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Review Article

Utilising Anticancer Properties of Soursop (*Annona muricata* L.) for MCF-7 Breast Cancer Treatment

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Abstract: *Breast cancer is the most common malignancy for Indonesian women. Treatments for this particular cancer have experienced major progress yet there are still loose ends that do not benefit patients. One of the most prominent drugs used in chemotherapy is doxorubicin, acting as an inhibitor of topoisomerase II causing DNA fragmentation in cancer cells. Indonesia is a country with vast cultures of consuming regional endemic fruits as a source of illness prevention and soothing symptoms. One often consumed through fruits and boiled leaves is soursop (Annona muricata L.) known for its abundance of acetogenins. 15-acetylguanacone is a kind of acetogenin known for possessing potent anti-proliferative and anti-metastatic properties by inhibiting vascular endothelial growth factor 2 (VEGF2). 15-acetylguanacone from soursop is extracted through silica gel fractionation. Literature studies show that loading doxorubicin and 15-acetylguanacone to PDEN derived from soursop promises potent abilities to inhibit proliferation of MCF-7 breast cancer cells. Loading these materials involve incubation or electroporation. Assays like cell cycle analysis, intracellular ROS analysis, and SA-β Gal senescence-based assay show that the interaction of 15-acetylguanacone and doxorubicin increased proliferative inhibition rate and cytotoxicity. This is a literature study written to show potentials of loaded soursop PDEN as alternative treatment for MCF-7 breast cancer cells.*

Keywords: *15-acetylguanacone; doxorubicin; MCF-7 breast cancer; PDEN; soursop*

Abstrak: *Kanker payudara merupakan kanker yang paling sering menyerang wanita Indonesia. Namun, pengobatan kanker masih memiliki sifat invasif yang merugikan pasien. Salah satu obat kemoterapi yang umum digunakan adalah doxorubicin yang mampu menginhibisi aktivitas topoisomerase II sehingga terjadi fragmentasi DNA sel kanker. Indonesia merupakan negara yang mengayomi budaya mengkonsumsi buah endemik melalui konsumsi buah dan daunnya guna mencegah penyakit dan mengurangi gejala. Salah satu buah yang umum dikonsumsi adalah sirsak (Annona muricata L.) karena kandungan asetogenin yang tinggi. 15-acetylguanacone merupakan salah satu bentuk asetogenin yang memiliki sifat antiproliferatif dan antimetastatik melalui penghambatan faktor pertumbuhan endotelial vaskuler 2 (VEGF2). 15-acetylguanacone dapat diekstrak dari buah sirsak melalui fraksinasi gel silika. Studi literatur menunjukkan bahwa PDEN sirsak yang berisi ekstrak 15-acetylguanacone dan doxorubicin memiliki potensi menghambat proliferasi sel kanker payudara. 15-acetylguanacone dan doxorubicin dapat di-loading ke PDEN melalui metode inkubasi atau elektroporasi. Uji seperti analisa siklus sel, analisa kadar ROS pada intraseluler, dan uji SA-β Gal senescence-based menunjukkan bahwa interaksi antara 15-acetylguanacone dan doxorubicin mampu menghambat laju proliferasi sel kanker melalui penurunan kadar ROS serta peningkatan kadar sitotoksisitas sel kanker. Studi literatur ini dilakukan untuk melihat potensi PDEN berisi 15-acetylguanacone dan doxorubicin sebagai pengobatan alternatif untuk kanker payudara MCF-7.*

Kata kunci: *15-acetylguanacone; doxorubicin; kanker payudara MCF-7; PDEN; sirsak*

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1. Introduction

According to Cooper and Adams (2022), cancer is an illness where certain or multiple genes mutate and cause out of control proliferation creating a mass or tumour. Cancer commonly starts in a certain body part (tissues, cells, nodes, etc) then spreads to adjacent systems in a process called metastasis (Park *et al*., 2022). Cancer may be caused by the consumption of physical, chemical, and biological carcinogens such as radiation exposure (physical), pollution and asbestos (chemical), and virus (biological) (Blackadar, 2016). Carcinogens act as initiating agents that not only destructs DNA and cause mutation, but also stimulates cancer cells proliferation through C kinase protein activation (Cooper and Adams, 2022).

