



Role of the Red Fruit (*Pandanus conoideus* LAM) Ethyl Acetate Fraction on the Induction of Apoptosis vs. Downregulation of Survival Signaling Pathways in Cervical Cancer Cells

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Authors' contributions

This work was carried out in collaboration among all authors. All authors designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Red fruit (*Pandanus conoideus* Lam) has been used as a traditional herbal medicine in Papua and also as an agent against malignant diseases empirically. To elucidate the detailed mechanisms producing such an activity, the characterization and determination of the molecular mechanisms of its antitumor effects were conducted. The inhibitory activities against cervical cancer cell proliferation and the expression levels of corresponding molecules were investigated using human cervical cancer cells treated with the ethyl acetate fraction of red fruit extract (EtOAc RF). The EtOAc RF possessed strong anti-proliferation activities against HeLa and CaSki cells. Furthermore,

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the down regulation of the expression of phosphorylated Akt and mTOR in the cells was induced shortly after treatment with the fraction, followed by the activation of caspase-8, caspase-9, caspase-3, p53 serine 46 and PARP along with the suppression of the expression of Bcl-2 family proteins and Akt-mTOR, leading to apoptotic cell death. Taken together, these results suggest that red fruit extract could be a promising agent against cervical cancer cells.

Keywords: Red fruit; cervical cancer cells; apoptosis.

1. INTRODUCTION

Cervical cancer is the second most common gynecological cancer worldwide, carrying a high mortality rate for women. Death due to cervical cancer is mostly found in third-world countries such as Indonesia, where 90% mortality is found [1]. Of the estimated 500,000 new cases of cervical cancer diagnosed each year, 80% occur in developing countries, with the highest rates occurring in Africa, Asia, and Central and South America [2,3]. Cervical cancer is a major problem in Indonesia. In its early stages, the disease often does not cause symptoms or complaints, so most patients visit the health center already at a later stage [4]. The data show that 60% of patients who visited our center (2007–2011) were diagnosed with cervical cancer out of a total of 11,434 gynecological cancer patients; among them, 72% already had end-stage cervical cancer [5,6].

Red fruit (RF) or *Pandanus conoideus* Lam, commonly known as Buah Merah, is a medicinal plant that originated from Papua (Fig. 1A, B). It is popular among Indonesians due to its wide array of medicinal properties. Some studies have demonstrated that the fruit extracts of RF have properties against degenerative diseases, such as hypertension, diabetes, heart disease and cancer [7]. Moreover, its potency as an anticancer agent has been known for generations. Many studies have been conducted concerning the extraction, isolation, and characterization of RF's bioactive constituents (compounds) as potential sources of anticancer agents [7-9]. In this study, the *Pandanus conoideus* Lam extract prepared from the ethyl acetate fraction of red fruit (EtOAc RF) was investigated *in vitro*, and its antitumor effects on cervical cancer cells were elucidated together with the determination of the molecular mechanisms involved and apoptosis- and survival-signaling pathways. Our results suggest that EtOAc RF, as a natural compound, may provide the possibility of an application for future cancer prevention and therapy.

2. MATERIALS AND METHODS

2.1 Cell Lines and Culture Conditions

Two human cervical cancer cell lines, HeLa and CaSki, were purchased from the American Type Culture Collection (Manassas, VA). HeLa cells were maintained in Eagle's Minimum Essential Medium (EMEM) obtained from Sigma Chemical Co. (St. Louis, MO), supplemented with 2 mM L-glutamine, 1.0 mM sodium pyruvate, and 10% heat-inactivated fetal bovine serum (FBS) obtained from Gibco (BRL, Grand Island, NY). CaSki cells were maintained in RPMI-1640 medium (Gibco) supplemented with HEPES (Sigma) and 10% FBS.

2.2 Plant Materials

Pandanus conoideus Lam or red fruit (RF) was purchased from the Merdey district, Teluk Bintuni, West Papua, Indonesia. The plant species was identified by the laboratory of Plant Taxonomy staff at State University of Papua, Manokwari, West Papua, Indonesia. A voucher of the specimen was deposited at the Herbarium of the Bandung Institute of Technology, Bandung, Indonesia.

2.3 Extraction and Isolation (Fig. 1C)

The fresh fruits of RF (900 gr) was subjected to MeOH extraction. The MeOH extract (600 gr) was partitioned among EtOAc, *n*-hexane and water to afford an active EtOAc extract (16 gr). Four grams of EtOAc was evaporated (Fig. 1D) to yield 3.2 gr of EtOAc, which was then dissolved in 0,05% DMSO. After serial testing, we determined the IC₅₀ EtOAc BM for CaSki cells (after 32x dilution from the stock) to be 3.3 mg/ml.

2.4 Inhibition Concentration (IC) Assay

An IC assay for each compound was conducted in the presence of serially diluted compounds as described previously [10-12]. Cell viability was then measured with the aid of a cell counting kit