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In Vitro Studies of Antiproliferative Properties of Aqueous leaf Extracts of *Moringaoleifera* and *Globimetulabrauniion* on the Human Pancreatic Cell Line AsPC-1

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Abstract

Cancer of the pancreas is the fourth deadliest form of cancer in the world as symptoms do not manifest until the advanced stages. Conventional methods of treatment have less than 10% rate of success. Therefore the need exists to develop anticancer agents from medicinal plants to support conventional methods. The objective of this study was therefore to determine the anti-proliferative effect of aqueous Moringa oleifera and Globimetula braunii leaf extracts on the human pancreatic cancer AsPC-1 cell line. Human pancreatic (AsPC-1) cells were seeded (1.07 x 10^4 cells/well) into 96 well plates and exposed to graded (0 – 10000 µg ml^{-1}) concentrations of each extract for 24 hours, thereafter the anti-proliferative effect of the extracts were evaluated in -vitro using the MTT [3-(4,5-dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. Leaf extracts of Moringa oleifera and Globimetula braunii exhibited dose dependent inhibition of AsPC-1 cell proliferation, with IC_{50} values of 156.25 µg ml^{-1} and 312.50 µg ml^{-1}, respectively. Of both plant extracts tested on AsPC-1, the aqueous leaf extract of had a higher inhibitory effect. This result suggests the presence of key phytochemical or a group of phytochemical compounds with anti-proliferative properties such as flavonoids, tannins or cyanogenic glycosides in the tested extracts.

Key words: Anti-proliferative, MTT, AsPC-1, *M. oleifera*, *G. braunii*

INTRODUCTION

Pancreatic cancer is the fourth commonest cause of cancer-related mortality across the world (1), and the most common gastrointestinal malignancy in Lagos, Nigeria (2). Palliative therapy through administration of gemicitabine and capecitabine at appropriate therapeutic level has less than a 10% rate of success (3). Poor management of the disease in Nigeria is due to the cost of treatment which most people in this part of the world cannot afford and the lack of specialists in the field (4).

According to the World Health Organization (5), about three quarter of the world's population currently use herbs and other forms of traditional medicines to treat diseases and more than 80% of people in developing countries depend on traditional medicine for their primary health needs. *Moringa oleifera* (6; 7) and Globimetula braunii (8; 9; 10) are major medicinal plants used in traditional medicine in Nigeria and around the globe, thus it is necessary to determine their anti-proliferative potential on cancer cell lines and to explore their possibility for use in the treatment of pancreatic cancer.

METHODOLOGY

Plant materials and preparation of crude extracts

The plants used for this study; *Globimetula braunii* and *Moringa oleifera* were purchased from Oyingbo market in the Lagos Metropolis of Nigeria, authenticatedat the Forestry Research Institute of Nigeria Herbarium, Ibadan and at the University of Lagos Herbarium with voucher numbers FHI 107741 and LUH 4558, respectively. The plant samples were air dried in the shade for seven (7) days, pulverized and kept in storage containers at 4°C prior to use. The plant derived aqueous extracts used in this study were prepared by soaking 245.00g of *Moringa oleifera* and 145.00g of *Globimetula braunii* powdered leaves in 950mL and 1200mL of distilled water respectively with intermittent stirring for 3 days at room temperature. The mixtures were then filtered twice through a sterile filter paper into a sterile beaker, concentrated under vacuum using a rotary evaporator at 50°C, freeze dried and stored at 4°C until use. The yield obtained after extraction was 6.02g of Moringa oleifera and 0.33g of Globimetula braunii. Stock solutions (100 mg/ml) of the extracts in culture medium were prepared and 0.22micron membrane filter sterilized before use.

In vitro assay for cytotoxic activity

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Cell lines and culture conditions

Human pancreatic cancer AsPC-1 cell line procured from Cell Line Services (CLS), Germany were cultured in RPMI 1640 medium supplemented with 4.5 g/L glucose, 2mM L-glutamine, 1 mM sodium pyruvate, 10% fetal bovine serum, 100 units/ml penicillin and 100 µg/ml streptomycin. Cells grown in T75 culture flask were maintained at 37°C in a 5% CO2 atmosphere with 95% humidity and passaged at 75% confluency.

