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Biochemical characterization of *Momordica charantia* (leaf and fruit) and effect of soluble extract on MCF-7 breast cancer cell lines

A.A. ANSARI[1,♥], J. SINGH[2], M. AMINUDDIN[3]
[1]Department of Biology, Faculty of Natural Sciences, University of Guyana, Georgetown, Guyana. ♥email: abdullah.ansari@uog.edu.gy
[2]School of Forensic and Applied Science, University of Central Lancashire. Preston, UK
[3]Texila American University. Georgetown, Guyana

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Abstract. *Ansari AA, Singh J, Aminuddin M. 2019. Biochemical characterization of* Momordica charantia *(leaf and fruit) and effect of soluble extract on MCF-7 breast cancer cell lines. Cell Biol Dev 3: 1-5.* Cancer is a serious worldwide problem targeted by various treatments, including traditional medicinal plants. One known medicinal plant is bitter gourd (Momordica charantia), which has been investigated for its anti-cancerous properties. This study was carried out to explore the biochemical analysis of different components of *M. charantia* (leaf and fruit) and the effect of alcoholic extract of *M. charantia* to investigate their potential effect on MCF-7 breast cancer cell line in comparison to cisplatin, a commercial anti-cancer drug. The different components (leaf and fruit) were separated, dried, and converted to powdered form. MCF-7 (human mammary primary epithelial cancer cells) breast cancer cell line was treated with different concentrations (8 - 800 µg/mL) of the soluble extract and cisplatin (all dissolved in DMSO and diluted in the incubating medium) for 48 hours. Initial time course experiments established that maximal cell death occurred between 24-48 hours. Cell viability (cell death) was measured using an established method. The results have shown that with the MCF-7 cell line, the extract at a high concentration (800 µg/mL) was more effective in killing the cancer cells when compared to cisplatin. The present results have clearly shown that the soluble ethanol extract of *M. charantia*, especially at high doses, can be used effectively to treat breast cancer.

Keywords: Breast cancer, cell viability, cisplatin, *Momordica charantia*

INTRODUCTION

Medicinal plants have been used to treat acute diseases like diabetes and cancer. Research has focused on using tropically grown plants like bitter gourd (Momordica charantia), which has been part of the human diet for centuries (Heinrich and Bremner 2006). Bitter gourd is cultivated throughout South America, Asia, and Africa, including Guyana, for food and medical values (Singh et al. 2004). All the components (fruit, leaves, and stem) are known for potential medical values. In recent decades, many studies have been conducted for anti-cancer, anti-diabetic, anti-viral, anti-helminthic, antioxidant, and anti-bacterial properties (Ahmed et al. 1999; Basch et al. 2003; Alessandra et al. 2008; Lee et al. 2014). The universal properties of bitter gourd may be due to the presence of many biologically active phytochemical constituents such as triterpenes, proteins, steroids, alkaloids, inorganic lipids, and phenolic compounds (Zhu et al. 1990; Murakami et al. 2001; Parkash et al. 2002; Grover et al. 2004).

Cancer is a complex disease of uncontrolled growth of cells due to signaling failure of oncogenic expressions resulting in many different types of cancers based on the origin of tumors in specific organs. Breast cancer is one of the most common cancers, emphasizing mammary gland epithelial cell cancer. The most common form of cancer affecting the human population worldwide is breast cancer, especially among females and is the major cause of mortality and is caused by aging, pollution, exposure to chemicals and ionizing radiations, genetic causes, lifestyle, and many other reasons (Torre et al. 2015; Bai et al. 2016). There are potentially many medicinal plants with therapeutic properties that have been used traditionally in many countries and are also being researched by various groups in the form of extracts against different types of cancer for possible treatments (Dandawate et al., 2016; Singh et al., 2016). *Momordica charantia* (bitter melon) is a common vegetable used as a source of food and medical values for treating many diseases as part of indigenous knowledge. All the parts of bitter melon, such as fruits, leaves, and stems, are known to have anti-cancer, antipyretic, anti-diabetic, anti-hypertensive, and multiple other positive effects on human health (Manoharan et al. 2014; Dandawate et al. 2016; Singh et al. 2016). Many researchers have investigated the effect of water, alcohol, and other organic solvent-based extracts on cancer cells that would inhibit or arrest growth by releasing cytochrome c, apoptosis induction, interference in the cell cycle, autophagy, and stem cell growth inhibition. Many bioactive compounds such as cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, essential oils, saponins, fatty acids, and proteins present in bitter melon may have a role in anti-cancer properties (Manoharan et al. 2014; Dandawate et al. 2016; Singh et al. 2016).

