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RESEARCH ARTICLE

Biocompatibility assessment and antiproliferative activity of *Detarium microcarpum* Guill. and Perr. fruit pulp extracts.

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Abstract

The consumption of tropical fruits rich in chemopreventive compounds are required to prevent cells carcinogenesis and proliferation. This study was designed to assess the biocompatibility of *Detarium microcarpum* fruit extract on normal fibroblasts and its antiproliferative potentiality on human osteosarcoma MG-63 cells. Primary dermal fibroblasts and human osteosarcoma MG-63 cells were treated with different concentrations of hexane, chloroform, ethyl acetate and methanol extracts of *D.microcarpum* fruit pulp for 24h, 48h and 72h. The biocompatibility property of extracts on the normal fibroblasts and its antiproliferative activity on the human osteosarcoma cells were evaluated by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assay. The biocompatibility study of *D.microcarpum* fruit pulp showed that the chloroform extract has exhibited the highest cytotoxic effect on normal fibroblasts followed by the ethyl acetate extract. Hexane extract wasn't cytotoxic at concentrations of 125 and 250 µg/mL but caused more than 80 % of cell death at a concentration of 500 µg/mL. Methanol extract didn't show a significant cytotoxic effect. Furthermore, chloroform and ethyl acetate extracts showed the best antiproliferative activity on osteosarcoma cells. A complete cell death was observed when osteosarcoma cells were treated with ethyl acetate extract at all concentrations while chloroform extract at concentrations of 250 and 500 µg/mL caused a complete cells death. Methanol extract exhibited any antiproliferative activity. Chloroform and ethyl acetate extract of fruits pulp of *D. microcapum* are potent source of anticancer phytomolecules and have potential to be a promising anti-osteosarcoma extract.

Keywords: Antiproliferative; Biocompatibilty; Detarium microcarpum; Fibroblast; Osteosarcoma.

Introduction

Osteosarcoma is the common malignant borne tumor and is responsible of many cancer-related death due to its high metastasis [1]. Osteosarcoma metastasis-derived tumors includes lungs, prostate, head, neck and ovarian cancer and are responsible of more than 40% of osteosarcoma-related death [2]. Environmental factors such as physical (ultraviolet and ionizing radiations), chemicals (methylcholanthrene, chromium salt, aniline dyes) and biological (simian virus 40) agents have been suggested for osteosarcoma [3]. For a long times, the chemotherapeutic treatment of osteosarcoma cells uses cytostatic agents including methotrexate [4], doxorubicin [5], ifosfamide and cisplatin [6]. The development of resistance and the several side

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effects on surrounded normal cells make in failure the use of these anticancer systemic drugs. Modern treatments of osteosarcoma combine neoadjuvant chemotherapy, surgery and radio - therapy [7]. Unfortunately the last 5-years survival rate for adolescent and adult osteosarcoma -diagnosed patients remains less than 70% [8]. Hence, development of new target therapeutic agents becomes a great interest for researchers and anticancer herbs and plants were thus extensively screened in the last decade.

D. microcarpum is belong to the family of Caesalpiniaceae and widely grown in the savanna region of West Africa [9]. The fruit pulp of the plant is commonly consumed crude or cooked. It is extensively studied for its nutritional and therapeutic properties. In West Africa countries, fruit is frequently used in the folklore medicine to treat meningitis and skin infections and tuberculosis [10]. Previous phytochemical studies showed that the fruit pulp contains a high amount of ascorbic acid as well as carbohydrates and oligo-element [11, 12]. Other studies demonstrated that the fruit is rich in total flavonoids and total phenolic contents positively correlated to its free radical scavenging potent against DPPH and $ABTS^+$ and its ferric reducing power [13]. The pharmacological properties of the fruit includes antibacterial [11], antioxidant [13], enzymes inhibition [14], DNA protection and DNA repair activities [15]. Four new bioactive clerodane diterpenes (3,4-epoxyclerodan-13E-en-15-oic acid, 5α,8α-(2-oxokolavenic acid), 3,4-dihydroxyclerodan-13E-en-15-oic acid and 3,4-dihydroxyclerodan-13Z-en-15-oic acid) were isolated from the dichloromethane extract of the fruit pulp which exhibited a great inhibitor activity on acetylcholinesterase enzyme [14]. Regarding to the multiple health benefit properties of the fruit pulp of D. microcarpum demonstrated in the literature, this study was designed to evaluate the antiproliferative activity of the fruit pulp against osteosarcoma MG63 as well as its biocompatibility property on normal fibroblasts.