Cytotoxicity screening using MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide)] assay

Cells grown as monolayer in T75 culture flask were washed twice with phosphate buffered saline (Ca2+ and Mg2+ free), trypsinized (0.05% trypsin/EDTA) and suspended in culture medium. The cell count was determined using a counting chamber (haemocytometer) while viewing the cells through an inverted microscope and the cells were then seeded in 96-well plate at 1.07 x 104cells/well except the cells in the 12th column which received 100µl of medium only, serving as the blank. After 24hrs incubation at 37^{o}C, the medium was replaced with fresh medium or medium containing graded (0 - 10,000 µg ml^{-1}) concentrations of plant extracts (in triplicates) and the cells incubated for another 24hrs, after which 10µl (5 µg ml^{-1}) of (MTT) solution was added into each well. The incubation was continued at 37°C for another 3hrs then the medium was carefully removed from each well, 100µl of solubilizing agent (acidic isopropanol) was added, the plate was covered with a foil and agitated on an orbital shaker for 5mins to ensure complete dissolution of the formazan crystals. The optical density (OD) was determined at 492nm using a plate reader (Biotek® Synergy HT Spectrophotometer) against the blank. Finally, percent cell viability was calculated as follows:-

Percentage (%) cell viability
= (Optical density of the sample / Optical density of the control) ×100

The IC_{50} values were calculated using GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA).

RESULTS

Aqueous *Moringa oleifera* and *Globimetula braunii* leaf extracts reduced the viability of AsPC-1 cells in a dose-dependent manner with increasing extract concentrations. AsPC-1 cells were more susceptible to the treatment with *Moringa* extract (IC_{50}=156.25 µg ml^{-1}) than to *Globimetula* (IC_{50}= 312.50 µg ml^{-1}) (Figures 1 and 2). Treatment with *Moringa* extract at concentrations higher than 2500 µg ml^{-1} was significantly ($P<0.01$) toxic to the human pancreatic cancer cell line. At concentrations greater than 2500.00 µg ml^{-1}, the leaf extract of *Globimetula braunii* (Fig. 2) was significantly ($P<0.001$) toxic to the human pancreatic cancer cells.

