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Alpinia galanga Extract Inhibits MCF-7/HER2+ Cells by Inducing Apoptosis

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ABSTRACT

Background: Cancer is a disease caused by abnormal growth of body tissue cells. Cancer treatment strategy by induce apoptosis and inhibit proliferation.

Aim: This research aims to examine the anticancer effect of Alpinia galanga extract on MCF-7/HER2+ breast cancer cells.

Method: The MTT Assay cytotoxic test was carried out to determine the growth inhibitory activity. Apoptotic assay to determine the activity of compounds in apoptosis, as well as through a structural approach utilizing various virtual platforms to monitor its activity.

Result: Based on the MTT assay Alpinia galanga extract possessed cytotoxic effect in dose-dependent manner with IC50 value of 330.79 µg/mL. The extract induces apoptosis of MCF-7 cells up to 25.56%.

Conclusion: It can be concluded that Alpinia galanga extract demonstrated cytotoxic activity toward MCF-7/HER2+ breast through apoptosis induction. This extract had an opportunity to be developed as a potential anticancer agent to overcome breast cancer diseases.

Keywords: Cytotoxic, apoptosis, MCF-7/HER2+, Alpinia galanga

BACKGROUD

Breast cancer is a type of cancer with the highest prevalence in women and causes high mortality rates. In 2020, there will be 19.3 million breast cancer cases and 10.0 million leading to cancer deaths globally [1]. Chemotherapy has frequently resulted in various side effects in the treatment of cancer. It is difficult to develop new prospective anticancer agents that are selective to tumor cells while being non-harmful to normal cells [2]. Various natural resources, particularly medicinal plants, have become targets for the development of novel anticancer agents with minimal side effects [3]. Many active substances have been shown to induce apoptosis in various cancer cells[4–6]. Therefore, it is necessary to develop more effective and less harmful anticancer drugs as quickly as possible.

Alpinia galanga contains various anticancer secondary metabolites, including 1'acetoxychavicol acetate (ACA), galangin, caryophyllene-oxide and limonene [7]. ACA a major compound on Alpinia galanga extract has anti-cancer activity through induction of cell cycle arrest, apoptosis and inhibition of cell proliferation [7,8]. ACA also induces apoptosis by increasing caspase 3 activity, inhibiting activation of nuclear factor kappa-β (NF-kβ), and increasing ligands associated with apoptosis-associated tumor necrosis factor [9]. However, the role of Alpinia galanga extract on breast cancer HER2+ such as MCF-7 still unclear. Hence, current study was conducted to investigated the effect of Alpinia galanga extract on MCF-7 HER2+.

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METHODS

Alpinia galanga extraction procedure

The rhizome of Alpinia galanga were collected, peeled, dried for approximately 3 days at 40°C. The dried rhizome was powdered. A total of 500 mg of Alpinia galanga powder was extracted by maceration using 90% methanol solvent (7.5 L) for 3 days while stirring occasionally. The filtrate was macerated and evaporated using a rotary vacuum evaporator to obtain a thick extract [10].

MCF-7/HER2+ cell culture

The MCF-7/HER2+ cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) mixed with 10% Fetal Bovine Serum (FBS) (Sigma-Aldrich, Louis St, MO), 100 IU/mL penicillin, and 100 mg/mL of streptomycin (GibcoTM Invitrogen, NY, USA) and incubated under 37°C, 5% CO2, and 80% humidity condition. The culture medium was replaced in 3 days interval. After cells reached 80% confluency, the isolated cells were passaged using trypsin and propagated.

MTT cytotoxic assay

The cytotoxic assay was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay (Sigma-Aldrich). MCF-7/HER2+ breast cancer cell cultures were confluently harvested and then distributed into 96-wellplate wells. Cells given treatment with Alpinia galanga extract in various concentration 10-500 µg/mL in triples and incubated for 24 hours under 37°C and 5% CO2 incubator. The sample was discarded and given MTT reagent for 4 hours. Cells were added with DMSO stopper and incubated 15 minutes in dark room. The absorbance of cells was measured at 595 nm by a microplate reader. Untreated cells were counted with a hemacytometer and used for interpolating the absorbance.