The International Agency for Research on Cancer (IARC) stated that breast cancer is the most common cancer in women with 22,598 deaths from 66,271 cases in 2022. Breast cancer treatments

currently involve removal of lymph nodes and tissues through surgery, radiation, and chemotherapy (Mayo Clinic Staff, 2024). One of the most common drugs used for chemotherapy is doxorubicin (DOX) with abilities to inhibit topoisomerase II activity that causes DNA instability resulting in pieces of DNA binding to Top2 enzyme creating a Top2cc complex. This complex induces cancer cells apoptosis (Johnson-Arbor and Dubey, 2023). Unfortunately, these treatments possess side effects like hair loss, nausea, and other symptoms. Hence, there is a need for drug innovation providing higher safety and comfort for patients.

Plants of Indonesia are often consumed to soothe symptoms and prevent illnesses. A fruit possessing anti-cancer abilities is soursop (*Annona muricata* L.). Soursop is easily cultivated with low prices in Indonesia (Sarasati *et al*., 2023). Soursop carries active metabolites like acetogenins that show promise as anti-cancer bioactive compounds in proliferative activity inhibition through vascular endothelial growth factor 2 (VEGF2) inhibition (Mutakin *et al*., 2022; Jacobo-Herrera *et al*., 2019). Acetogenins have a structure involving α-terminal, bis-tetrahydrofuran (THF), and β-unsaturated ɣ-lactone ring. A kind of acetogenin that is abundant in soursop is 15-acetylguanacone.

Plant Derived Exosome-like Nanovesicles (PDEN) are nanovesicles with active constituents like proteins, DNAs, mRNAs, miRNAs, lipids, and metabolites (Sarasati *et al*., 2023). PDENs' performance dominates mammalian nanovesicles because PDENs have the ability to prevent zoonosis and possess higher bioavailability (Dad *et al*., 2021). PDENs can be made through isolating plant extracts by differential ultracentrifugation and density-gradient centrifugation methods. Choice of method depends on density factors, surface contents, size, and precipitation (Hadizadeh *et al*., 2022). Ultracentrifugation method disposes particles depending on size respectively (cells, cell wastes, apoptosis cells, then microvesicles) through force and duration of progressive centrifugation (Livshits *et al*., 2016). Unfortunately, this method leaves vesicle sediments, protein aggregates, and exosome aggregates (Szatanek *et al*., 2015). Density-gradient method differentiates PDEN size, mass and density among other constituents with sucrose or iodixanol (Chou and Ansel, 2016). Though this method produces PDEN with high purity, it can only be done in small batches tediously (Tauro *et al*., 2012).

PDENs primary property is antiproliferative abilities that prevent cancer cell growth and induce cancer cell apoptosis. In order to accurately deliver cargo to cancer cells, PDENs also possess abilities to modulate tumours' microenvironment so cancer cell walls are conditioned to be easily penetrated, have high circulatory ability to prevent early drug elimination, and are non-invasive (Chen *et al*., 2023). PDENs properties are to be seen below.

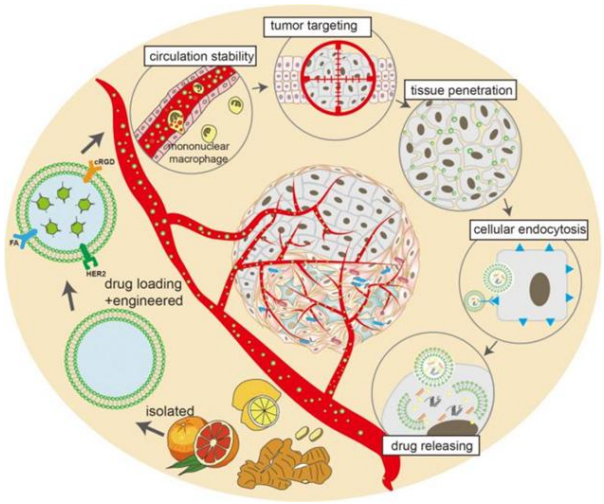

Figure 1. Properties of PDEN (Chen *et al*., 2023)

PDENs' efficacy can be heightened through loading doxorubicin and 15-acetylguanacone. This combination may act as an alternative treatment that lessens doxorubicin's invasive nature and improves drug delivery accuracy so that cells in the body do not experience side effects and eliminate cancer drugs. Literature studies are needed in order to understand characteristics of 15-acetylguanacone, interactions between loading materials doxorubicin and 15-acetylguanacone, and the effects of 15-acetylguanacone and doxorubicin-loaded PDEN towards MCF-7 breast cancer cells.