Several studies have been conducted on the effect of bitter gourd and have shown anti-cancer activity against lymphoid leukemia, lymphoma, choriocarcinoma,

melanoma, breast cancer, skin tumor, and prostatic cancer (Ganguly et al. 2000; Sun et al. 2001). The hot water extract of *M. charantia* was found to be inhibitory against inhibited uterine adenomyosis and mammary tumor growth in mice. The maximum effect was conferred by peel extract (Singh et al. 1998; Nagasawa et al. 2002). The anti-cancerous activity of water-soluble extract *M. charantia* has been reported through inhibition of DNA, RNA, and cellular protein synthesis (Licastro et al. 1980; Zhu et al. 1990; Tsao et al. 1990; Chang et al. 2008). The researchers show evidence of effective cancer treatment through *M. charantia*, and its extract controls cancer cell growth and tumor formation (Cunnick et al. 1990).

This study was designed to investigate the biochemical analysis of *M charantia* (different components-Leaf and fruit) and the anti-cancer properties of an alcoholic soluble extract of *M charantia* (different components-Leaf and fruit) on isolated breast cancer cell lines (MCF-7). In addition, the effect of cisplatin was investigated for comparison.

MATERIALS AND METHODS

Sample preparation

The leaves and fruits of *Momordica charantia* were collected locally from Guyana (Figure 1). These were shade dried and made into a coarse powder and stored in an air-tight container for biochemical analysis and testing against the growth of MCF-7 breast cancer cell lines.

Biochemical analysis of *M. charantia*

The biochemical analysis was carried out using ICAP-OES 7000 series spectrometer (inductivity coupled plasma-optical emission spectrometry) at the Chemistry lab (University of Central Lancashire, Preston, UK) from March to May 2018. 500 mg of each sample was digested with a mixture of 8 mL nitric acid and 2 mL hydrogen peroxide, and analysis was done using ICP-OES. The concentration (mg/g) of sodium, calcium, magnesium, potassium, manganese, ferrous, copper, and zinc were analyzed.

Preparation and application of *M. charantia* extract on MCF-7 breast cancer cell line

These experiments were conducted at the Biomedical Research lab (Tissue culture), University of Central Lancashire, Preston, the UK, from March to May 2018. First, 20 mg of each of the samples (leaf and fruit) of *M. charantia* was dissolved in 1 mL DMSO and 1 mL PBS by continuous stirring with a sonicator (stock solution). Next, this was followed by preparing different concentrations, namely 8, 80, and 800 µg/mL. Similarly, 5 mg of cisplatin was dissolved in 5 mL PBS and diluted to 8, 80, and 800 µL/mL concentrations. Next, the different concentration of extract in the cell medium was transferred in triplicate using a Gilson pipette to 96 well plates to give a final volume of 200 µL to the treated cell wells. Finally, the medium's equivalent volume of 200 µL was added to the control (untreated) well with cells. In this study, both time-course and dose-dependent experiments were performed. The time-course experiments were done initially for 48 hours to determine the time that produced maximal cell death. Then, dose-dependent experiments were done during the incubation period of 48 hours, either alone or combined. At the end of the treatment with either the extract or cisplatin, the viability of the cells was measured using an established fluorescent signal luminogenic ATP-assay method (Manoharan et al., 2014).