Materials and Methods

Chemicals

All chemical were an analytical grade. Hexane, chloroform, ethylacetate and methanol were purchased from Chemical Pro-Lab. Hank balanced salt solution (HBSS), Dimethyl sulfoxide (DMSO), Dulbecco's modified eagle medium (DMEM), 2-propanol, Trypsin-ethylenediamine tetraacetic acid solution, Fetal bovine serum (FBS) and Penicillin-neomycin-streptomycin were purchased by Sigma Aldrich, France.

Fruits collection and extraction

D. microcarpum (fruits) were collected in Gampela (15 km, East of Ouagadougou), Burkina Faso, in January 2017 (Coordinates: 12°, 25', 51" N and 1°, 22', 18" W). The botanical identification was performed by a local botanist (Pr Jeanne Millogo, University Ouaga JKZ, Burkina Faso) and a voucher specimen (IC: 15928) was kept at the UFR SVT herbarium, University Ouaga JKZ, Burkina Faso.

Fruit pulp of D. microcarpum was powdered and 100g were successively depleted in hexane, chloroform, ethylacetate and methanol solvent by maceration during 24 h. The resulting mixtures were filtered and concentrated to dryness with a vacuum evaporator (Büchi Labortechnik AG, Switzerland). All fruits extracts were kept in a freezer (4°C) until further investigations.

Cells culture

Cells cultured was performed as described previously [16]. Primary dermal fibroblasts or osteosarcoma MG63 cells (ATCC, Rockville, MD) were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin - neomycin - streptomycin (P/N/S), in a humidified atmosphere of 5% CO_2 at 37 °C. When the cells reached 80 % confluence, they were harvested with 0.25% trypsin containing 1 mM ethylenediamine tetraacetic acid (EDTA) and seeded on 96-well plates.

MTT cell viability assay

Fruits extracts were solubilized in DMSO and diluted in cells growth medium. Afterwards, extracts were sterilized by filtration through 0.22 μm of micro pore filter. One hundred microliters of different concentrations of each extract (125, 250 and 500 μg/ml) were put in contact with the same volume of 5000 cells/mL in each well of 96-well plates and incubated at 37 °C for 24, 48 and 72 hours in a humidified atmosphere 5% $C0_2$. Cells were incubated in the same conditions with the vehicle (DMSO 1% in DMEM) and served as control. At the end of the incubation time, cells viability was measured by using the standard MTT assay as described previously [5]. The medium was gently discarded and replaced with the 100 μL of MTT solution (5 mg/mL in Hank balanced salt solution) and plate was incubated for 3h. After the exposure time, MTT solution was aspirated and 100 μL of 2-propanol were added to dissolve the formazan crystals metabolized by the viable cells. The dissolution of the formazan was completed by shaking in the shaker for 20 min. The optical density of the formazan solution was measured spectrophotometrically at 570 nm with a Tescan Sunrise Plate Reader. The percentage of cell viability was calculated according to the following equation, where PCV is the percentage of cell viability, Abs (A1) is the absorbance of samples and Abs

(A0) is the absorbance of control.

$$PCV = \frac{Abs(A1)}{Abs\ (A0)} X\ 100$$

Statistical analysis

Data were presented as the mean value of three repeated independent experiments ± Std. One-way analysis of variance (ANOVA) followed by Newman-Keuls post-test was used for the statistical analysis to give significantly differences of cytotoxicity. Significantly differences were indicated as $^{*}P < 0.05$.

Results and discussion

The biocompatibility study of D. microcarpum fruit pulp extracts was carried out on the normal fibroblasts and results were showed in figure 1. Data showed that the chloroform extract has exhibited the highest cytotoxic effect on normal fibroblast followed by the ethyl acetate extract. Furthermore, the cytotoxic effects of extracts increased with the treatment duration. Hexane extract wasn't cytotoxic at concentrations of 125 and 250 µg/mL but caused more than 80 % the cell death at a concentration of 500 µg/mL. Methanol extract didn't show a significant cytotoxic effect at 24h and 48h when the viability of cells treated with methanolextract is compared to this of the vehicle ($p>0.05$). The cytotoxic effects of extracts were ranged in the following order of decreasing levels: chloroform extract > ethyl acetate extract > hexaneextract> methanolextract.

The anticancer properties of fruit pulp extracts were assessed by evaluating their antiproliferative effects on osteosarcoma MG-63 cells. Data were showed in figure 2. Chloroform and ethyl acetate extracts showed the best antiproliferative activity on osteosarcoma MG-63 cells. A complete cell death was observed when osteosarcoma were treated with the ethyl acetate extract for 24h, 48h and 72h at a concentration of 500 µg/mL. A similar result was observed when osteosarcoma cells were treated with the chloroform extract at a concentrations of 500 µg/mL for 48h and 72h. The antiproliferative activity of the chloroform extract increased in a concentration dependent manner and its cytotoxic effects intensified with the treatment duration. The methanol extract treatment compared to the vehicle treatment hasn't exhibited any antiproliferative activity ($p>0.05$). The antiproliferative activity of extracts were ranged in the following order of decreasing levels: chloroform extract > ethyl acetate extract > hexane extract > methanolextract. All these data demonstrated that chloroform and ethyl acetate fruit pulp extracts are potent sources of anticancer drugs but exhibit some cytotoxic effects on normal fibroblasts. Interestingly, at a short time of treatment, ethylacetate extract in a concentration of 500 µg/mL induces more cytotoxic effects on osteosarcoma MG63 cells (induces more than 90% of cells death) than normal fibroblast cells (induces less than 60% of cells death).

