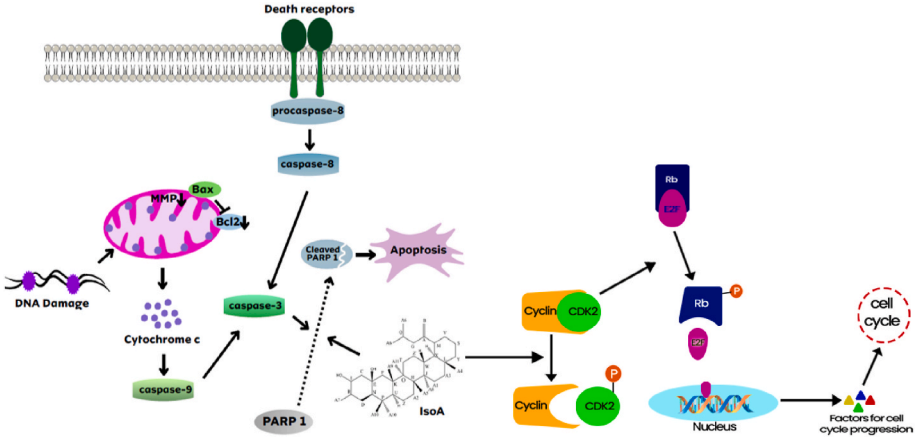

Figure 3. Proposed mechanisms of action of IsoA from WB results. IsoA induces the breaking of PARP1, which leads to irreversible commitment to apoptosis. The phosphorylation of Rb by cyclin-bound Cdk2 causes the release of E2F, which migrates to the nucleus to induce transcription factors for cell cycle progression. The phosphorylation of Cdk2 by IsoA prevents the binding of cyclins, thereby inhibiting the action of Cdk2. This prevents the release of E2F, leading to the arrest of the cell cycle.

of 5FU. Poly [ADP-ribose] polymerase 1 inhibition has been considered a potential cancer treatment strategy (Mergui-roelvink et al., 2009). This stems from the fact that a functioning PARP1 helps to drive tumour progression (Martí et al., 2020; Schiewer et al., 2012). The results of the WB analysis in the present study indicate that IsoA causes cell death through the induction of apoptosis via caspase-activated cleavage of PARP1. Cleavage of PARP1 upon caspase activation during apoptosis is an established process, and the generation of an 89 kDa fragment during catalytic cleavage is a characteristic of apoptotic cell death (Kaufmann, 1989; Kaufmann et al., 1993). Upon cleavage, the 89 kDa fragments translocate from the nucleus to the cytoplasm, activating the apoptosis-inducing factor (AIF) (Mashimo et al., 2021). In contrast, the necrotic cleavage of PARP1 results in the generation of a 50 kD fragment rather than an 89 kD fragment (Gobeil et al., 2001). The results from the present study are similar to findings from other studies showing that PARP inhibition or cleavage plays a crucial role in the anti-cancer effect of active agents through their involvement in the induction of apoptosis. Olaparib, a breast and ovarian cancer medication (Jones, 2010), for example, was shown to inhibit PARP1 by 90% compared to the baseline value in ovarian cancer patients (Mergui-roelvink et al., 2009), while cucurbitacin B (CuB) and its loaded nanoparticle (CuB-NP), both of which possess strong anti-cancer benefits (Chen et al., 2012), were found to cause the induction of apoptosis through PARP cleavage in breast adenocarcinoma (HTB-26) cells (Bakar-Ates et al., 2020). Inotodiol, a triterpenoid that has incredible activity, was also reported to induce apoptosis through a p53-dependent pathway, leading to the activation of caspase-3 and the subsequent PARP1 fragmentation in cervical cancer (HeLa) cells (Zhang et al., 2019).

Concerning H3 pSer10, no band was present or observed for phosphorylated histone 3 in any of the treatments. This implies that IsoA and 5FU do not induce cell cycle arrest at M-phase because phosphorylation of serine residues (Ser10) in histone H3 points to highly condensed chromosomes during mitosis with the cell cycle is unable to continue (Ramos-Alonso et al., 2017).

