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

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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 problem worldwide that has been targeted by variety of treatments that include the use of traditional medicinal plants. One such known medicinal plants are bitter gourd (*Momordica charantia*) that 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 effect of alcoholic extract of *M. charantia* to investigate their potential effect 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 established method. The results have shown that with MCF-7 cell line, the extract at high concentration (800 µg/mL) was more effective in killing the cancer cells when compared to cisplatin. The present results have clearly shown that either the ethanol soluble 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 for the treatment of critical diseases like diabetes and cancer. Researches have focused on use of tropically grown plants like bitter gourd (*Momordica charantia*) which has formed part of human diet for many centuries (Heinrich and Bremner 2006). Bitter gourd is cultivated throughout South America, Asia, and Africa, including Guyana that is used for food and medical values (Singh et al. 2004). All the components (fruit, leaves, and stem) are known for potential medical values. In the last 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 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 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. One of the most common cancer is breast cancer where emphasis is on mammary gland epithelial cell cancer. The most common form of cancer affecting the human population world wide is breast cancer especially among the 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 common vegetable used as source of food and medical values for treatment of many diseases as part of indigenous knowledge. All the parts of bitter melon such as fruits, leaves, and stem are known to have anti-cancer, anti-pyretic, anti-diabetic, anti-hypertensive and multiple other positive effects on human health are known (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 different types of cancer cells that seem to inhibit or arrest the growth by release of cytochrome c, apoptosis induction, interference in 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 which 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 anticancer 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. Maximum effect was conferred by peel extract (Singh et al. 1998; Nagasawa et al. 2002). 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 researches show that there are evidence of effective cancer treatment through the use of *M. charantia* and its extract have shown to control cancer cell growth and tumor formation (Cunnick et al. 1990).

This study was designed to investigate 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). 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 coarse powder and stored in an air-tight container for biochemical analysis and testing against 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 Chemistry lab (University of Central Lancashire, Preston, UK) during March-May 2018. 500 mg of each sample was digested with 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 Biomedical Research lab (Tissue culture), University of Central Lancashire, Preston, UK during March-May 2018. 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 sonicator (stock solution). 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. The different concentration of extract in 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. An equivalent volume of 200 µL of the medium 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 over a period of 48 hours to find out the time that produced maximal cell death. Dose-dependent experiments were done during incubation period of 48 hours either alone or when 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

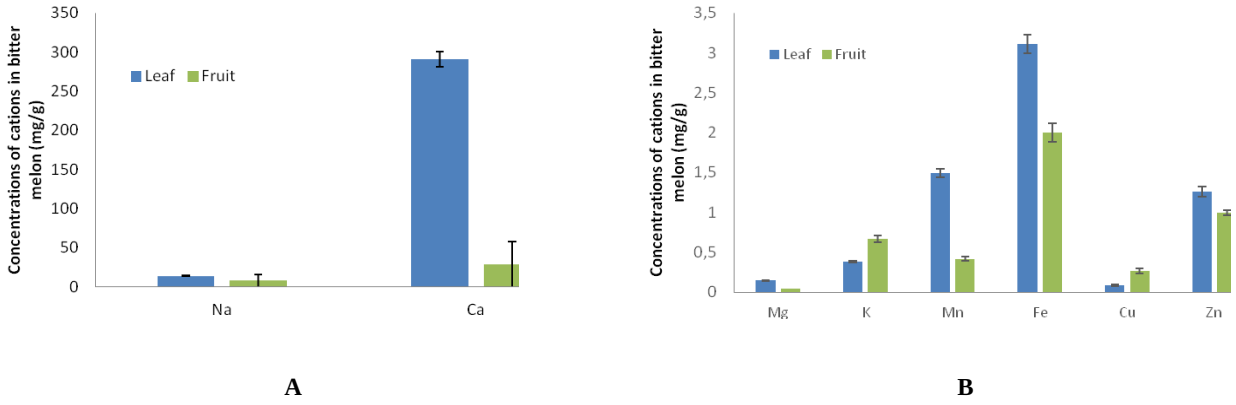

Figure 2. A. Metal analysis (sodium and calcium) of leaf and fruit samples, B. Metal analysis (magnesium, potassium, manganese, ferrous, copper and zinc) of leaf and fruit samples

