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THE POTENTIAL USE OF *Phaleria macrocarpa* LEAVES EXTRACT AS AN ALTERNATIVE DRUG FOR BREAST CANCER AMONG WOMEN LIVING IN POVERTY

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ABSTRACT

Cancer is recognized as global threat to human development and it was estimated that in 2030 there will be 22.2 million new cases of cancer and 12.7 million cancer-related deaths worldwide. Herbal medicine plant till this day all over in the word is still the main source and one of the most important fields of traditional medicine agents to fight breast cancer. The usage of plants is still important because has its own beaviour advantages such as low toxicity, easy to get, cheap and has less side effect if it is used in a right. One of this plant that has been used as a traditional medicine is a Phaleria macrocarpa (Scheff) Boerl) also known as Mahkota Dewa from Papua island. It has been used traditionally for treatment of cancers in Indonesia and also to cure many diseases. The therapeutic effect of P. macrocarpa is related to its bioactive compounds contents. There are many reported compounds from P. macrocarpa, which are found, reported and published such as Icariside, phalerin, mangiferin, gallic acid, 29-Norcucurbitacin, gentiobioside and glucoside. All compounds reported above have already published as anticancer agents,where anticancer activity were detected and showed against Hela cells, leukemia cells, cervical cancer, breast cancer, and L1210 cells. Research on P. macrocarpa fruit and leaves merit have been done and published, however, there are very limited research on the linked correlation between the concentration of compounds and its the cytotoxicity level. Because of that, research about correlation level of P. macrocarpa leaves extract still need to investigate to find the relationship between them in the goal to investigate alternative low cost herbal medicine agents to fight breast cancer especially useful for low income people. Cytotoxicity properties of samples against breast cancer cell lines was performed by using the MTT assay against MCF-7 cell line. The correlation between concentration of crude and cytotoxic activity was interpreted by statistical analyses. The study showed that P. macrocarpa.) leaves extracts showed cytoxicity activity against breast cancer MCF7 cell lines. Correlation between concentration of extract and cytotoxicity property (absorbance value) were founded in weak relationship (R = -0.37181). It could be effect of many different compounds in the P. macrocarpa leaves methanol extracts may cause the pharmacological interactions, so lower or higher concentration will be antagonistic effect on absorbance or cell viability.Further study on its mechanism pathway on revealing against breast cancer could be explored. Furthermore, the natural product derived from P. macrocarpa leaves extracts have potential use in cheap alternative agents against breast cancer.

Keywords: *Phaleria macrocarpa* (Scheff) *Boerl., MTT assay, MCF-7*

INTRODUCTION

Cancer is recognized as global threat to human development and it was estimated that in 2030 there will be 22.2 million new cases of cancer and 12.7 million cancer-related deaths worldwide (Bray *et al.*, 2012). In low and middle-income countries (LMIC) worldwide , where woman odds of survival in many low income countries, are as low as 10–25% (Sankaranarayanan, 2010) with more harsh consequences for women due to gender discrimination, stigma, and cultural taboos, which conspire to limit women's choices

to seek care even when it is available (Ginsburg, 2013). In among women world-wide breast cancer is currently the leading cause of cancer incidence which accounts for nearly 1 in 4 cases of cancer, with 55% of cases occurring in more industrialized countries and 45% in less industrialized countries (Krieger, 2002). Estimated In 2008 there were an 1.38 million new cases accounting for 23% of all new cancer cases and 458,000 deaths from breast cancer (Ferlay *et al*., 2010), with consistently ranks the breast cancer as the most common cancer in women in most countries. In low- and middle-income countries, incidence of breast cancer is rising rapidly with economic development, which approximately half of new cases and close to 60% of deaths occur (Jin *et al*., 1999).

Each year, more than 200,000 women are died from breast and cervical cancer which over 50% of deaths are from breast cancer and approximately 88% of deaths from cervical cancer occur in less developed regions, where gender discrimination and extreme poverty severely limit a woman's choice to seek care (Ginsburg, 2013). In LMI countries health systems are overwhelmed with competing priorities, while providers of care are unprepared to deal with the growing epidemic of cancer and most women with cancer will face additional burdens as the need to attend to a her personal health crisis always outweighed by primary responsibilities to the family (Ginsburg, 2013). In Indonesia more than 65% of Indonesian breast cancers were found already in late stage, with poor grade and with lymph nodes metastasis condition, therefore, research for study of clinicopathological features from Indonesian molecular subtypes need to be done, in order to determine more proper management and prognosis (Widodo *et al*., 2013).

There are several molecular subtypes of breast cancer with different biological behavior, epidemiological risk factor, natural histories, response against local and systemic treatment and also prognosis (Goldhirsch *et al*., 2011). Breast cancer is classified based on their molecular expression characteristics of ER, PR, Her-2 and Ki-67, an classified into subtype of luminal A, luminal B, Her-2+ and triple negative subtypes/basaL-like (Onitilo *et al*., 2009), and In Indonesia molecular subtypes of breast cancers are still unknown (Widodo *et al*., 2014).