Excessive proliferative signalling caused by the dysregulation of the cell cycle machinery, especially at the G1/S transition phase, has long been acknowledged as one of the six hallmarks of cancer cells (Fouad & Aanei, 2017). Therefore, there is an ignited interest in exploring the susceptibility of Cdk2 to bioactive agents that are potential antitumour agents because of their essential role in regulating the cell cycle (Tadesse et al., 2020). The present study revealed the elevation of Cdk2 pTyr15 by IsoA at various concentrations. This indicates that IsoA halts cell cycle progression at the G1/S phase. This is because the increase in Cdk2 pTyr15 is a hallmark of cell cycle arrest at the G1/S phase. Phosphorylation of Cdk2 at Tyr15 and/or Thr14 inhibits the activity of Cdk2 and causes a halt in cell cycle progression at the G1/S phase. This finding is consistent with other works that established the targeting of Cdk2 as the means of anti-cancer effects of bioactive agents. Tyagi and his colleagues, for example, demonstrated that the anti-cancer agent silibinin exerts its antiproliferative effect by inhibiting Cdk2, among others, which causes the hypophosphorylation of Rb/p107 and Rb/p130 in DU145 (human prostate cancer) cells (Tyagi et al., 2002).

Finally, while the present study offers valuable insight into the antiproliferative potential of IsoA and its mechanisms of action, it is important to recognize some notable limitations. The first limitation is the limited structural characterization of IsoA. Comprehensive identification and quantification of IsoA using techniques such as MS, GC-MS, FTIR, etc., could not be undertaken due to time and funding constraints, limiting the detailed understanding of IsoA's composition and bioactivity. Another limitation is the restricted mechanistic insights. Advanced techniques such as flow cytometry and Western blot analysis of a larger panel of protein markers (e.g., caspases, Bcl-2 family proteins, cyclins, and CDKs) were not employed due to resource limitations, restricting the depth of exploration into IsoA's apoptotic pathways and cell cycle modulation. Further research addressing these limitations will enhance our understanding and provide a more comprehensive overview of IsoA's effects as a good anti-cancer candidate.

5. Conclusion

This study highlights the novel antiproliferative potential of IsoA, a bioactive fraction derived from the leaves of *Ceiba pentandra*, particularly against HepG2 and HeLa cancer cell lines. IsoA's dual mechanisms of action underscore its therapeutic promise, halting the cell cycle at the G1/S checkpoint through upregulation of Cdk2 pTyr15 and inducing apoptosis via PARP1 cleavage. These findings provide strong evidence of IsoA's capability to disrupt cancer cell proliferation through simultaneous cell cycle regulation and apoptosis initiation. This dual mechanism not only distinguishes IsoA as a unique antiproliferative agent but also emphasizes its potential for advancing cancer treatment strategies.

CRediT authorship contribution statement

Bright Selorm Addy: Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation. **Caleb Kesse Firempong:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Gustav Komlaga:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Patrick Addo-Fordjour:** Writing – review & editing, Supervision, Project administration. **Seth Agyei Domfeh:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Oluwatomisin Deborah Afolayan:** Writing – review & editing, Investigation, Data curation. **Eric Nana Yaw Nyarko:** Writing – review & editing, Visualization, Validation, Software, Investigation. **Benjamin Obukowho Emikpe:** Writing – review & editing, Validation, Supervision, Conceptualization.

Ethics approval

This study did not require ethical approval.

Funding

This project was funded by the authors with support from the KNUST Research Fund (KReF), Ghana.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

The authors thank Prof. Mohamed Mutocheluh for donating the cell lines and granting access to his cell culture laboratory. We also thank Dr. Patrick Narkwa for his support during the cell culture experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2025.119363.

Glossary

ACTB	Beta actin
AIF	Apoptosis-inducing factor
ANOVA	Analysis of variance
Cdk2 pTyr15	Phosphorylated Cdk2 (phosphotyrosine 15)
Cdk2	Cyclin-dependent kinase 2
CO_2	Carbon dioxide
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
ECL	Enhanced Chemiluminescence

EDTA	Ethylenediaminetetraacetic acid
EtOAc	Ethyl acetate
FBS	Foetal bovine serum
IC_{50}	Half-maximal inhibitory concentration
MeOH	Methanol
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
n-BuOH	n-Butanol
NEAA	Nonessential amino acids
NMR	Nuclear Magnetic Resonance
PARP1	Poly [ADP-ribose] polymerase 1
PVDF	Polyvinylidene difluoride
Rb	Retinoblastoma
RIPA	Radio immunoprecipitation assay
SD	Standard deviation
TBST	Tris-buffered saline with Tween-20
WB	Western blot
5FU	5-fluorouracil

Data availability

Data will be made available on reasonable request.