A **B**

Figure 1. *Momordica charantia.* A. Fruit and leaves, B**.** Flower

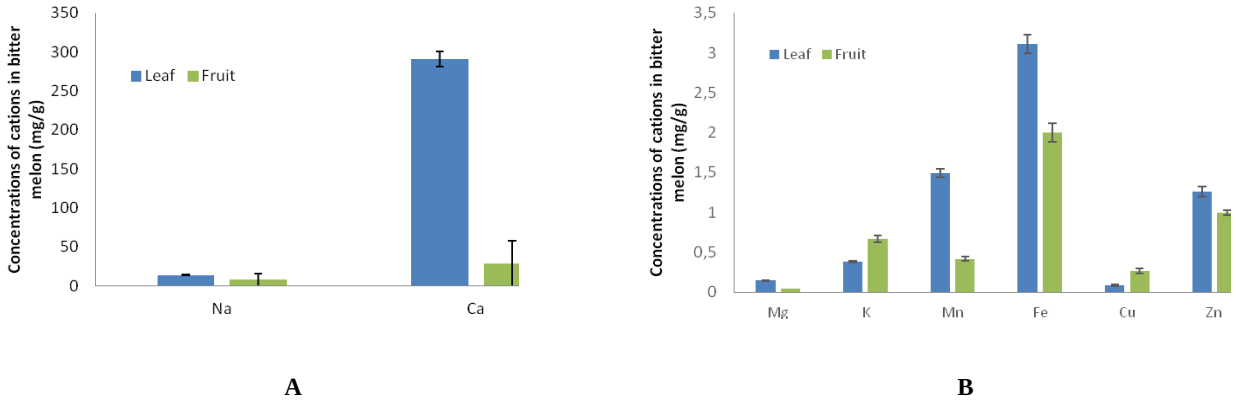

Figure 2. A. Metal analysis (sodium and calcium) of leaf and fruit samples, B. Metal analysis (magnesium, potassium, manganese, ferrous, copper, and zinc) of leaf and fruit samples

Time-course effects of the crude water-soluble extract of *M. charantia* on cell viability

The time-course effects (12, 24, 36, and 48 hours of 800 µg/mL of the extract of *M. charantia* on the viability of MCF-7 were initially done to establish the time that produced maximal cell death. From these initial time-course experiments, it was established that cell death increased to its maximal level after 24-48 hours of incubation with the alcohol-soluble extract of *M. charantia*. Therefore, the incubation time of 48 hours was employed in all dose-dependent and combined dose experiments in this study.

RESULTS AND DISCUSSION

The biochemical composition of bitter melon (*M. charantia*) suggests it is a good source of essential elements like Ca, Mg, Mn, Cu, and Zn. The results of the biochemical analysis are illustrated in Figure 2.A-B, which substantiate the vital role of bitter melon in providing essential nutrients.

The concentration of calcium (290.87±9.86, 28.96±2.2) was highest, followed by sodium (13.89±0.46, 7.76±0.52) and ferrous (3.11±0.12, 1.99±0.11) in both leaf and fruit samples respectively. The least concentration was recorded concerning copper (0.085±0.01) for leaf samples, whereas minimum magnesium concentration (0.044±0.002) was observed for fruit samples. Next, the Na, Mg, Ca, Mn, Fe, and Zn concentrations were higher in leaf than in fruit samples, whereas K and Cu were greater in fruit than in leaf samples. Single-factor ANOVA at p=0.5 suggests that the variation in concentration of metal ions in leaf and fruit samples was significant (leaf p=3.62E-23; fruit p=3.24E-17).

Figure 3.A-C shows MCF-7 cancer% cell death at different concentrations (8, 80, and 800 µg/mL) of the extract or cisplatin compared to untreated cells. The results show that low and moderate doses of the extract induce a lower death rate of the MCF-7 cells where are a high dose of 800 µg/mL) maximizes the death of MCF-7 cancer cells. Maximum cell death was recorded at all concentrations of leaf extract when compared to fruit extract or cisplatin.

At 8 mg, the death rate of MCF cells after 24 h of incubation was 0.53% with cisplatin. Further death of 8.49% was recorded after 48 h. That was less effective compared to leaf and fruit extract. With leaf extract, the death rate was 39.88% after 24 h incubation. 19.2% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (2.78% after 24 h and 8.87% after 48 h).

At 80 mg, the death rate of MCF cells after 24 h of incubation was 0.69% with cisplatin. Further death of 20.4% was recorded after 48 h. That was less effective compared to leaf and fruit extract. With leaf extract, the death rate was 60.46% after 24 h incubation. 37.74% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (3.73% after 24 h and 9.6% after 48 h).