Apoptosis annexin V-PI assay

Cells of $2x10^5$ cells/ml were distributed into 6-well plates and incubated until normal conditions. Cells was treated with a series concentration of *Aplinia galanga* extract in culture medium and incubated for 24 hours in an incubator of 5% CO2, 37°C. After 24 hours of incubation, apoptotic cell was analyzed with Annexin V and propidium iodide reagents (BD Annexin V-FITC-Apoptosis Detection Kit). Percentage of apoptotic analysis was read with a DB C6 flow cytometer.

Statistical analysis

One way Analysis of Varian (ANOVA) was carried out to determine statistical significance and P value of less than 0.05 was considered as statistically significant**.**

RESULTS

Alpinia galanga extract production

A total of 500 mg of dried Alpinia galanga powder was macerated with 7.5 L of 90% methanol as a solvent at room temperature for 72 hours to produce a yield of 11.7%.

Cytotoxic effect of Alpinia galanga extract on MCF-7/HER2+

Cytotoxic test was performed using MCF-7/HER2+ breast cancer cells. Results showed that by addition of Alpinia galanga in MCF-7/HER2+ cell culture, number of MCF-7/HER2+ cells were shown less when treated with higher concentration of Alpinia galanga extract (Figure 1A). Alpinia galanga extract mild possessed cytotoxic effect with IC50 value of 330.79 µg/mL (Figure 1B).

Apoptosis analysis of Alpinia galanga extract on MCF-7/HER2+

To identify the different pathways of cell death-either necrosis or apoptosis in treated cancer cells with extracts, staining with annexin V and propidium iodide (PI) were used for determination of the early stages of apoptosis and both necrosis and late apoptosis, respectively [11–13]. In MCF-7/HER2+ breast cancer cells, extracts increased significantly the apoptotic cells in dose-dependent manner than untreated cells. Alpinia galanga extract induce cell death up to 25.56% in high concentration (IC_{50}) (Figure 2A-B).