References

Addy, B.S., Firempong, C.K., Komlaga, G., Addo-fordjour, P., Domfeh, S.A., Afolayan, O., Emikpe, B.O., 2024. In vitro antiproliferative activities of some Ghanaian medicinal plants. Clinical Phytoscience 10 (19), 1–10.

Alipanah, H., Farjam, M., Zarenezhad, E., Roozitalab, G., Osanloo, M., 2021. Chitosan nanoparticles containing limonene and limonene-rich essential oils: potential phytotherapy agents for the treatment of melanoma and breast cancers. BMC Complementary Medicine and Therapies 21, 1–10.

Aloke, C., Nwachukwu, N., Idenyi, J.N., Ugwuja, E.I., Nwachi, E.U., Edeogu, C.O., Ogah, O., 2010. Hypoglycaemic and hypolipidaemic effects of feed formulated with Ceiba Pentandra leaves in alloxan induced diabetic rats. Australian Journal of Basic and Applied Sciences 4 (9), 4473–4477.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M., Rollinger, J.M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E.A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G.K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., Supuran, C.T., 2021. Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery 20 (3), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Bakar-Ates, F., Ozkan, E., Sengel-Turk, C.T., 2020. Encapsulation of cucurbitacin B into lipid polymer hybrid nanocarriers induced apoptosis of MDAMB231 cells through PARP cleavage. International Journal of Pharmaceutics 586 (April), 119565. https://doi.org/10.1016/j.ijpharm.2020.119565.

Barba-Ostria, C., Carrera-Pacheco, S.E., Gonzalez-Pastor, R., Heredia-Moya, J., Mayorga-Ramos, A., Rodríguez-Pólit, C., Zúñiga-Miranda, J., Arias-Almeida, B., Guamán, L.P., 2022. Evaluation of Biological Activity of Natural Compounds: Current Trends and Methods. Molecules 27 (14), 1–35. https://doi.org/10.3390/molecules27144490.

Berridge, M.J., 2014. Module 9: Cell Cycle and Proliferation. Cell Signalling Biology 6, 1–45. https://doi.org/10.1042/csb0001009.

Câmara, J.S., Perestrelo, R., Ferreira, R., Berenguer, C.V., Pereira, J.A.M., Castilho, P.C., 2024. Plant-Derived Terpenoids: A Plethora of Bioactive Compounds with Several Health Functions and Industrial Applications—A Comprehensive Overview. Molecules 29 (16). https://doi.org/10.3390/molecules29163861.

Castañeda, A.M., Meléndez, C.M., Uribe, D., Pedroza-Díaz, J., 2022. Synergistic effects of natural compounds and conventional chemotherapeutic agents: recent insights for the development of cancer treatment strategies. Heliyon 8 (6), e09519. https://doi.org/10.1016/j.heliyon.2022.e09519.

Chaitanya, G.V., Steven, A.J., Babu, P.P., 2010. PARP-1 cleavage fragments : signatures of cell-death proteases in neurodegeneration. Cell Communication and Signaling 8 (1), 1–11. https://doi.org/10.1186/1478-811X-8-31.

Chang, M.Y., Shieh, D.E., Chen, C.C., Yeh, C.S., Dong, H.P., 2015. Linalool induces cell cycle arrest and apoptosis in leukemia cells and cervical cancer cells through CDKIs. International Journal of Molecular Sciences 16 (12), 28169–28179. https://doi.org/10.3390/ijms161226089.

Chen, X., Bao, J., Guo, J., Ding, Q., Lu, J., Huang, M., Wang, Y., 2012. Biological activities and potential molecular targets of cucurbitacins: a focus on cancer. Anti-Cancer Drugs 23 (8), 777–787. https://doi.org/10.1097/CAD.0b013e3283541384.

Doan, P., Musa, A., Candeias, N.R., Emmert-Streib, F., Yli-Harja, O., Kandhavelu, M., 2019. Alkylaminophenol induces G1/S phase cell cycle arrest in glioblastoma cells through p53 and cyclin-dependent kinase signaling pathway. Frontiers in Pharmacology 10 (APR), 1–17. https://doi.org/10.3389/fphar.2019.00330.

