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In Vitro Evaluation of Anticancer Activity of *Moringa Peregrina* Seeds on Breast Cancer Cells

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Abstract: *Moringa peregrina* have long been used traditionally to treat many diseases in Arabian Peninsula folklore medicine. The objective of this study was to determine the anticancer activity of *Moringa peregrina* seed ethanolic extract (MPSE) on the breast cancer cell. The 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay was used to determine the anticancer effect of MPSE treatment at concentrations ranging from 25 to 400 µg/ml against AU565 breast cancer cells. The statistical values were determined employing the statistical test of one-way analysis of variance. The results show that the MPSE inhibits the proliferation of AU565 breast cancer cells with IC₅₀ values 201.7µg/ml. These findings suggest that MPSE has anti-proliferative effect on AU565 breast cancer cells can be used to treat and prevent cancer diseases, the outcomes is promising and paving the way for develop new natural drug for treatment of breast cancer disease.

Keywords: *Moringa peregrina*, Cytotoxicity Activity, AU565 cell.

Introduction

Moringa peregrina (Forssk) Fiori can be found in Asia and Africa. Traditionally, this plant is used for the treatment of hypertension, stomach disorder, malaria, burns and diabetes (Albaayit et al., 2018; Senthilkumar et al., 2018). *M. peregrina* seeds contain tocopherols, flavonoid and phenolic compounds, which help to reduce cancer mortality (Koheil et al., 2018; Vijayalakshmi et al., 2013). Seeds of *M. peregrina* have been validating pharmacology to have Anticancer activity (Elsayed et al., 2016) as well as antidiabetic, anti-inflammatory and antimicrobial activities (Koheil et al., 2011), however, there is limited knowledge about the anti-proliferation effect of *M. peregrina* seed against AU565 cells. AU565 is an epidermal growth factor positive breast cancer cell line (Her-2+) used in medical research for screening the anticancer drugs in vitro (Huang et al., 2010).

Breast cancer is the most common cancer in women after lung cancer worldwide. In Saudi Arabia and Malaysia, breast cancer was the highest type of cancer among woman (Elsayed et al., 2015). Although developments in breast cancer therapy, these chemotherapys associated side effects and often patients are concerned to use it, there have been interesting to use herbal medicine as antitumor remedy (Hadadi et al., 2018). Thus, the present study was undertaken to evaluate the activity of *M. peregrina* seed extract, which might provide natural drug for cancer therapeutic with less side effect to the normal system, against AU565 breast adenocarcinoma cells.

Method

Moringa Peregrina Ethanol Extracts Preparation (MPSE)

100g of powdered dried seeds were immersed in 500 mL of ethanol for 3 days. After that the mixture was filtered by filter paper. The liquid filtrate was subject to rotary evaporator at (45°C - 50°C) in order to obtain the ethanol extract, whereas the residues were introduce to further immersion with ethanol (Albaayit et al., 2014; Albaayit et al., 2014).

Cell Lines

AU565 (Human breast cancer, which positive Her-2) cell lines was purchased from ATCC. Cells were cultured at DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and incubated at 37C in a humidified atmosphere of 5% CO₂.

Anti-proliferative assay on AU565

Inhibitory effects of MPSE on the growth of breast cancer were examined using the MTT assay. The AU565 cells were seeded in a 96-well plate at a density of 5.0×10^3 cells/well, and then treated with different concentrations of MPSE ranging from 25 to 400 µg/ml and incubated the plate for 48hr. 20 µL of MTT solution (0.5 mg/mL) was added to each well and incubated the plate at 37C for 4 hours. The purple formazan crystal was dissolved in 150 µL of dimethyl sulfoxide (DMSO) and absorbance was measured at 570 nm wavelength using a microplate reader (Tecan, Austria). Each experiment was carried out three times with triplicate wells for each concentration (Albaayit et al., 2020; Albaayit et al., 2015).

Statistical Analysis

Graph Pad prism 6.0 analysis software was used to determine statistical significance $p < 0.001$ compare to untreated cell, data were considered as mean \pm standard error.

Results and Discussion

In the pharmaceutical industry, natural product has become more interesting for the preparation of potential safe drug because of their wonderful medicinal properties (Albaayit et al., 2020; Al-Bahran et al., 2020; Elsayed et al., 2016). Traditionally, seeds of *M. peregrina* have been used to treat skin problems, abdominal pain and diabetic (Albaayit & Ozaslan, 2019; Al Dhaberi et al., 2016). Although only a few studies available on its pharmacology efficacy (Senthilkumar et al., 2018), the activity of the seed against cancer was limited interest. Accordingly, we interest to evaluation the antiproliferative efficacy of MPSE against breast cancer cell line that may be paved as a potential cancer therapeutic drug. In this study, the cytotoxicity assay of MPSE on the viability of AU565 cells is 69%.at 400µg/ml with IC₅₀ values 201.7µg/ml Figure1.

The inhibited level in breast cancer cell (AU565) culture might be due to antioxidant capacity provided by MPSE as an inverse relationship between antioxidants and the growth of cancer cells (Albaayit & Maharjan, 2018; Koheil et al., 2018; Saeidnia & Abdollahi, 2013). This plant has been reported to contain high amounts of flavinoid, tocopherols and phenolic compounds, which are attributed to the antiproliferative properties of extract on cancer cells (Huang et al., 2010). The present study outcome is in consist with previous study by Adebayo et al who reported that *M. oleifera* has a significant cytotoxic effect against the breast cancer cell.

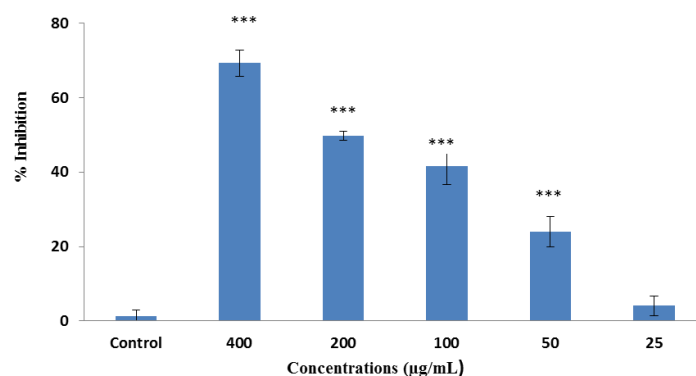


Figure 1. Inhibition effects of Moringa perigrena seeds on AU565

Conclusion

In conclusion, ethanol extract of *Moringa peregrina* seed has cytotoxic activity against AU565 breast cancer cells. This study has suggested seeds of *Moringa peregrina* as good anticancer agents, may be paved for further research study to understand the mechanism of action of this plant.

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