

Cytotoxicity of Fractions Derived from Crude Water Soluble Extract (CWSE) of *Momordica charantia* (Linn) Leaves on Lung, Cervical and Breast Cancer Cells

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ABSTRACT

The in vitro cytotoxicity of five fractions derived from the crude water-soluble extract (CWSE) of Momordica charantia, a known medicinal herb was tested on four cancer cell models; MDA-MB 436 and 231 (breast cancer cell lines), HeLa (Cervical cancer cell lines) and A549 (Lung cancer cell lines). N-hexane (D4) < Dichloromethane (D5) < Ethyl acetate (D3) < Butanol (D2) < Aqueous (D6) fractions, all obtained via solvent partitioning in their order of increasing polarity were administered at varied concentrations (50, 75, 100, 125, 150 and 200 µg/mL) for 24 h.

Cell viability was detected using the MTT assay. Cells treated with the Butanol and Aqueous fractions respectively showed survival rates as high as 96.11% and 85.75% for MDA-MB 231; 93.59% and 88.28% for MDA-MB 436; 101.62% and 96.76% for HeLa and 89.55% and 81.40% for A549 even at 200 µg/mL, the highest considered concentration.

Whereas, at tested concentrations, cells treated with the N-hexane, Dichloromethane and Ethyl acetate fractions showed marked cytotoxic effects with the Inhibitory concentration (IC₅₀) ranging between 100 µg/mL and 125 µg/mL. We also observed that the ethylacetate fraction displayed the highest cytotoxicity having its approximate IC₅₀ at 100 µg/mL, especially for MDA-MB 231 (~51%), HeLa (~54%) and A549 (~43%). The percentage viability for MDA-MB 436 at 100 µg/mL is about 64.2%, albeit at 125 µg/mL percentage viability dropped to 34.7%

This paper may provide cytological evidence for the inhibitory effect of Momordica charantia on certain cancer cell proliferation.

Keywords: *Momordica charantia*, cancer cell lines, cytotoxicity, MTT assay and crude water-soluble extract (CWSE).

Introduction

Evidence abound now of the important role played by popular medicinal plants in the treatment and management of ailments (Tauseef and Shahabuddin, 2013). One of the most feared ailments of our time is cancer which in 2012 accounted for up to 8.2 million deaths of the total 14.1 million cases reported (Ferlay *et al.*, 2013). The medicinal plant *Momordica charantia* (MC) has been named as one of such potent medicinal plants especially in relation to its usefulness in the treatment and management of cancers (Kubola and Siriamornpun, 2008). Quite a number of literatures have affirmed the antiproliferative effects of the plant on cancer cell lines (Lee-Huang *et al.*, 2000; Yasui *et al.*, 2005; Ray, *et al.*, 2010; Fang *et al.*, 2012). In our Lab, a number of preliminary tests carried out on this plant suggest it may be a potent inducer of apoptosis; the death a cell caused by its own self (Odewusi *et al.*, 2010; Ehigie *et al.*, 2013). Many types of cancers, numbering more than 100 different types have been named. In 2008, approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers) (Jemal *et al.*, 2011) and in 2010 nearly 7.98 million people died (Lozano *et al.*, 2012). Cancers as a group account for approximately 13% of all deaths each year with the most common being: lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths) (WHO on “Cancer”, 2010) This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world (Jemal *et al.*, 2011). Over half of these cases occur in the developing world (Jemal *et al.*, 2011). The Hallmarks of cancer include uncontrolled cell division, evasion of apoptosis, non-dependence on growth regulation, angiogenesis, immortality and invasion and metastasis. Carcinogenesis is the actual formation of a cancer, whereby normal cells are transformed into cancer cells. The process is characterized by a progression of changes at the cellular, genetic and epigenetic level

that ultimately reprogram a cell to undergo uncontrolled cell division, thereby forming a malignant mass (Fearon and Vogelstein, 1990). We now know that some herbs may have inhibitory effects on the proliferation of cancer cell lines (Chen and King, 2005) a much desired weapon in the war against cancer cell growth. A greater understanding of the pivotal events associated with carcinogenesis will facilitate the use of natural products/medicinal plants as a key strategy to prevent cancer and initiate cancer cell death. Indeed, experimental evidence indicates that phytochemicals can modulate the complex multistage process of carcinogenesis at each of the three recognized stages of initiation, promotion and malignant progression (Chen and King, 2005). Working on the premise that MC is a potent pro-apoptotic agent (Ehigie *et al*, 2013) and most likely possessive of anti-proliferative properties, we assayed to determine its likely anti-cancer properties. Hence, the experiments leading to this publication which exhibits our findings on the cytotoxicity of fractions derived from the CWSE of MC in Breast, Lung and Cervical cancer cell lines.