In the recent times complementary and alternative medicine (CAM) is common use among breast cancer patient world wide, its impact on use is estimated to be between 48% and 70% in the United States (Nahleh, 2003). In a study of breast cancer patients showed that more common used CAM in broad modalities such as natural products, botanical supplements, special dietary methods (Greenlee *et al*., 2009), herbal remedies, and spiritual healing (Lee *et al*., 2000). Definitions of CAM currently categorizes into five modalities: mind-body medicine (mind), manipulative and body-based practices (body), biologically based products (nature) include herbs/botanicals, probiotics, and high-dose vitamins, energy medicine (energy), and whole medical systems (WMS)(Matsuno *et al*., 2012)

Source of natural product as sources of anti-oxidant coumpounds from secondary metabolites to fight against disease is very broad area such as sponges (Zalilawati *et al*., 2015) and seaweeds from sea (Andriani *et al*., 2016), wetlands forest plant (Eldeen *et al*., 2016), coastal area plant (Andriani *et al*., 2015), tropical forest plant (Mohamad *et al*., 2015; Andriani *et al*., 2005), ordinary plant (Andriani *et al*., 2008), bacteria (Radzi *et al*., 2015) and fungi (Andriani *et al*., 2007).

Herbal medicine plant till this day all over in the word is still the main source and one of the most important fields of traditional medicine agents to fight breast cancer. The usage of plants is still important because has its own beaviour advantages such as low toxicity, easy to get, cheap and has less side effect if it is used in a right dose (Andriani *et al*., 2011). One of this plant that has been used as a traditional medicine is a *Phaleria macrocarpa* (Scheff) Boerl) also known as Mahkota Dewa from Papua island. It has been used traditionally for treatment of cancers in Indonesia and also to cure many diseases like lever, heart, diabetic, skin diseases, rheumatism, anti histamine, and lower the cholesterol level. This therapeutic effect is directly related to the antioxidant activity which are major contribution from its Phenolics contents (Heo *et al*., 2007: Jacobo, 2009). From the fruit, seed and leaves of *P. macrocarpa* antioxidant activity and potencies has been reported (Lisdawati, 2006; Andriani, 2011) and phytochemical and biological activities investigation have been done (Purwantini, 2002).

The therapeutic effect of *P. macrocarpa* is related to its bioactive compounds contents. There are many reported compounds from *P. macrocarpa* have been found, reported and published such as icariside, phalerin, mangiferin (Oshimi ,2009), gallic acid (Faried, 2007), isomer of phalerin

(Mae, 2005; Tambunan 2006), 29-Norcucurbitacin, together with its derivatives (Kurnia, 2008), *gentiobioside and glucoside* (Andriani, 2014). All compounds reported above have already published as anticancer agent, wich anticancer activity were detected and showed against Hela cells (Sumastuti, 2002), leukemia cells (Lisdawati, 2006), cervical cancer (Mudahar, 2005), breast cancer (Harahap, 2007), and L1210 cells (Winarno, 2009) .

Research on *P. macrocarpa* fruit and leaves merit have been done and published. However, there are very limited research on the correlation between the concentration of compounds and its cytotoxicity level. Because of that, research about correlation level of *P. macrocarpa* leaves extract still need to investigate to find the relationship between them in the goal to investigate alternative low cost herbal medicine agents to fight breast cancer especially useful for low income people, thus, information about *P. macrocarpa* leaves become more complete.

RESEARCH METHOD

Sample collection and extraction

Samples of *P. macrocarpa* leaves were collected from the City Bengkulu area, Sumatera Island, Indonesia. Firstly, the leaves samples were sliced and air-dried for 14 days. The dried leaves were ground into powder. Crude extract was obtained by maceration using methanol, i.e. the samples of dry powder (500g) were soaked in methanol for 5 days, filtrated, and concentrated by using a rotary evaporator to obtain a concentrated methanol extract. The actives crude fraction extract were be continued for further Cytotoxicity/ MTT assay.

Cytotoxicity/ MTT assay from crude extract

Cytotoxicity properties of samples against breast cancer cell lines were performed using the MTT assay. Cytotoxicity activity from *P. macrocarpa* leaves crude extract were determined by MTT (Microtetrazolium) assay against MCF-7 (hormone-dependent breast carcinoma cells) cell line. MTT or 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide assay was prepared as previously described method was used by Mosmann (1983) with some modifications. The MTT assay relies on the production of a colored formazan by the action of mitochondrial enzymes on MTT. The MTT assay test was performed using sterile flat-bottomed 96 well plates. About 100 µL of exponentially grown MCF-7 cell suspensions was seeded, harvested, counted and inoculated at a density of 5×10^3 cells/well in into three 96-well micro-titre plates. The plate was incubated at 37 °C in 5 % (v/v) CO_2 incubator for 24 hours. After 24 hours, 100 µL of the prepared concentrations of *P. macrocarpa*. leaves crude extract (60; 30; 15; 7,5; 3,75; 1,875; 0,9375; and 0 µg/mL) of each sample was added into each well and the plates were further incubated for 72 hours at 37 °C in 5 % (v/v) CO_2 .