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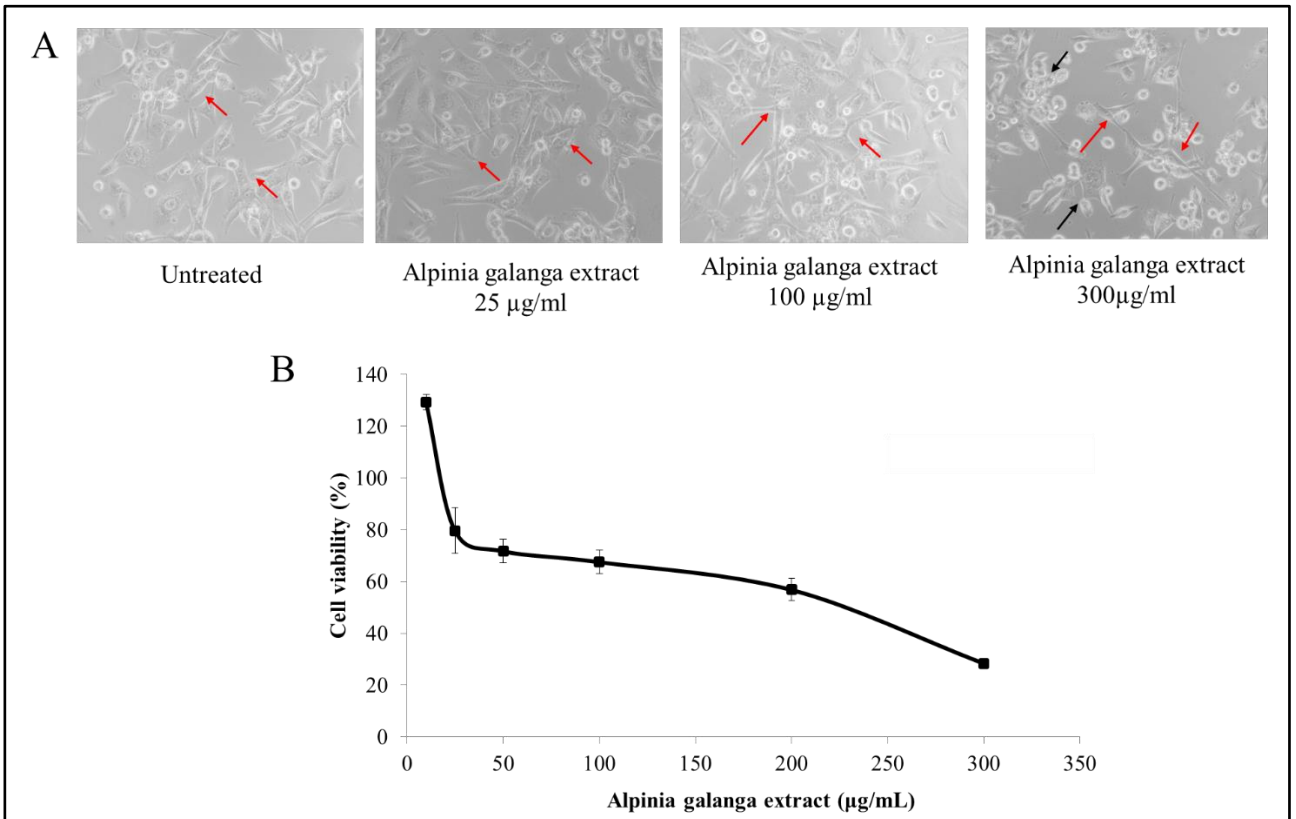

Figure 1. (A) Morphological MCF7/HER2+ cells treated with serial concentration of Alpinia galanga extract. (B) Cell viability were measured with MTT assay, an interpolated number of viable MCF-7/HER2+ cells after treated with Alpinia galanga extract.

DISCUSSION

Cancer cells death by apoptosis, necrosis, or a combination of the two when they are damaged or aged. In other words, cell death in cancer cells can occur through the stimulation of extrinsic or intrinsic pathways, which are the two basic mechanisms of apoptosis [3]. Extrinsic signaling pathways, also known as death receptor pathways, are activated when transmembrane death receptors connect with ligand to trigger cell death effectors, whereas the intrinsic signaling system utilizes internal signals regulated by the mitochondria. Understanding the molecular pathways of apoptosis can lay the groundwork for innovative targeted treatments that lead to the arrest of cancer cells[14]. Many traditional Asian compounds have been used to treat cancer [15]. In this study, we report the effect of Alpinia galanga extract on the growth of MCF-7/HER2+ cells. Alpinia galanga extract strongly blocked the growth of MCF-7/HER2+ cells in a doses-dependent manner, suggesting that Alpinia galanga could be a useful anti-cancer agent against HER2-expressing breast cancer. The growth inhibition induced by Alpinia galanga extract was accompanied by an increase in apoptotic cells.