Materials and methods

Chemicals

The culture media and fetal bovine serum were purchased from Hyclone (Logan, UT, USA). The CCK8 cell counting kit was from Dojindo Laboratories (Kumamoto, Japan). Cell-culture Petri dishes were from BD Biosciences (San Jose, CA, USA). N-hexane, Dichloromethane, Ethyl acetate and Butanol were obtained from BDH chemicals Ltd, (Poole UK). All other reagents were manufactured in China and of analytical grade.

Plant material

Fresh, healthy leaves of *Momordica charantia* were collected from the Botanical gardens of the Obafemi Awolowo University, Ile- Ife Campus, Nigeria and authenticated at the Faculty of Pharmacognosy Herbarium of the same Institution with the plant herbarium No. FPL-1783. The petioles were separated, weighed, rinsed with clean water, drained and air -dried. A total of 5.4 kg was pulverised using a manual blender and subsequently soaked in 9.0 litres of water. It was left for 12 hours at room temperature with occasional agitation. The macerate was filtered, concentrated *in vacuo* at 65°C to small volume using the rotary evaporator before being freeze-dried to obtain a powdery extract. The yield was about 4.87% of the raw material.

Fractionation of the extract

The powdery extract, was subsequently adsorbed on silica gel (ratio of 1:1) with little methanol, transferred to a Buchner funnel, where it was successively eluted under vacuum using solvents of different polarities to obtain n-hexane (D4), dichloromethane (D5), ethyl acetate (D3), butanol (D2) and aqueous (D6) fractions respectively. The solvents of elution were only switched when it was clear to daylight and by TLC. Each fractions obtained during partitioning was subjected to concentration *in-vacuo* and later freeze dried to obtain powdery samples. After freeze drying, the different fractions were preserved at low temperature (4°C).

Cell culture

The human breast cancer cells MDA-MB-231, MDA-MB-436, the human cervical cancer cells HeLa and the human lung cancer cells A549 were all obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and were cultured under conditions recommended by ATCC.

Cell viability assessment using the 3-(4, 5-Dimethyl thiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) Assay

The cytotoxicity induced by the five fractions of the crude water-soluble extract of *Momordica charantia* was determined using the MTT Assay, which is based on the reduction of water-soluble tetrazolium salt

by mitochondrial reductases of metabolically active cells. Briefly, cells already plated in 96-well plates were treated with 50-200 $\mu\text{g/ml}$ of the five fractions for 24 h at 37 °C in a humidified incubator at an atmosphere of 95% air and 5% CO_2 . After drug treatment, the old culture medium is removed and the cells were incubated with 100 μl of 10% MTT solution for 2 h at 37 °C, after which the solution was replaced with DMSO, which solubilized the formazan crystals formed by MTT to give a purple /violet solution whose absorbance was measured at 490 nm using a microplate Reader (Thermo LabSystems) Spectrophotometer. Cell viability is expressed as percentage of MTT reduction.

Data analysis

The difference between the control groups and the experimental groups were determined using the one-way Analysis of Variance (ANOVA) and Student's t-test. P-values less than 0.05 were considered as significant. All data were analyzed using Microsoft Office Excel, 2007 and GraphPad Prism Computer software packages.