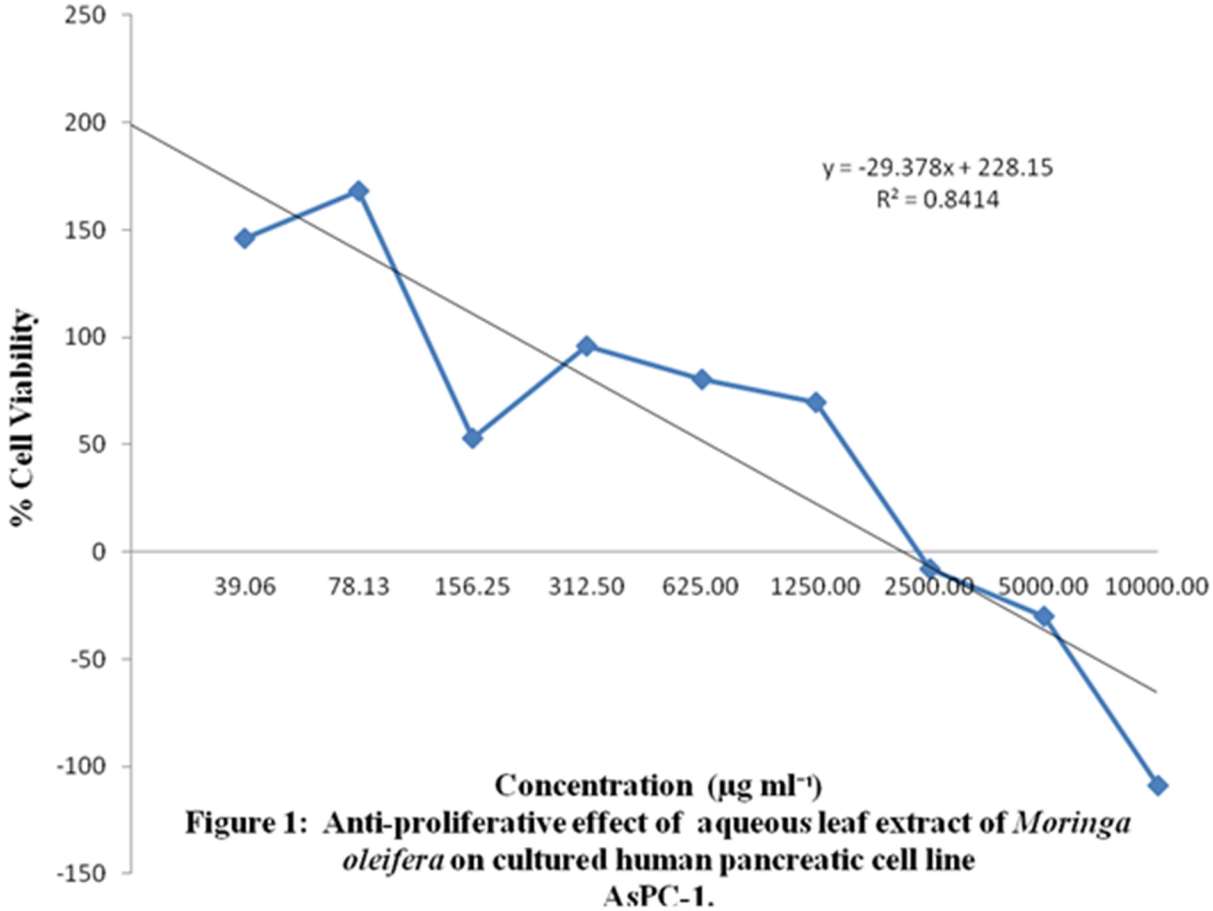

Figure 1: Anti-proliferative effect of aqueous leaf extract of *Moringa oleifera* on cultured human pancreatic cell line AsPC-1.