At 800 mg, the death rate of MCF cells after 24 h of incubation was 7.91% with cisplatin. Further death of 29.26% was recorded after 48 h. That was less effective when compared to leaf and fruit extract. With leaf extract, the death rate was 65.9% after 24 h incubation. 55.84% more cells died after 48 h. Fruit extract was less effective in controlling the growth of MCF-7 cells (5.77% after 24 h and 14.58% after 48 h).

The highest cell death of 65.9% at 24 h and 55.84% at 48 h are observed for leaf extract at 800 mg concentration when compared to fruit extract (5.77% at 24 h and 14.58% at 48 h) and cisplatin (7.91% at 24 h and 29.26% at 48 h). Furthermore, two-way ANOVA shows statistical significance between the different treatments - cisplatin, leaf, and fruit extracts (p=0.001 at 24 h and p=0.03 at 48 h) on% cell death at different concentrations (8 to 800 mg) at 24 hours and 48 hours of the incubation period.

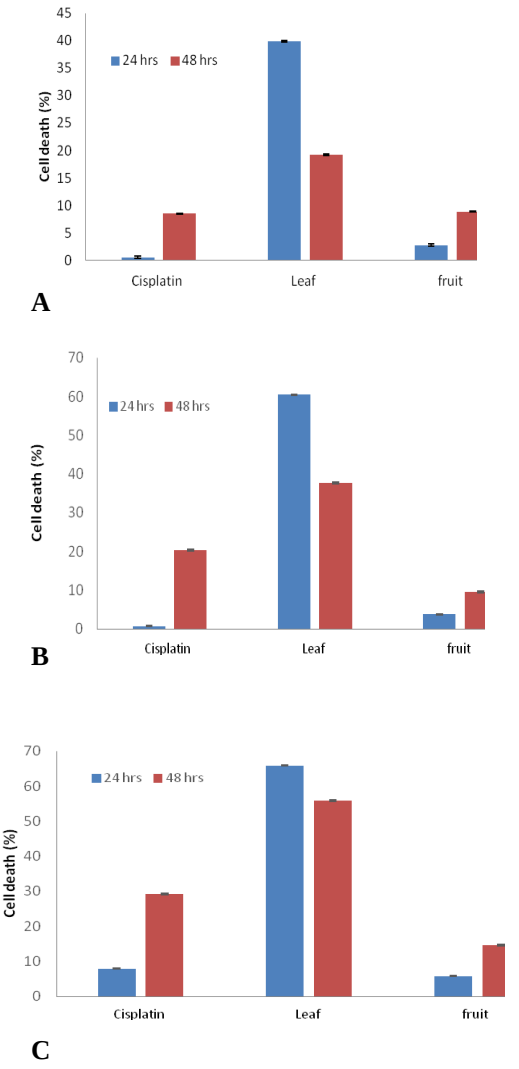

Figure 3. A. Cell death (%) at 8 mg concentration of leaf and fruit extracts, B. Cell death (%) at 80 mg concentration of leaf and fruit extracts, C. Cell death (%) at 800 mg concentration of leaf and fruit extracts

The results presented in this study have demonstrated that either the bitter melon extract or cisplatin, especially at high doses, can elicit marked and significant decreases in cell viability (cell death). Furthermore, low doses of either the extract or cisplatin seemed to cause a proliferation of MCF-7 cancer cells. In contrast, high doses, especially at 800 µg/mL, killed the MCF-7 cells compared to untreated cells (Figure 3c). While a moderate dose (80 µg/mL) of either the extract (Figure 3b) or a low dose (8 µg/mL) of cisplatin only kills about 8.49% of the MCF-7 cancer cells (Fig 3a). Reports by various researchers (Chuang et al. 2006; Nerurkar and Ray 2010; Nhiem et al. 2012; Cao et al. 2015; Bai et al. 2016) suggest that bitter melon contains cations and bio-active compounds (triterpenoids, glycosides, saponins, alkaloids, oils, protein and steroids) are probably responsible for anti-tumor effects.

In conclusion, the results indicate that cations and bio-active compounds present in leaf and fruit extracts are at desired levels and may have a potential role in effectively controlling cancer cells (MCF - cells). The different extracts of leaf and fruit components of bitter melon are more effective in controlling the growth of MCF-7 cells when compared to control-cisplatin. Furthermore, compared to fruit, the leaf was better in reducing the number of cancer cells, which supports the use of plant-based treatments for different cancers.

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