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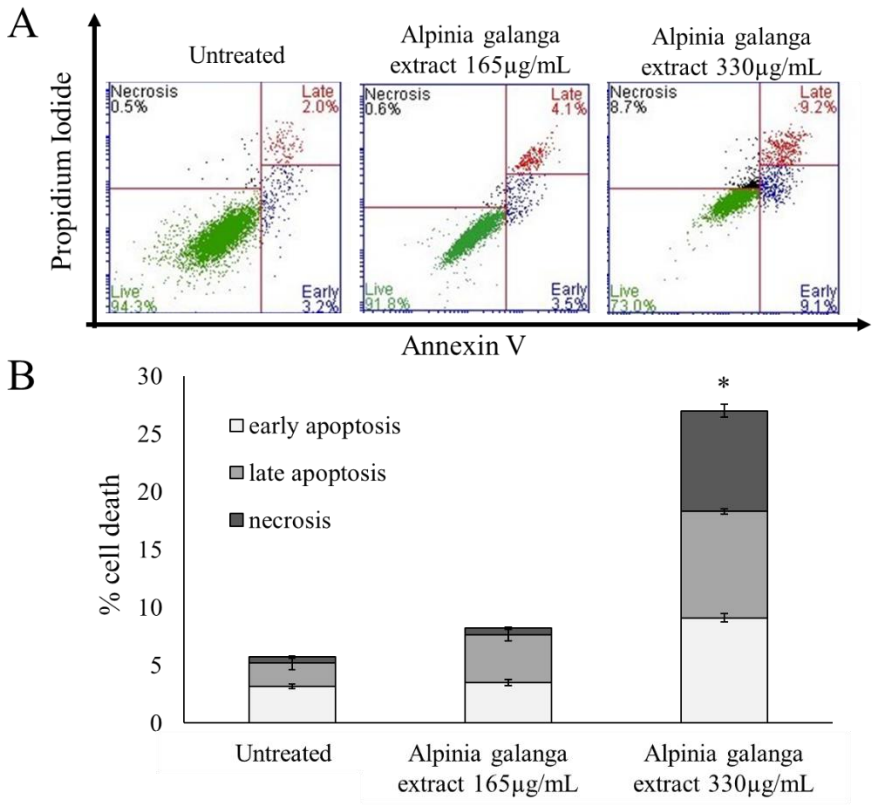

Figure 2. (A) Apoptosis profile of MCF-7/HER2 cells treated with Alpinia galanga extract under flow cytometry analysis. (B) The percentages of cells in different stages of apoptosis were labeled with early apoptosis, late apoptosis, and necrosis. Significant decrease of live cells by Alpinia galanga extracts in cancer cells in comparison with untreated cells. *Significant increase of stages of apoptosis than control

About 20–25% of invasive breast cancers contain HER2 gene amplification. A normal breast cell possesses 20,000 HER2 receptors, but a breast cancer cell might have up to 1.5 million. HER2 belongs to the HER/ErbB2/Neu protein family, which also includes HER1/EGFR, HER3, and HER4 [16]. HER2 interacts with the estrogen receptor (ER) signaling pathway [17]. The content of total flavonoid compound in the Alpinia galanga had been reported in the earlier study in which the ethanolic extracts of rhizome contained total flavonoid were 10.55 mg QE/g dried extract [18]. This flavonoid contains may be associated with the anticancer effect. Previous study reported that Alpinia galanga extract has cytotoxic effect on PC-3 cells through DNA fragmentation induction [19]. Natural chemicals' ability to interfere with cancer metabolism is not limited to Alpinia galanga. Other studies have found that other natural chemicals have a similar impact. Citrus extract have high flavonoid compound has been shown to have anticancer and chemo preventive action in breast cancer via promoting cell cycle arrest and apoptosis by elevating reactive oxygen species (ROS) effect [13],[20],[21]. Taken together, Alpinia galanga extract showed the capability to halt HER2-overexpressing breast cancer cell proliferation and this was associated with the induction of apoptosis. Therefore, it is interesting to further explore the activity of Alpinia galanga and its active compounds in cancer as a target to inhibit cancer cell proliferation.

CONCLUSION

In conclusion, Alpinia galanga extract showed the capability to halt HER2-overexpressing breast cancer cell proliferation and this was associated with the induction of apoptosis. Although further investigation is still needed to clarify the mechanism of Alpinia galanga extract cancer death induction.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

AUTHORS' CONTRIBUTION

SI contribution in conceived and designed the analysis, wrote the paper, collected the data and contributed analysis tools.

FUNDING

None

AUTHOR DETAILS

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