Duronio, R.J., Xiong, Y., 2013. Signaling pathways that control cell proliferation. Cold Spring Harbor Perspectives in Biology 5 (3), 1–12. https://doi.org/10.1101/cshperspect.a008904.

Einbond, L.S., Soffritti, M., Esposti, D.D., Park, T., Cruz, E., Su, T., Wu, H.-A., Wang, X., Zhang, Y.-J., Ham, J., Goldberg, I.J., Kronenberg, F., Vladimirova, A., 2009. Actein activates stress- and statin-associated responses and is bioavailable in Sprague-Dawley rats. Fundamental & Clinical Pharmacology 23 (3), 311–321. https://doi.org/10.1111/j.1472-8206.2009.00673.x.

Fouad, Y.A., Aanei, C., 2017. Revisiting the hallmarks of cancer. American Journal of Cancer Research 7 (5), 1016–1036.

Gao, S., Shan, Y., Wang, Y., Wang, W., Li, J., Tan, H., 2024b. Polysaccharides from Lonicera japonica Thunb.: Extraction, purification, structural features and biological activities—A review. International Journal of Biological Macromolecules 281, 136472. https://doi.org/10.1016/j.ijbiomac.2024.136472.

Gao, S., Tan, H., Gang, J., 2024a. Inhibition of hepatocellular carcinoma cell proliferation through regulation of the Cell Cycle, AGE-RAGE, and Leptin signaling pathways by a compound formulation comprised of andrographolide, wogonin, and oroxylin A derived from Andrographis Paniculata(Bu. Journal of Ethnopharmacology 329, 118001. https://doi.org/10.1016/j.jep.2024.118001.

Ghosh, S., 2020. Triterpenoids: structural diversity, biosynthetic pathway, and bioactivity. Studies in Natural Products Chemistry 67, 411–461.

Gobeil, S., Boucher, C.C., Nadeau, D., Poirier, G.G., 2001. Characterization of the necrotic cleavage of poly (ADP-ribose) polymerase (PARP-1): Implication of lysosomal proteases. Cell Death and Differentiation 8 (6), 588–594. https://doi.org/10.1038/sj.cdd.4400851.

Id, R.J., Chaudhry, G., 2019. Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Advanced Pharmaceutical Bulletin 9 (2), 205–218. https://doi.org/10.15171/jcvtr.2015.24.

Jain, P.G., Patel, B.D., 2019. Medicinal chemistry approaches of poly ADP-Ribose polymerase 1 (PARP1) inhibitors as anticancer agents - A recent update. European Journal of Medicinal Chemistry 165, 198–215. https://doi.org/10.1016/j.ejmech.2019.01.024.

Jones, P., 2010. Chapter 14 - Development of Poly(ADP-Ribose)Polymerase (PARP) Inhibitors in Oncology. In: Macor, M.C. (Ed.), Annual Reports in Medicinal Chemistry, 45. Academic Press, pp. 229–243. https://doi.org/10.1016/S0065-7743(10)45014-9. J. E. B. T.-A. R.

Kaufmann, S.H., 1989. Induction of endonucleolytic DNA cleavage in human acute myelogenous leukemia cells by etoposide, camptothecin, and other cytotoxic anticancer drugs: a cautionary note. Cancer Research 49 (21), 5870–5878.

Kaufmann, S.H., Desnoyers, S., Ottaviano, Y., Davidson, N.E., Poirier, G.G., 1993. Specific proteolytic cleavage of poly (ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Research 53 (17), 3976–3985.

Kłos, P., Chlubek, D., 2022. Plant-Derived Terpenoids: A Promising Tool in the Fight against Melanoma. Cancers 14 (3), 502.

Koklesova, L., Liskova, A., Samec, M., Buhrmann, C., Samuel, S.M., Varghese, E., Ashrafizadeh, M., Najafi, M., Shakibaei, M., Büsselberg, D., Giordano, F.A., Golubnitschaja, O., Kubatka, P., 2020. Carotenoids in cancer apoptosis—the road from bench to bedside and back. Cancers 12 (9), 1–41. https://doi.org/10.3390/cancers12092425.

Kumar, S., Gautam, V., Singh, B.P., Kumar, D., 2023. Editorial: Investigating the impact of bioactive metabolites and extracts in human health and disease. Frontiers in Molecular Biosciences 10 (August), 1–4. https://doi.org/10.3389/fmolb.2023.1244316.