Results

The percentage cell viabilities of the Model cell lines (MDA-MB 231&436, HeLa and A549) treated with the five fractions (D2-D6) of CWSE of MC at different (50-200 $\mu\text{g/ml}$) concentrations over a period of 24 h were obtained. In Figure 1, the percentage cell viability of MDA-MB 231 cells treated with D2 and D6 were as high as 96.11% and 85.75% respectively at 200 $\mu\text{g/ml}$, the highest tested concentration, rendering these two fractions non cytotoxic. However, for D3, D4, and D5 more than 95% of cells died at this same concentration. D3 being the most potent fraction, presenting only 50.56% cell viability at 100 $\mu\text{g/ml}$, 33.61% viability at 125 $\mu\text{g/ml}$ and 20.66% at 150 $\mu\text{g/ml}$ compared with D4 and D5 which cells' viability were as high as 96.54% and 77.16% at 100 $\mu\text{g/ml}$, 65.85% and 45.63% at 125 $\mu\text{g/ml}$ and 58.70% and 39.01% at 150 $\mu\text{g/ml}$ respectively. In Figure 2, assessing the effect of MC on MDA-MB 436; the percentage cell viability for D2 and D6 were as high as 93.59% and 88.28% respectively at 200 $\mu\text{g/ml}$, the highest tested concentration, rendering them non cytotoxic, while at least 85% of cells died at this same concentration when treated with D3, D4 and D5, with D3 being the most potent extract presenting 64.19% cell viability at 100 $\mu\text{g/ml}$, 34.71% viability at 125 $\mu\text{g/ml}$ and only 12.29% viability at 150 $\mu\text{g/ml}$ compared with D4 and D5 which cells' viability were as high as 74.36% and 69.75% at 100 $\mu\text{g/ml}$, 46.08% and 57.04% at 125 $\mu\text{g/ml}$ and 22.42% and 43.00% at 150 $\mu\text{g/ml}$ respectively. A similar trend was observed for the Lung cancer (A549) cells (Figure 3) as D2- and D4-treated cells had percentage cell viability as high as 89.55% and 81.40% respectively at 200 $\mu\text{g/ml}$, the highest tested concentration whereas, up to about 95% cells died in the D3-treated group while for D4 and D5 about 75% of cells died at this same concentration. D3 being the most potent extract presenting only 42.76% cell viability at 100 $\mu\text{g/ml}$, 31.64% viability at 125 $\mu\text{g/ml}$ and 21.53% at 150 $\mu\text{g/ml}$ compared with D4 and D5 which cells' viability were 49.15% and 53.71% at 100 $\mu\text{g/ml}$, 31.60% and 45.62% at 125 $\mu\text{g/ml}$ and 24.58% and 32.80% at 150 $\mu\text{g/ml}$ respectively. For the HeLa cells (Figure 4), percentage cell viability were as high as 101.62% and 96.76% for D2- and D4- treated cells, respectively at 200 $\mu\text{g/ml}$. However, for D3, D4, and D5 more than 90% of cells died at this same concentration. D3, the most potent extract presented only 54.10% viability at 100 $\mu\text{g/ml}$, 38.58% viability at 125 $\mu\text{g/ml}$ and 27.55% at 150 $\mu\text{g/ml}$ compared with D4 and D5 which cells' viability were as high as 72.65% and 85.38% at 100 $\mu\text{g/ml}$, 66.00% and 48.22% at 125 $\mu\text{g/ml}$ and 43.47% and 42.05% at 150 $\mu\text{g/ml}$ respectively.

Discussion

Plant-derived herbal medicines have been used for a long time in several countries. Anticancer activity is one among all the effects been reported by studies *in vitro* using natural herb extracts (Kandaswami *et al.*, 2005; Cheng *et al.*, 2010). *M. charantia*, also called bitter melon, is a popular vegetable of great medicinal value. It has been named as one of such potent medicinal plants especially in relation to its usefulness in the treatment and management of cancers (Kubola and Siriamornpun, 2008). For instance, Kuguacin J and momorcharins, phytochemicals derived from the plant were effective against human

prostate cancer (Xiong *et al.*, 2009; Pitchakarn *et al.*, 2011). These findings suggest that *M. charantia* has great potential as a health food or a source for new drug development.

In the current study, we determined the cytotoxicity of five fractions derived from the crude water-soluble extract of *M. charantia* on four selected cancer cell lines at varying concentrations.

The observed effects of three of the fractions obtained from the crude water soluble extract of *Momordica charantia* depict that these fractions are potent cytotoxic agents against the selected cancer cells. For example, the Ethylacetate fraction (D3) which is believed to be rich in flavonoids (Xu and Chang, 2007) exhibited consistently high cytotoxicity against all cancer cell lines, even at the least tested concentration of 50 µg/ml. Likewise D4 and D5 albeit, at a lower toxicity level. On the contrary, D2 and D6, two of the fractions obtained from the extract showed no significant cytotoxic effects, as concentration of viable cells were still very high (93.59% and 88.28% respectively) even at the highest concentration of 200µg/ml.

Thus, D3 was adjudged the most potent fraction presenting a cell viability of less than 10% in all cell lines at the highest tested concentration.

