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Cell sensitivity assay and cell viability of Breast cancer cell (MCF7) using MTT assay of Ashitaba (*Angelica Keiskei*) leaf extract

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Abstract

The 3-(4,5-dimethylethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay was used to investigate Angelica keiskei Koiz mi (Ashitaba in Japanese), a perennial plant belonging to the Umbelliferae family, for its cytotoxic effect against breast cancer cells or MCF, to an insoluble purple formazan by the action of mitochondrial reductase. Formazan is then solubilized and the concentration determined by optical density at 570nm. The plant extract is not cytotoxic to breast cancer cells and has no impact even at high concentrations. These findings indicate that the plant extract has a detrimental effect on the cell. Furthermore, prior to testing, the components of the plant extract must be extracted.

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Introduction

Cancer is the leading cause of mortality in both men and women, taking nearly 6 million lives each year globally. In 2012, over 14.1 million new cancer cases were diagnosed worldwide. Lung cancer, prostate cancer, colorectal cancer, and stomach cancer are the most frequent cancers in men. Colorectal cancer, lung cancer, cervical cancer, and breast cancer are the most prevalent forms among females. Breast cancer is the most frequent invasive cancer in women across the world. It affects roughly 12% of women globally.

Angelica keiskei Koizumi (Ashitaba in Japanese) is a perennial plant in the Umbelliferae family that grows naturally on the Izu islands and the Izu, Bouso, and Miura located along Japan's Pacific Coast (Monma *et al.*, 1990). 1990). Flavonoids, coumarins, and chalcones are the primary bioactive elements of this plant (Kozawa *et al.*, 1978). It has diuretic, laxative, analeptic, and galactagogue properties. Many studies have found that this plant offers a variety of physiological effects, including antibacterial, anticancer, antiulcer, and antithrombotic activity, as well as vasodilative action (Inamori *et al.*, 1991; Kimura *et al.*, 2003; Kimura *et al.*, 2004; Kyogoku *et al.*, 1979; Fujita *et al.*, 1992)

Ashitaba constituents are believed to have anticancer properties including the prevention of carcinogen-induced preneoplastic lesions, anti-angiogenic action, anti-proliferative and cytotoxic activity, and anti-invasive activity. Furthermore, chalchones extracted from ashitaba have been shown to cause apoptosis in a variety of cancer cells. Although it has been hypothesised, there is no conclusive proof that keiskei prevents coronary heart disease, hypertension, or cancer. It has been reported that chalcone derivatives, such as xanthoangelol and 4-hydroxyderricin, have been identified as key components of yellow compounds from this root (Kozawa *et al.*, 1977; Kozawa *et al.*, 1978).

Mosmann devised a semi-automated colorometric test in 1983 based on the assumption that the mitochondria of live cells convert the tetrazolium salt MTT to formazan. A modified version of this is now being used effectively by the National Cancer Institute USA to assess the chemosensitivity of novel medications on cell lines (Alley *et al.*, 1988). Therefore the study aim to the cytotoxic effect of Angelica keiskei against breast cancer cells or MCF7 was assessed using the MTT test.

Materials and methods

Plant Extraction

Ashitaba dried leaves and stems were crushed and immersed for 24 hours in a flask holding 200 ml of 95% ethanol. The plant extract was extracted from the filter using Whatman No.1 filter paper. Rotary evaporation was used to concentrate the filtrate (ROTAVAP). The extract was put in a sanitised amber container and kept at 4°C until needed.

MTT Assay

The MTT cytotoxicity assay performed in this study was adapted from Mosmann (1983). In detail, MCF7 cells were seeded at $6x10^4$ cells/mL in sterile 96-well microtiter plates. The plates were incubated overnight at 37 °C and 5% CO^2.

Four concentrations of the extract were prepared as treatment: 50µg/mL, 25µg/mL, 12.5µg/mL and 6.25µg/mL. Doxorubicin (DOXO) served as positive control while dimethyl sulfoxide (DMSO) served as negative control. Following incubation, cells were treated with 10µL of each extract dilution. The treated cells were again incubated for 72 hours at 37 °C and 5% CO^2.

After incubation, the media was removed and 20µL 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 5 mg/mL PBS was added. The cells were again incubated at 37 °C and 5% CO^2 for 4 hours. After which, 150µL DMSO was added to each well. Absorbance was read at 570nm. Trials were performed in triplicates. All cells were viewed and photomicrographs were captured Using Carl Zeiss AxioVert Microscope; Magnification is 100x (10x for eyepiece, 10x for objective).