Cancer is a world health crisis [17]. For a long-time, chemotherapy of cancer employed high toxicity of systemic adjuvants to kill malignant cells. The development of resistance of certain cancer with high probability of metastasis and the high cytotoxicity of the used anticancer chemicals on the surrounded normalcells made in challenge this therapeutic option. Osteosarcoma chemotherapy combines neoadjuvant chemotherapy, surgery and radiotherapy [7]. Unfortunately, the percentage of osteosarcoma-related death remains raised [2]. In recent years, researches focused on the screening of dietary anticancer compounds or their metal complex to improve the sensitivity of anticancer drugs without negatively affect the normal cells, and wild edible fruits have been extensively studied. Wild edible fruits contain many bioactive compounds such as flavonoids, terpenes, anthocyanins and phenolacids. These bioactive compounds possess divers pharmacological properties including antioxidant, anti-inflammatory, antibacterial and anticancer activities [18]. Previous studied identified many dietary anticancer compounds and their complex capable to reduce osteosarcoma cells proliferation, differentiation and invasion. Indeed, chrysin-vanadium complex exhibited antitumor activity on osteosarcoma spheroids models and xenograft tumor in mice in vitro and in vitro respectively [19]. Flavonoids like quercetin, curcumin, hyperoside, silibilin and hesperidin suppressed human osteosarcoma cells MG63 migration and invasion in relation with the downregulation of oncogenes expression [20–24]. Some phenol acids such as ferrilic acid and caffeic acid promoted osteosarcoma cells apoptosis through PI3K/Akt pathways blockage and caspase activation respectively [25, 26]. Moreover, diterpenes such as sclareol enhanced osteosarcoma cellapoptosis followed by G1-phase cell cycle arrest [17]. Fruit pulp D. microcarpum was identified as a potent source of bioactive phenolic and diterpenes compounds known as promising anticancer compounds against osteosarcoma cells [13, 14]. In this study, chloroform and ethyl acetate extract of D. microcarpum fruit pulp in a concentration game of 125-500 µg/mL induced human osteosarcoma cells MG-63 apoptosis suggesting that ethyl acetate and chloroform extracts contain effective anticancer phytomolecules. Interestingly, ethylacetate in a concentration of 500 µg/mL was more cytotoxic on osteosarcoma than normal fibroblasts. This finding suggested that anticancer compounds of ethylacetate extract exercise targeted cytotoxic effects on human osteosarcoma and hereby could promote the development of new effective and selective anticancer drugs against osteosarcoma.