2. Methodology

2.1. Information and Data Collecting

Literature studies are done across 45 (forty-five) sources that are narrowed down to 27 (twenty-seven) sources due to high similarity of objectives between sources. Sources include journal articles

from PubMed, PMC, NIH, Wiley, and Elsevier, and chemical compound databases. Keywords used were PDEN, exosome, nanovesicle, doxorubicin, MCF-7 breast cancer, phytochemistry of soursop, cancer prognosis, TNM classification, and acetogenin. Data collection involves literature study to broaden understanding of existing problems and contemplate problem-solving models, secondary data as comparative study, and databases to support discussion and comparative study.

2.2. Data Analysis

Data analysis involves grounded theory, where analysis is performed inductively after data collecting in order to find patterns and categories. Concepts and theories are developed following the findings of patterns and categories.

2.3. Conclusion Gathering

Conclusions are drawn out according to derived problems and the purpose of this literature study. Conclusions are to represent the discussion, with advice adjacent to recommending further studies.

3. Result

3.1. Characteristics of 15-Acetylguanacone

Studies show that the molecular weight of 15-acetylguanacone is measured with the EI-MS (Electron Ionization Mass Spectrophotometry) method, showing a result of 662.1 g/mol. 15-acetylguanacone binds with VEGF2 with a lowest binding energy (LBE) of -8.5 kcal/mol (Agu *et al.*, 2017). The negative value of LBE shows the binding strength between enzymes and ligands (Terefe and Ghosh, 2022). The isolation and elucidation process of 15-acetylguanacone involves using an ethyl acetate mixture. Therefore, it can be concluded that 15-acetylguanacone is soluble in ethyl acetate (Agu *et al.*, 2017; Agu *et al.*, 2018). The structure of 15-acetylguanacone can be seen below.

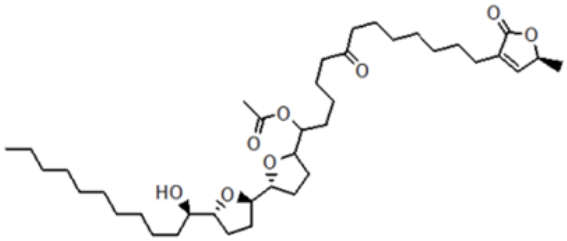

Figure 2. Structure of 15-acetylguanacone (Olubodun and Agu, 2022)

3.2. Interactions of 15-Acetylguanacone and Doxorubicin (DOX)

The study of 15-acetylguanacone and doxorubicin interactions involves assays like cell cycle analysis, intracellular ROS analysis, and SA-β Gal senescence-based assay. Cell cycle analysis uses a BD Accuri C6 flow cytometer to analyse the distribution of the cycle in each cell and determine the percentage of cell death at each stage of the cell cycle. Cell cycle analysis of this combination tested on 4T1 mammalian breast cancer cells showed that induction of cell cycle interruption occurred in the G2/M phase, while 15-acetylguanacone alone only interrupted the cycle in the G1 phase (Salsabila *et al.*, 2021). Intracellular ROS analysis assay used the DCFDA staining assay to measure ROS rate in this combination tested in 4T1 mammalian breast cancer. It showed that the combination and sole 15-acetylguanacone lowered ROS at a similar rate (Ng and Ooi, 2021; Salsabila *et al.*, 2021). SA-β Gal senescence-based assay detected galactosidase activity in the lysosomal fraction of cells under low pH conditions. This assay, through galactosidase activity detection by X-Gal, showed that the combination did not increase deterioration rate by much (Niedernhofer *et al.*, 2017; Salsabila *et al.*, 2021). This can be caused by lowered ROS levels.

4. Discussion

4.1. Physical and Chemical Properties of 15-Acetylguanacone

Properties of 15-acetylguanacone show its potential to act as cargo for soursop PDEN. Binding strength, shown by the Lowest Binding Energy (LBE) value, indicates the potential of 15-acetylguanacone to bind with cancer cell growth factors and utilise its own properties to inhibit cancer cell growth and induce apoptosis.

4.2. 15-Acetylguanacone and Doxorubicin-loaded Soursop PDEN Potentials as a Drug Delivery System

Engineered exosomes are natural nanoparticles, derived from cells, that have been modified to encapsulate therapeutic agents and target specific disease sites, such as tumours, representing a promising advancement in the field of drug delivery (Zhang *et al.*, 2020). This modification of exosomes aims to reach tumour cells without being eliminated by the body or attacking healthy cells.