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 was 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*. 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 that it is a good source of essential elements like Ca, Mg, Mn, Cu, and Zn. The results of biochemical analysis are illustrated in Figure 2.A-B, which substantiate 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 with reference to copper (0.085±0.01) for leaf samples whereas minimum magnesium concentration (0.044±0.002) was observed for fruit samples. The concentration of Na, Mg, Ca, Mn, Fe and Zn were higher in leaf compared to fruit samples whereas K and Cu were greater in fruit compared to 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 either the extract or cisplatin when compared to untreated cells. The results show that low and moderate doses of the extract induce 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 period was 0.53% with cisplatin. Further death of 8.49% was recorded after 48 h. This was less effective when 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 found to be 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 period was 0.69% with cisplatin. Further death of 20.4% was recorded after 48 h. This was less effective when 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 found to be 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 period was 7.91% with cisplatin. Further death of 29.26% was recorded after 48 h. This 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 found to be less effective in controlling the growth of MCF-7 cells (5.77% after 24 h and 14.58% after 48 h).

Highest cell death of 65.9% at 24 h and 55.84% at 48 h is 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). 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 incubation period.

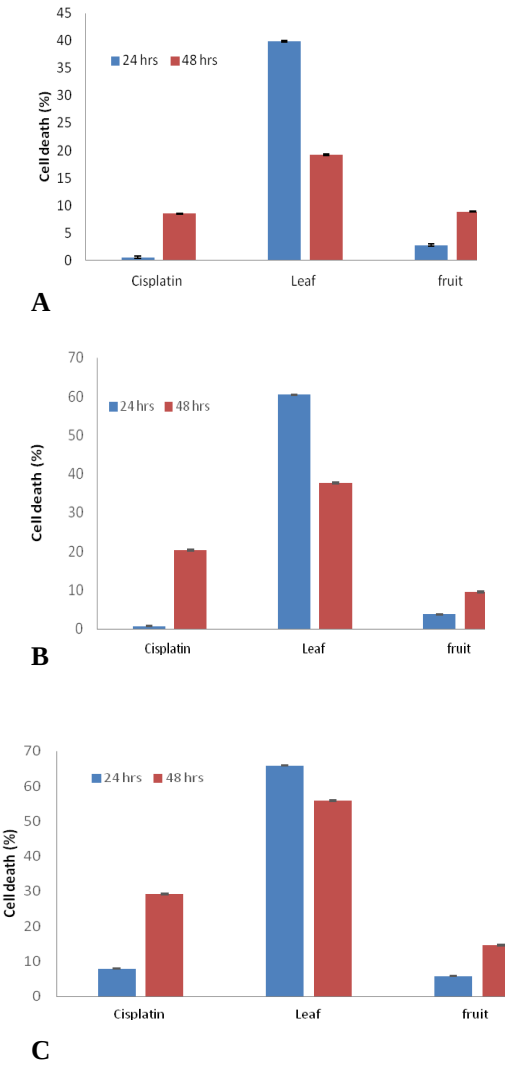

Figure 3. A. Cell death (%) at 8 mg concentration of Leaf and fruit extracts, B. Cell death (%) at 80 mg concentration of Leaf and fruit extracts, C. Cell death (%) at 800 mg concentration of Leaf and fruit extracts

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). Low doses of either the extract or cisplatin seemed to cause a proliferation of MCF-7 cancer cells whereas at high doses, especially at 800 µg/mL, killed the MCF-7 cells compared to untreated cells (Figure 3c). In contrast, a moderate dose (80 µg/mL) of either the extract (Figure 3b) or with a low dose (8 µg/mL) of cisplatin only kill 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) suggests that bitter melon contain 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 potential role in effective control of cancer cells (MCF - cells). The different extracts of leaf and fruit component of bitter melon are more effective in controlling the growth of MCF-7 cells when compared to control-cisplatin. The leaf, when compared to fruit, was better in reducing the number of cancer cells which supports the use of plant-based treatments for different types of cancers.

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