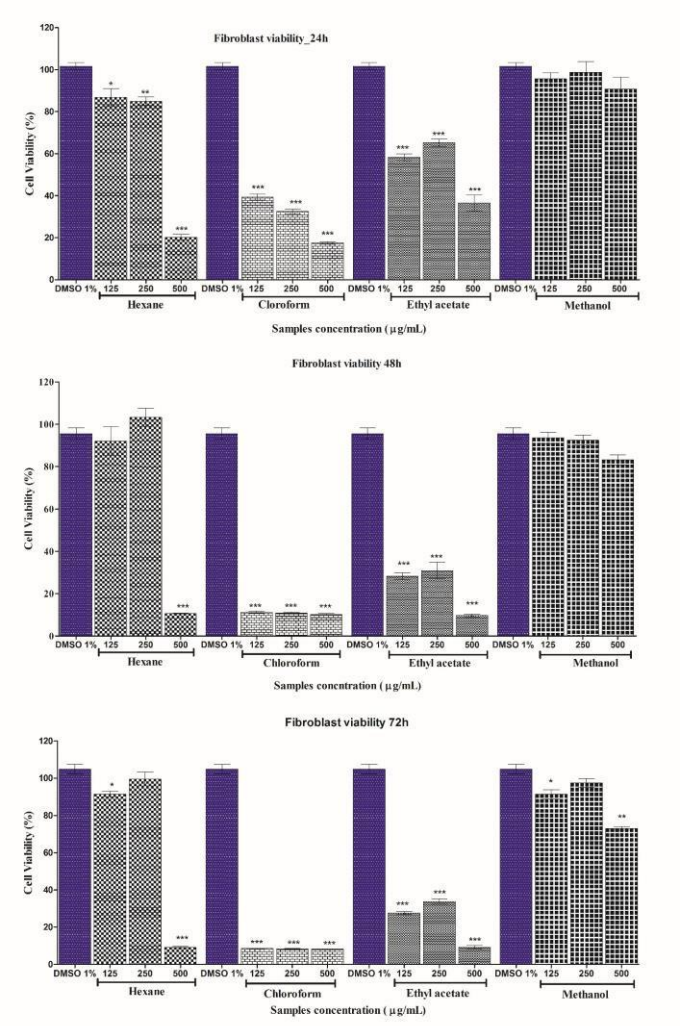

Figure 1 The biocompatibility assessment of D. microcarpum fruit pulp extracts. Normal exponential growth fibroblasts were kept in contact with different concentrations of hexane, chloroform, ethyl acetate and methanol fruit pulp extracts and the viability of cells was measured at different times by using MTT assay. Data were expressed as the mean ± Std of three independent experiments. Significant difference from the vehicle (DMSO 1% in DMEM); *P< 0.05; **P < 0.01; ***P < 0.001.

Conclusion

Fruit pulp of D. microcarpum are a potent source of anticancer phytomolecules. These anticancer compounds are more extractible by chloroform and ethyl acetate than hexane and methanol solvents. However, the extractible anticancer molecules were also cytotoxic on normal fibroblasts. However, ethyl acetate extract was more cytotoxic on osteosarcoma MG63 cells than normal fibroblast cells. Further phytochemical investigations are necessary to isolate anticancer drugs of the ethyl acetate extracts from D. microcarpum fruit pulp in sight of to develop new effective target anticancer agents.

Author's contributions: Ablassé Rouamba, Bernabé Lucien Nkono Ya Nkono design and conduct the experiments. Liliana Verestiuc and Iona Alexandra Duceac valid and supervise the experiments. Ablassé Rouamba, Vincent Ouédraogo writes the first draft of the manuscript. Maurice Ouédraogo, Moussa Compaoré and Martin Kiendrebeogo check and correct the grammar. All author read and approve the final paper.

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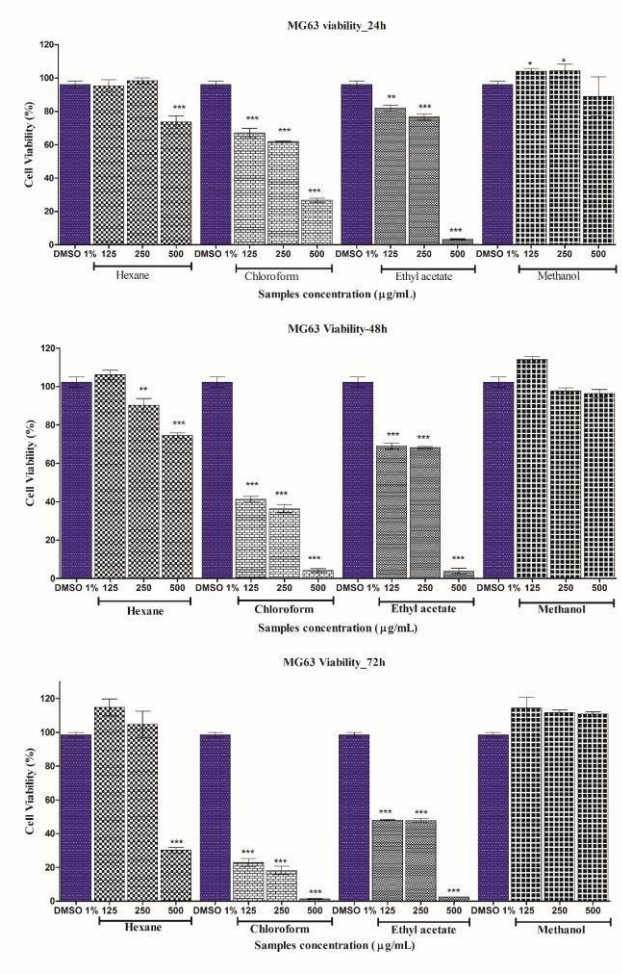

Figure 2 The antiproliferative activity of D. microcarpum fruit pulp extracts. Exponential growth osteoblasts were incubated with different concentrations of hexane, chloroform, ethyl acetate and methanol fruit pulp extracts and the percentages of viable cells were recorded at different times by using MTT assay. Data were expressed as the mean ± Std of three independent experiments. Significant difference from the vehicle (DMSO 1% in DMEM); *P< 0.05; **P < 0.01; ***P < 0.001.

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Conflict of interest statement: All authors declare that no conflict of interest exists.

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