Drug delivery systems (DDS) have emerged as a critical area of research, focusing on the development of vehicles that can transport therapeutic substances safely and effectively to their destination. This helps introduce therapies with lower levels of toxicity, longer half-life in systemic circulation, and higher efficacy through controlled release rates at their target (Tiwari *et al.*, 2012). One promising example of DDS is PDEN (Plant Derived Exosome-like Nanovesicle). These nanoparticle carriers offer a natural and biodegradable alternative to synthetic drug delivery systems, potentially improving patient outcomes and reducing the environmental impact of pharmaceutical waste (Gao *et al.*, 2023).

The combination of 15-acetylguanacone and doxorubicin will be loaded onto soursop PDEN through incubation or electroporation methods (Zeng *et al.*, 2023). The incubation method depends on the co-incubation of exosomes and other small molecules over a period of time. A doxorubicin and 15-acetylguanacone mixture is poured into test tubes containing soursop PDEN and incubated for two hours at 4°C (Wang *et al.*, 2022). The electroporation method loads cargo into exosomes using high-voltage (220V, 350μF, 4.5ms) short pulses that break down the lipid bilayer membrane, making it semi-permeable so that cargo can be loaded; the broken-down membrane is then restored through incubation (Zeng *et al.*, 2023). Other loading methods are shown in the figure below.

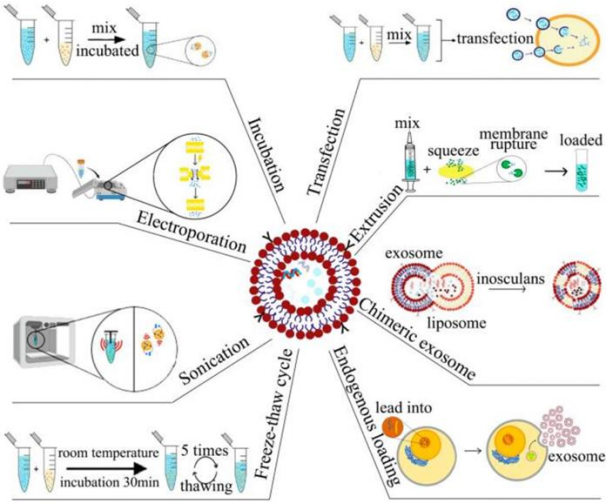

Figure 3. PDEN loading methods (Zeng *et al.*, 2023)

Pairing 15-acetylguanacone with doxorubicin shows high promise as cargo, as shown by assay results such as increased antiproliferative activity, cell cycle interruption at the G2/M phase, and lower ROS levels indicating a low rate of proliferation. This low rate of proliferation may explain why deterioration activity could not be recorded and analysed properly.

4.3. Soursop PDEN Effects Towards MCF-7 Breast Cancer

There are precedent PDENs for MCF-7 breast cancer treatment, such as *Citrus limon* PDEN and *Camellia sinensis* PDEN. *Citrus limon* PDEN showed proliferative inhibition through induction of cell cycle interruption at the G2/M phase and inhibition of cell transition from G0 to G1 and G2 to M by lowering the expression of cell cycle proteins CyclinB1 and CyclinB2 and increasing the expression of the cell cycle inhibitor Cdkn1a (p21) (Chen *et al.*, 2023). Doxorubicin-loaded *Camellia sinensis* PDEN showed pro-apoptotic activity and high levels of cytotoxicity through amplified ROS production (Chen *et al.*, 2022). Higher ROS levels destroy mitochondria and tumour cell DNA, halt the cell cycle, and inhibit proliferation, migration, and invasive tumour cell activity (Yi *et al.*, 2014). Chen *et al.* (2023) stated that after four hours of incubation, ROS levels in MCF-7 and 4T1 cells were 14.2 and 9.3 times higher, respectively, than in cells that were not treated with PDEN.

The proliferative activity inhibition and ROS-level-lowering abilities demonstrated by the assays show that soursop PDEN loaded with 15-acetylguanacone and doxorubicin may serve as a form of alternative treatment for breast cancer patients. This would allow patients to experience fewer side effects, since PDENs are non-invasive. Patients are also expected to have better outcomes, since PDEN offers natural targeting and high circulatory ability, meaning fewer drugs are eliminated from the body and more drugs are able to penetrate cancer cell walls. Although this PDEN formulation has

not yet undergone wet-lab experiments or clinical trials, we hope this paper may bring newer and brighter ideas for the betterment of patients and the field of medicine.

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