Results and discussion

The colorimetric test using 3-4,5-dimethylthiazol2-yl-2,5-diphenyl tetrazolium bromide (MTT) was used to

quantify mammalian cell survival and proliferation, and was later employed to identify cancer cell viability. MTT is a yellow tetrazolium salt that is transformed into a blue or purple formazan by living cell dehydrogenases. The test is based on the idea that the amount of formazan generated is proportional to the number of living cells.

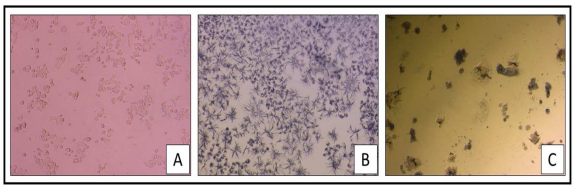

Fig. 1. Images of the MCF7 in A) Medium prior to treatment; B) exposed to DMSO—the negative control; C) exposed to DOXO—the positive control.

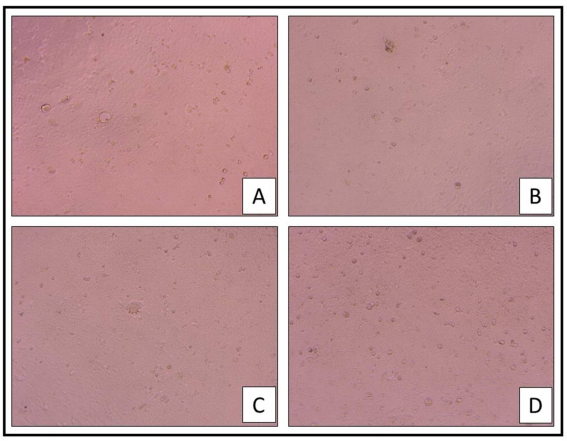

Fig. 2. Images of treated cells exposed to different concentration of Ashitaba extract. A) 6.25µg/mL; B) 12.5µg/mL; C) 25µg/mL; D) 50µg/ml.

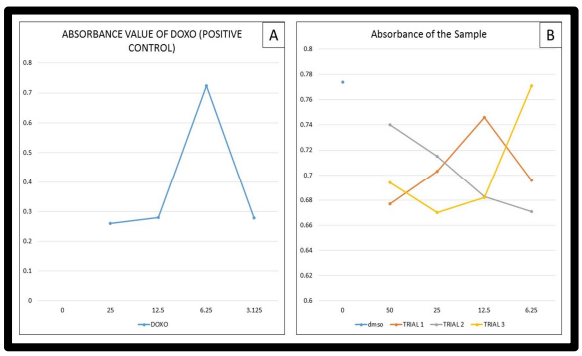

Fig. 3. Comparison of the absorbance value of the positive control and the Ashitaba extract.

The comparison of the cell before exposure to the treatments is shown in Fig. 1. It is the positive control because the DOXO's colour changes show that the chemical is hazardous to the MCF7 in a positive way. Fig. 2 illustrates the cytotoxicity against MCF7 in the cells subjected to plant treatments. Fig. 3's comparison of the absorbance in DOXO and plant extract serves as proof of this. A lower absorbance value is displayed by the DOXO with a lower concentration. but greater values in larger concentrations are shown by the plant extract. Hence the poor outcome. The chalcones are connected to this negative. Researchers attempted to identify various chalcone types that were found in ashitaba (Nishimura *et al.*, 2007). six chalcones were identified and investigated in neuroblastoma cells. The neuroblastoma cells exhibits cytotoxic to four of the cells, whereas the other two have no impact.

Five chalcones were extracted from the stems of *Angelica keiskei* (Umbelliferae) for a different investigation by the Akihisa group. Regarding their inhibitory effects on the induction of Epstein-Barr virus early antigen (EBV-EA), which is known to be a primary screening test for antitumor promoters, these compounds were assessed. With the exception of three compounds, all other compounds tested showed potent inhibitory effects on EBV-EA induction. Through this, they concluded that the effectivity of the different chalcones is not the same in different cell lines. Zhang *et al.,* 2013, did a review of a work. Al, The MTT or MTS test was often used to track cell cytotoxicity. When cells are exposed to chalcones, the viability of the cells might drop, and the cells may then quickly necrotize or initiate a programmed death (apoptosis). Leukemia, hepatoma, breast cancer, colorectal cancer, stomach cancer, prostate cancer, and epidermoid carcinoma are the most common malignancies against which chalcones show cytotoxicity. Despite the fact that chalcones have a high level of activity, they may lack the proper selectivity between normal and malignant cells. Furthermore, further research is needed to fully understand the underlying pathways that lead to cell death.

Conclusion

Ashitaba extract exhibits high absorbance values in co mparison to DOXO and did not exhibit any inhibition on MCF7, resulting in an unfavourable outcome. Additionally, according to some study, certain chalcones have varying impacts on cancer cells.

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