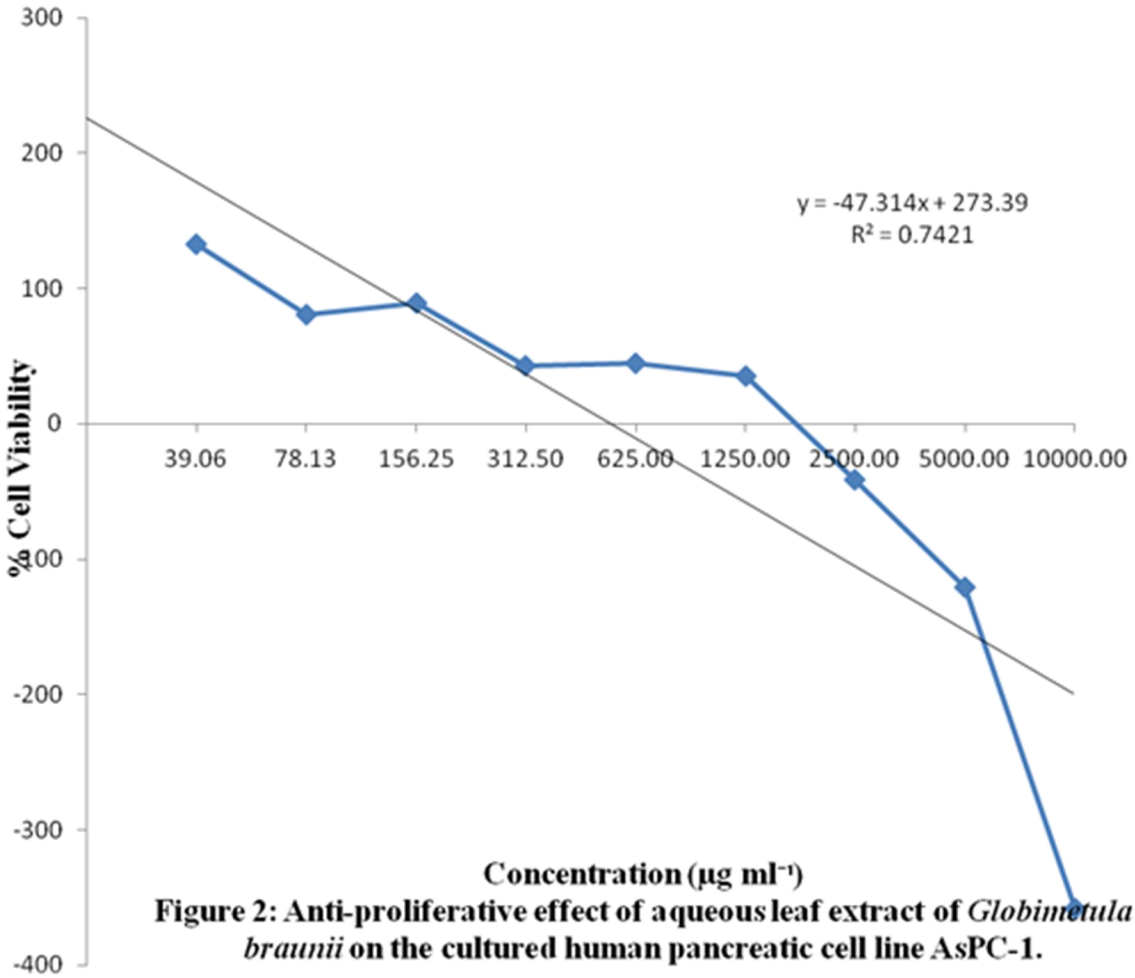

Figure 2: Anti-proliferative effect of aqueous leaf extract of *Globimetula braunii* on the cultured human pancreatic cell line AsPC-1.

DISCUSSION

The degree of reduction of MTT is an indicator of the functional integrity of mitochondria and hence, of cellular respiratory viability (11). The findings from this study indicate that *Moringa oleifera* aqueous leaf extract is cytotoxic to and have high inhibitory effects on the pancreatic cell line ASPC-1 at concentrations greater than 1250.00 µg ml^{-1}. Previous studies also observed that aqueous Moringaoleifera leaf extract inhibited the growth of the pancreatic cancer cell lines (Panc-1 and COLO-357) in a concentration-dependent manner (12). The results of this study lay credence to the anti-proliferative effects of aqueous leaf extract of *M. oleifera* plant on human pancreatic cancer (Aspc-1) cell lines.

This result is in agreement with that of Parvathay and Umamaheswari (13), which attributed the anticancer properties of the plant to the presence of flavonoid or alkaloid group of compounds similar to vincristine or vinblastin in the *Moringa* leaves.In addition, *Moringa oleifera* leaves contain glucosinolates, isothiocyanates, niazimicin, niaziminin and quercetin which may confer anticancer effect (14; 15; 16). The extract of *M. oleifera* had a more potent toxic effect on pancreatic carcinoma cells (ASPC-1) thus inferring a greater potential for the treatment of pancreatic cancer than the extract of *Globimetula braunii*. Various parts of the plant have been observed to

exhibit medicinal properties such as anti-cancer properties due to the presence of different bioactive ingredients (17; 18; 19). This implies the presence of a key phytochemical or a group of phytochemical compounds in the extract of *M. oleifera* that are absent in the *G. braunii* counterpart. Further research on the anti-cancer effects of specific bioactive metabolites derived from *Moringa oleifera* and *Globimetula braunii* is necessary.

CONCLUSION

The results of this study support the folk use of *M. oleifera* and *G. braunii* as anticancer plants. More rigorous study is required in order to achieve a level of proof required for full biomedical endorsement of *Moringaoleifera* and *Globimetulabraunii* as plants possessing anticancer properties.

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