We have thus been able to show that though the medicinal plant of interest has cytotoxicity against cancer cells, the solvents in which the plant is extracted play a key part in its effectiveness in the killing cancer cells. Also, the concentrations at which these fractions of MC are administered matter. Generally, the higher the concentration, the more cytotoxic the fractions become. Our observation is further underscored by the finding that MC has anti-leukemia and antiviral activities (Ng *et al.*, 1994), inhibits the growth of several cancer cell lines, including prostate adenocarcinoma, (Clafin *et al.*, 1978), human colon cancer (Caco-2 cells) (Yasui, *et al.*, 2005), the highly metastatic breast cancer cell lines MDA 231(Lee-Huang *et al.*, 2000).

Conclusively, we submit that the effective drugs are D3, D4, D5. There are differences in the sensitivity of the different cell lines to the different drugs for example, MDA-MB 436 cells differ slightly in their sensitivity to the tested drugs as seen in the cell viability assay results; while D3 at 100µg/mL showed percentage cell viability as low as 50.57%, 42.76% and 54.10% for MDA-MB 231, A549 and HeLa respectively, the percentage cell viability at this concentration for MDA-MB 436 was as high as 64.19%, short of the standard IC₅₀. We also deduced that the most efficacious drug concentration for the fractions are between 100 and 125µg/ml while the most potent of the fractions is D3.

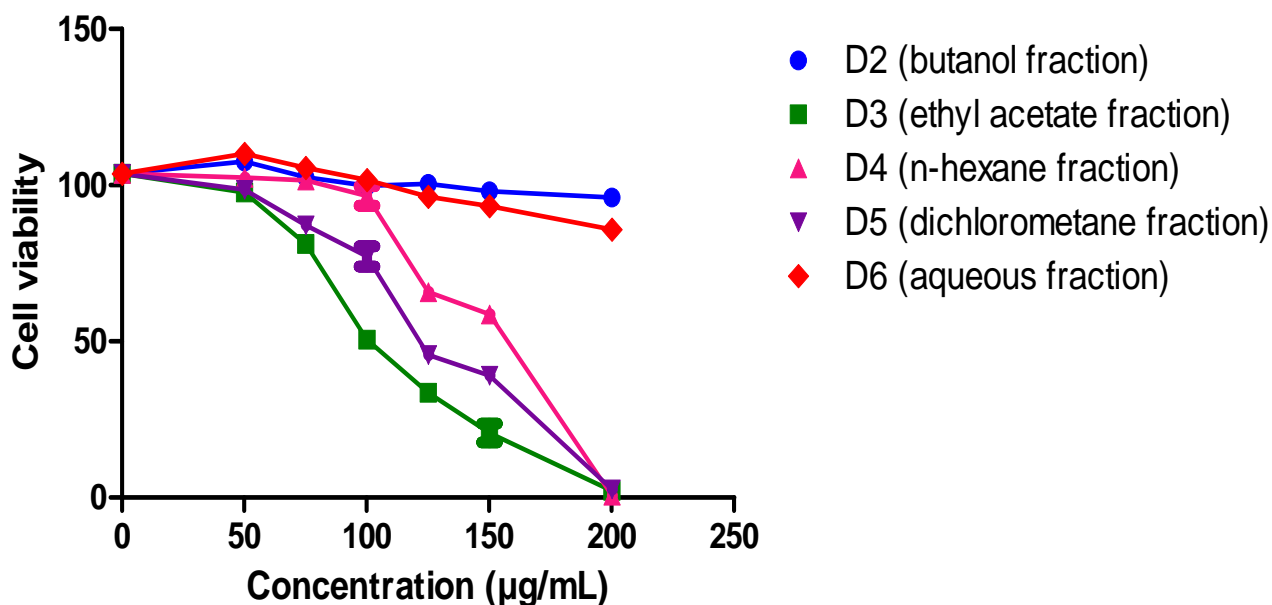


Figure 1: The cell viability of MDA-MB 231 cells at different (50-200 µg/ml) concentrations of the five fractions (D2-D6) at 24 h. Data are presented as mean ±SEM (n=5 per group). Level of significance was taken at *p<0.05