Lee, Y., Lahens, N.F., Zhang, S., Bedont, J., Field, J.M., Sehgal, A., 2019. G1/S cell cycle regulators mediate effects of circadian dysregulation on tumor growth and provide targets for timed anticancer treatment. PLoS Biology 17 (Issue 4). https://doi.org/10.1371/journal.pbio.3000228.

Li, M., Tao, H., 2024. Enhancing structural diversity of terpenoids by multisubstrate terpene synthases. Beilstein Journal of Organic Chemistry 20, 959–972. https://doi.org/10.3762/bjoc.20.86.

Lin, Z.P., Zhu, Y.L., Ratner, E.S., 2018. Targeting cyclin-dependent kinases for treatment of gynecologic cancers. Frontiers in Oncology 8 (AUG). https://doi.org/10.3389/fonc.2018.00303.

Mani, J., Johnson, J., Hosking, H., Hoyos, B.E., Walsh, K.B., Neilsen, P., Naiker, M., 2022. Bioassay Guided Fractionation Protocol for Determining Novel Active Compounds in Selected Australian Flora.

Martí, J.M., Fernández-Cortés, M., Serrano-Sáenz, S., Zamudio-Martinez, E., Delgado-Bellido, D., Garcia-Diaz, A., Oliver, F.J., 2020. The multifactorial role of PARP-1 in tumor microenvironment. Cancers 12 (3). https://doi.org/10.3390/cancers12030739.

Mashimo, M., Onishi, M., Uno, A., Tanimichi, A., Nobeyama, A., Mori, M., Yamada, S., Negi, S., Bu, X., Kato, J., Moss, J., Sanada, N., Kizu, R., Fujii, T., 2021. The 89-kDa PARP1 cleavage fragment serves as a cytoplasmic PAR carrier to induce AIF-mediated apoptosis. Journal of Biological Chemistry 296, 100046. https://doi.org/10.1074/jbc.RA120.014479.

Matthews, H.K., Bertoli, C., de Bruin, R.A.M., 2022. Cell cycle control in cancer. Nature Reviews Molecular Cell Biology 23 (1), 74–88. https://doi.org/10.1038/s41580-021-00404-3.

Mergui-roelvink, M., Mortimer, P., Ph, D., Swaisland, H., Sc, B., Lau, A., Ph, D., Connor, M.J.O., Ph, D., Ashworth, A., Ph, D., Carmichael, J., Kaye, S.B., Schellens, J. H.M., Ph, D., Bono, J. S. De, Ph, D., 2009. Inhibition of Poly(ADP-Ribose) Polymerase in Tumors from BRCA Mutation Carriers. New England Journal of Medicine 361 (2), 123–134.

Miao, R., Wei, J., Zhang, Q., Sajja, V., Yang, J., Wang, Q., 2008. Redifferentiation of human hepatoma cells (SMMC-7721) induced by two new highly oxygenated bisabolane-type sesquiterpenes. Journal of Biosciences 33 (5), 723–730. https://doi.org/10.1007/s12038-008-0092-x.

Moskot, M., Jakóbkiewicz-Banecka, J., Kloska, A., Piotrowska, E., Narajczyk, M., Gabig-Cimińska, M., 2019. The role of dimethyl sulfoxide (DMSO) in gene expression modulation and glycosaminoglycan metabolism in lysosomal storage disorders on an example of mucopolysaccharidosis. International Journal of Molecular Sciences 20 (2), 1–18. https://doi.org/10.3390/ijms20020304.

Nkouam, G.B., Adjoh, G., Leudeu, C.B.T., Kouebou, C., Tchiegang, C., Kapseu, C., 2017. Local uses of kapok (Ceiba pentandra Gaertn.) Tree from the Northern Part of Cameroon. International Journal of Environment, Agriculture and Biotechnology 2 (4), 2214–2219. https://doi.org/10.22161/ijeab/2.4.82.

Osuntokun, O., Ajayi, A.O., Adeoye, M., Odufunwa, A., 2017. Assessment of Antimicrobial and Phytochemical Properties of Crude Leaf and Bark Extracts of Ceiba Pentandra on Selected Clinical Isolates Found in Nigerian Teaching Hospital. Journal of Bacteriology & Mycology: Open Access 4 (1). https://doi.org/10.15406/jbmoa.2017.04.00079.

