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TITLE: HPLC Isolate from the Leaves of Kaffir Lime (*Citrus hystrix* DC) Exhibits Selective Cytotoxicity Against Human Breast Adenocarcinoma Cell Line, MCF-7**Name:** Fatima Mariz Almenario, M.Sc.**Affiliation:** Head of the Biology Department, Dona Remedios Trinidad Romualdez Medical Foundation**Country:** Philippines**Email ID:** fatzalmenario@gmail.com**ABSTRACT (up to 300 words)**

The increasing mortality and morbidity rate caused by breast cancer warrants the need to screen for compounds ideal for chemotherapeutic application. This study evaluated the cytotoxicity of an edible and native plant, *Citrus hystrix*, utilizing a bioactivity-guided fractionation scheme. Using various chromatographic techniques, the fractions were subjected to MTT [3-(4, 5-dimethylthiazol-2-yl)-2, diphenyltetrazolium bromide] assay to determine cytotoxicity and bioassays to confirm apoptosis as the mode of cell death. The results revealed that the crude extract was moderately cytotoxic against human breast adenocarcinoma (MCF-7) but not to other cancer cell lines tested. Solvent partitioning of the crude extract revealed that hexane partition exhibited the highest cytotoxicity (IC₅₀: 23 µg/mL) to MCF-7. Sequentially, vacuum liquid chromatography (VLC) of the hexane partition separated VLC 7 (IC₅₀: 17.2 µg/mL) with the highest percent yield. Hence, VLC 7 was subjected to gravity column chromatography (GCC), where GCC 7.4 exhibited promising cytotoxicity to MCF-7 (IC₅₀: 14.6 µg/mL). Selectivity index (SI) indicated that GCC 7.4 was highly selective against MCF-7 (SI > 2), relative to non-cancer cell lines NIH3T3, HDFn, and AA8. Interestingly, GCC 7.4 induced apoptosis as confirmed by several markers including phosphatidylserine (PS) membrane translocation, mitochondrial membrane depolarization, nuclear condensation, and DNA fragmentation. To further purify the components

present in GCC 7.4, high-performance liquid chromatography (HPLC) was performed. HPLC 7.4.5 was active against MCF-7 (IC₅₀: 5.5 µg/mL) without being cytotoxic against the non-cancer cell line NIH3T3. Overall, the results demonstrated that *C. hystrix* leaves possess promising compounds that are highly selective and cytotoxic against MCF-7 by inducing apoptosis.

BIOGRAPHY (up to 200 words)

Fatima Mariz Almenario has completed her Master of Science in Biology at the age of 26 years old from University of the Philippines Diliman, the country's premier state university. As a young researcher, Fatima Mariz Almenario has recently published research articles from peer-reviewed scientific journals. She is currently the head of the Biology Department of Dona Remedios Trinidad Romualdez Medical Foundation, one of the recognized medical schools in the Philippines. She has been one of the board members of two (2) reputable scientific organizations in the Philippines, the Philippine Society for Developmental Biology (PSDB) and Biology Teacher's Association of the Philippines (BIOTA). She has participated and won several research presentations in both oral and poster category. Her research interests include cancer cell biology, drug discovery on plant natural products, and stem cell biology.



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