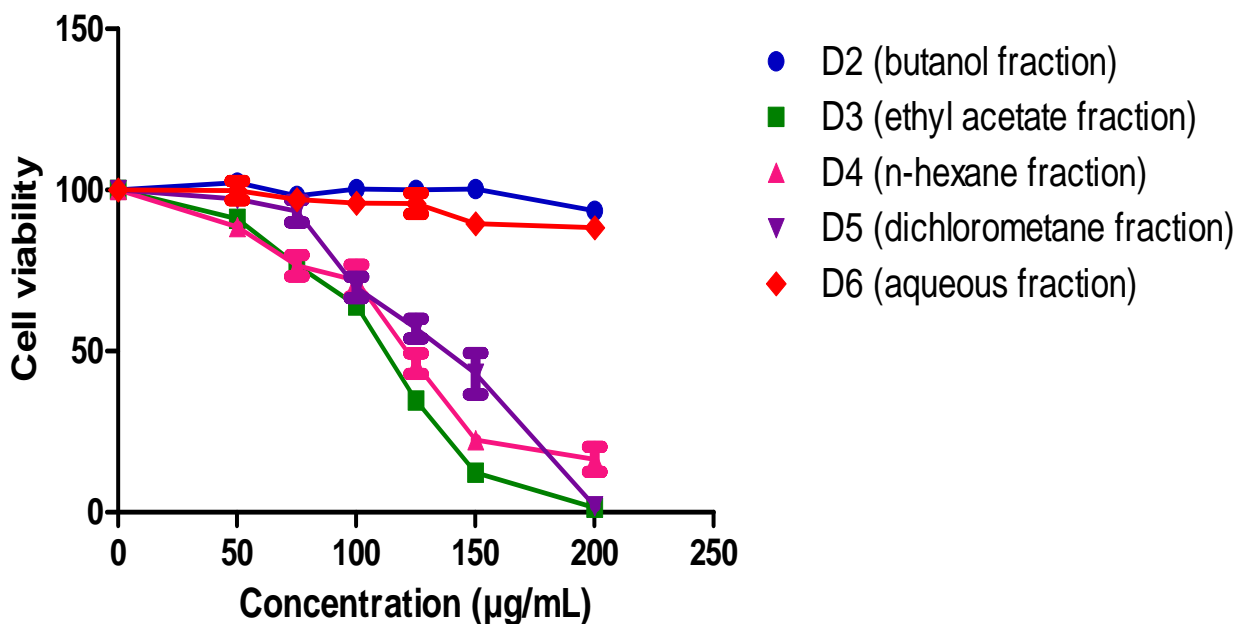


Figure 2: The cell viability of MDA-MB 436 cells at different (50-200 µg/ml) concentrations of the five fractions (D2-D6) at 24 h. Data are presented as mean ±SEM (n=5 per group). Level of significance was taken at *p<0.05

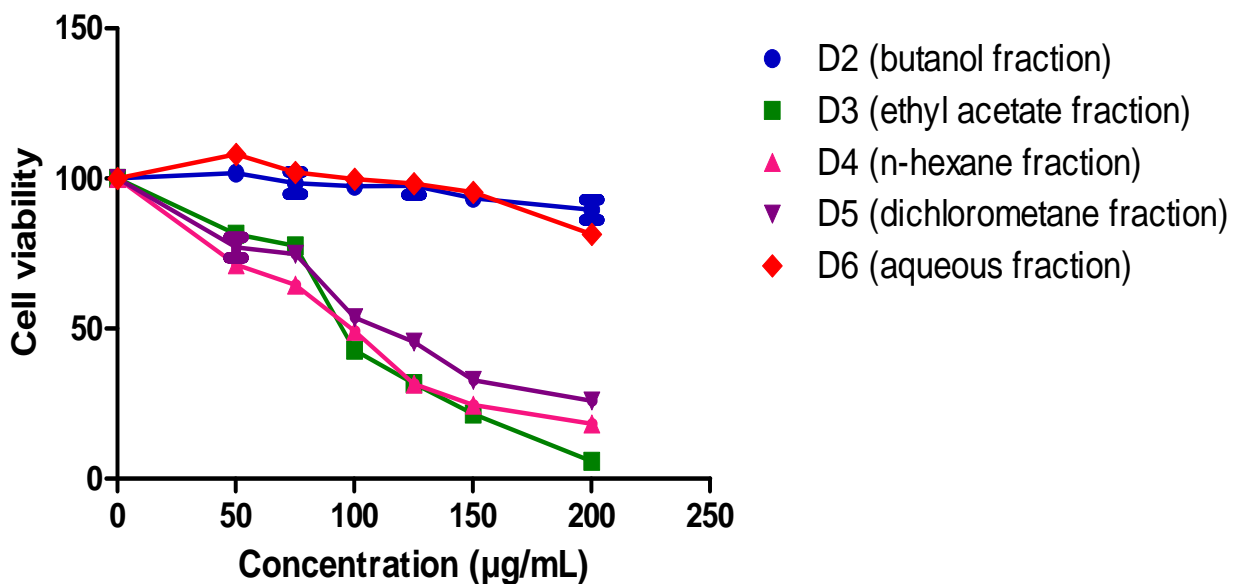


Figure 3: The cell viability of A549 cells at different (50-200 µg/ml) concentrations of the five fractions (D2-D6) at 24 h. Data are presented as mean ±SEM (n=5 per group). Level of significance was taken at *p<0.05