Park, W., Wei, S., Kim, B.S., Kim, B., Bae, S.J., Chae, Y.C., Ryu, D., Ha, K.T., 2023. Diversity and complexity of cell death: a historical review. Experimental and Molecular Medicine 55 (8), 1573–1594. https://doi.org/10.1038/s12276-023-01078-x.

Qi, H., Wei, L., Han, Y., Zhang, Q., Lau, A.S.-Y., Rong, J., 2010. Proteomic characterization of the cellular response to chemopreventive triterpenoid astragaloside IV in human hepatocellular carcinoma cell line HepG2. International Journal of Oncology 36 (3), 725–735. https://doi.org/10.3892/ijo_00000548.

Rabi, T., Bishayee, A., 2009. Terpenoids and breast cancer chemoprevention. Breast Cancer Research and Treatment 115 (2), 223–239. https://doi.org/10.1007/s10549-008-0118-y.

Ramos-Alonso, L., Holland, P., Le Gras, S., Zhao, X., Jost, B., 2017. Mitotic Chromosome Condensation Resets Chromatin to Safeguard Transcriptional Homeostasis during Interphase, 120. Proceedings of the National Academy of Sciences, p. 2017. https://doi.org/10.1073/pnas.

Ramteke, A., Ganatra, S., Gour, P., Jasudkar, D., Tulankar, A., 2021. Anti-cancer inhibition studies of naturally occurring Terpenes using in-vitro technique. Journal of Physics: Conference Series 1913 (1). https://doi.org/10.1088/1742-6596/1913/1/012081.

Sarfo, A., Abotsi, W.K.M., Ekuadzi, E., Woode, E., 2022. Ceiba pentandra (L.) Gaertn hydroethanolic leaf extract exhibits anticonvulsant properties in mouse models. Phytomedicine Plus 2 (2), 100263. https://doi.org/10.1016/j.phyplu.2022.100263.

Schiewer, M.J., Goodwin, J.F., Han, S., Brenner, J.C., Augello, M.A., Dean, J.L., 2012. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discovery 2 (12), 1134–1149. https://doi.org/10.1158/2159-8290.CD-12-0120.Dual.

Tadesse, S., Anshabo, A.T., Portman, N., Lim, E., Tilley, W., Caldon, C.E., Wang, S., 2020. Targeting CDK2 in cancer: challenges and opportunities for therapy. Drug Discovery Today 25 (2), 406–413. https://doi.org/10.1016/j.drudis.2019.12.001.

Tareau, M.-A., Greene, A., Odonne, G., Davy, D., 2021. Ceiba pentandra (Malvaceae) and associated species: Spiritual Keystone Species of the Neotropics. Botany 100 (2), 127–140. https://doi.org/10.1139/cjb-2021-0099.

Tyagi, A., Agarwal, C., Agarwal, R., 2002. The cancer preventive flavonoid silibinin causes hypophosphorylation of Rb/p107 and Rb2/p130 via modulation of cell cycle regulators in human prostate carcinoma DU145 cells. Cell Cycle 1 (2), 122–127. https://doi.org/10.4161/cc.1.2.115.

Valdespino-Gómez, V.M., Valdespino-Castillo, P.M., Valdespino-Castillo, V.E., 2015. Cell signalling pathways interaction in cellular proliferation: Potential target for therapeutic interventionism. Cirugia y Cirujanos (English Edition) 83 (2), 165–174. https://doi.org/10.1016/J.CIRCEN.2015.08.015.

Zhang, S.D., Yu, L., Wang, P., Kou, P., Li, J., Wang, L.T., Wang, W., Yao, L.P., Zhao, X.H., Fu, Y.J., 2019. Inotodiol inhibits cells migration and invasion and induces apoptosis via p53-dependent pathway in HeLa cells. Phytomedicine 60 (March). https://doi.org/10.1016/j.phymed.2019.152957.

Zhong Xu, Yong, Kanagaratham, Cynthia, Youssef, Mina, Danuta Radzioch, 2016. New Frontiers in Cancer Chemotherapy — Targeting Cell Death Pathways. In: New Insights, Prof. Stevo Najman. InTech. https://doi.org/10.5772/61260.

Zhou, X., Fu, F., Li, Z., Dong, Q., He, J., Wang, C., 2009. Escin, a natural mixture of triterpene saponins, exhibits antitumor activity against hepatocellular carcinoma. Planta Medica 75 (15), 1580–1585. https://doi.org/10.1055/s-0029-1185838.