104/A

Anticancer effects of *Plumeria rubra* in HER2 positive breast cancer cell lines

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Natural products have been used for centuries in the treatment of many diseases, and plants in particular have been heavily used for this purpose all over the world including in Sri Lanka. The chemical diversity of plants has played significant roles in drug discovery. Cancer has been a global burden and the search for novel drugs is yet a priority aim for cancer therapy due to the resistance mechanisms and high toxicity associated with current cancer chemotherapy drugs. Thus, it is important to discover and identify novel molecules from plants that will help to discover pharmaceutical agents for combating cancer. At present, around 50% of drugs used in cancer chemotherapy are natural-product derived. Previous research has shown that certain plant species in the Apocynaceae family possess anticancer activities. Therefore, the anticancer effect of leaves and stems of *Plumeria rubra,* which belongs to this family, was investigated. The ethanol extracts were prepared from dried leaves and stems. It was found that the leaf extract inhibits the growth of breast cancer cell lines, especially HER2 positive SKBR3 cells, by MTT assay (GI_{50}= 17.45 µg/ml ± 2.05). MTT assays were used to investigate the *in vitro* growth inhibitory effects of the plant extracts on cancer cell lines. However, the stem extract was not active in the cancer cell lines tested compared to the leaf extract. Thus, further investigations were carried out to determine the mechanism of the *Plumeria rubra* leaf extract. It was found that the 2x GI_{50} concentration of the leaf extract completely inhibited SKBR3 colony formation in the clonogenic assay ($p<0.0001$). The clonogenic assay was used to investigate whether single cancer cells are able to survive the challenge of the plant extracts and retain proliferative capacity to form progeny colonies. Subsequently, cell cycle assay was used to measure the percentage of cellular accumulation at a particular phase of the cell cycle in the presence of the plant extract. Interestingly, the leaf extract was also able to block the G1 phase in SKBR3 cells (84%) compared to untreated cells (75%) ($p<0.0001$). Apoptosis assays provides an estimate of viable cells and apoptotic cells, after treatment with the plant extract, as such a significant apoptotic population was also observed in SKBR3 cells that were treated with the leaf extract (7%), compared to control cells (3%) ($p<0.0001$). This agent was able to cause a minor amount of DNA double strand breaks by gamma H2AX analysis in SKBR3 cells ($P<0.05$). There is evidence indicating that reactive oxygen species can act as cancer suppressors. As such, it was found that the leaf extract was able to generate high levels of reactive oxygen species compared to SKBR3 control cells ($P<0.001$). All experiments were repeated ≥ 3 times and Graphpad Prism 7.0 was used for analysis. These results are promising and further studies will be conducted to isolate chemical compounds from this plant species for cancer therapy.

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