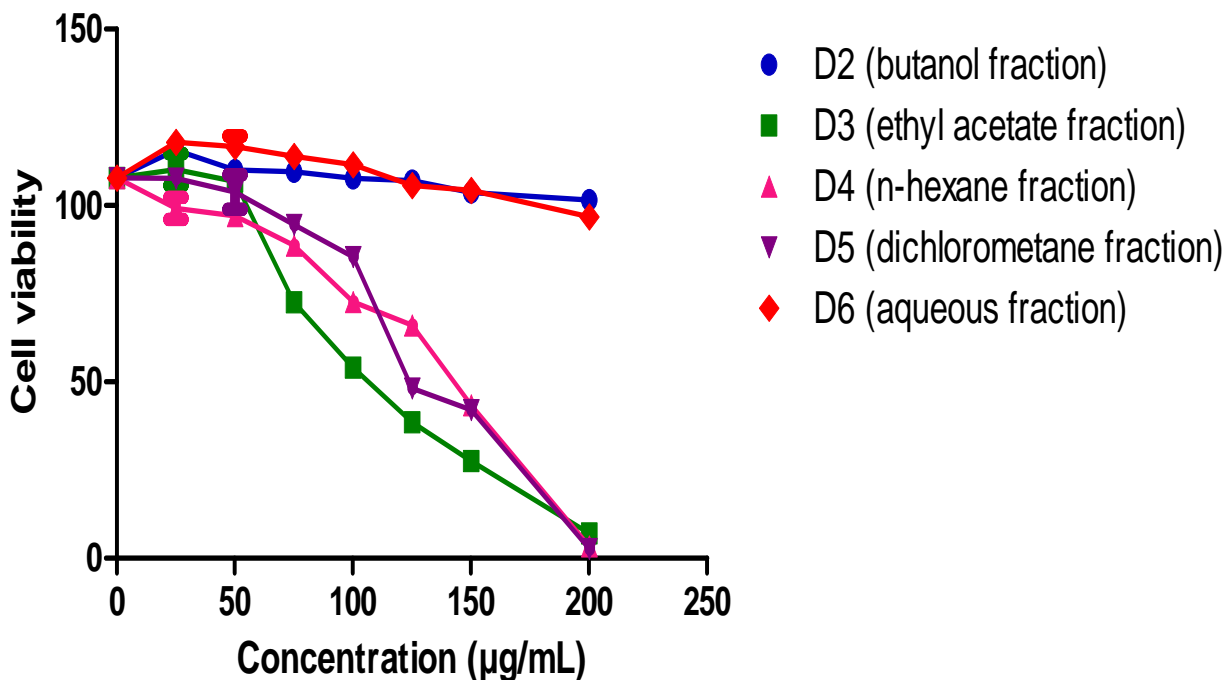


Figure 4: The cell viability of HeLa cells at different (50-200 µg/ml) concentrations of the five fractions (D2-D6) at 24 h. Data are presented as mean ±SEM (n=5 per group). Level of significance was taken at *p<0.05

Conclusion

This present work of ours aims at evaluating the cytotoxicity of the plant *Momordica charantia* on selected cancer cell lines, the crude water-soluble extract was partitioned into five fractions; N-hexane, Dichloromethane, Ethyl acetate, Butanol and Aqueous fractions. We gathered that of all the fractions, D3 was the most potent as it resulted into the least (<10%) cell survival after a 24-hour treatment on all the cell lines i.e for MDA-MB 231 (<1%), MDA-MB 436 (<1%), A549 (5.83%) and HeLa (7.01%) at the optimal tested concentration (200µg/ml), though D3 and D4 also elicited convincing cytotoxic responses. Further work is underway to elucidate the components of the most potent fraction D3, as well as determine the probable mechanism of action underlying the observed cytotoxicity.

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