The assay at each concentration was performed in triplicates and untreated cells were designated as control. After72 hours, the viability of cells was measured by the amount of blue formazan crystals formed after 20 µL fresh MTT solution (5 mg/mL in PBS) was added to each well. The plate then was incubated for four hours at 37 °C, 5% CO_2. Subsequently, 170 µL of medium was removed and 100 µL of Dimethyl sulphoxide (DMSO) was added into each well and mix thoroughly by pipettes (10-20 times) in order to dissolve the blue formazan crystals. The plate was left for 30 minutes before measuring the Optical Density (OD) by using ELISA reader at 595 nm reference wavelengths. The activity was expressed as IC_{50} (the concentration that inhibits 50% of cell growth). Cytotoxic activity was expressed as fifty-percent Inhibition concentration (IC50), i.e. the concentration that yields 50% inhibition of the treated cells compared to untreated cell control. Sample exhibiting cytotoxic index LC50 < 30 µg/mL, was considered to have significant cytotoxic activity. Cytotoxic activity was expressed as fifty-percent Inhibition concentration (IC50), i.e. the concentration that yields 50% inhibition of the treated cells compared to untreated cell control. The criteria of non toxic activity for the IC50 value of the sample if it is yielded more than 30 µg/ mL. (Andriani *et al*., 2011), and if sample which exhibit cytotoxic index LC50 < 30 µg/mL, were considered to have significant cytotoxic activity

Data analysis

Data collected from MTT assay was statistically analyzed using correlation method to measure the degree of association between the crude concentration and cytotoxic.

RESULTS AND DISCUSSION

Cytotoxicity assay

In this research, MCF7 cell lines was used to predict of the toxicity. Result in Table 1 show that absorbance from MTT assay test having IC50 value was counted. Table 1 also shows that methanol extract from *P. macrocarpa*.) leave exhibited the lowest absorbance in MCF7 cells produced by MTT assay at concentrations around 15 µg/ml. Lowering or increasing the extract concentration from 15 µg/ml, however, would improve the absorbance and, thus, reducing MCF7 cell viability. Correlation between the extract concentration and the absorbance was relatively low (R = -0.37181). These results suggested that various compounds contained in the *P. macrocarpa*. leave methanol extracts might pharmacologically interact resulting an antagonistic effect on absorbance or cell viability.

Figure 1 depicts 50% inhibitory activity (IC50) value of *P. macrocarpa*.) leave methanol extracts was obtained approximately at 15 µg/ml and considered as toxic concentration. Andriani *et al*. (2011) reported that cytotoxicity property of sample with the IC50 value less than 30µg/mL was considered as toxic. In this study, methanol extract from *P. macrocarpa*. leave exhibited a toxic activity against breast cancer MCF 7 cell line and offered further investigation. Nevertheless, the presence of antioxidants compound in the *P. macrocarpa*. leave extracts may potentially reduce cell number, since reactive oxygen radicals play an important role in carcinogenesis like breast cancer like MCF7.

Figure 1 also shows that lowering the concentration of methanol extract of *P. macrocarpa.* leave could substantially reduce the viability of MCF7 cells, even at concentrations lower than 30µg/mL. Further study is needed to elucidate the effect of lower concentrations (less than its IC50 value, ±15

Table. 1. Correlation between concentration and absorbance of each MCF7 cell lines for cytotoxicity assay of *Phaleria macrocarpa* (Scheff) Boerl.) leaves extracts.

Concentrations (µg/mL)	Absorbance	Mean	Correlation
60	0,298, 0,230, 0,222, 0,213, 0,222, 0,250, 0,233, 0,213, 0,260, 0,229, 0,229, 0,236	0,23625	R = -0.37181
30	0,208, 0,192, 0,239, 0,335, 0,168, 0,177, 0,168, 0,163, 0,230, 0,198, 0,155, 0,155	0,1990	
15	0,160, 0,106, 0,211, 0,153, 0,144, 0,245, 0,277, 0,140, 0,382, 0,266, 0,148, 0,138	0,1975	
7,5	0,190, 0,220, 0,217 :0,180, 0,190, 0,327, 0,287, 0,217, 0,242, 0,377, 0,180, 0,307	0,2445	
3,75	0,197, 0,283, 0,188, 0,204, 0,197, 0,263, 0,241, 0,188, 0,221, 0,286, 0,204 0,227	0,224917	
1,875	0,229, 0,303, 0,290, 0,275, 0,229, 0,281, 0,308, 0,275, 0,341, 0,334, 0,204, 0,204	0,27275	
0,9375	0,213, 0,249, 0,262, 0,286, 0,213, 0,312, 0,217, 0,217, 0,310, 0,240, 0,224, 0,224	0,24725	
0	0,186, 0,194, 0,215, 0,305, 0,305, 0,247, 0,282, 0,282, 0,261, 0,265, 0,307, 0,236	0,257083	

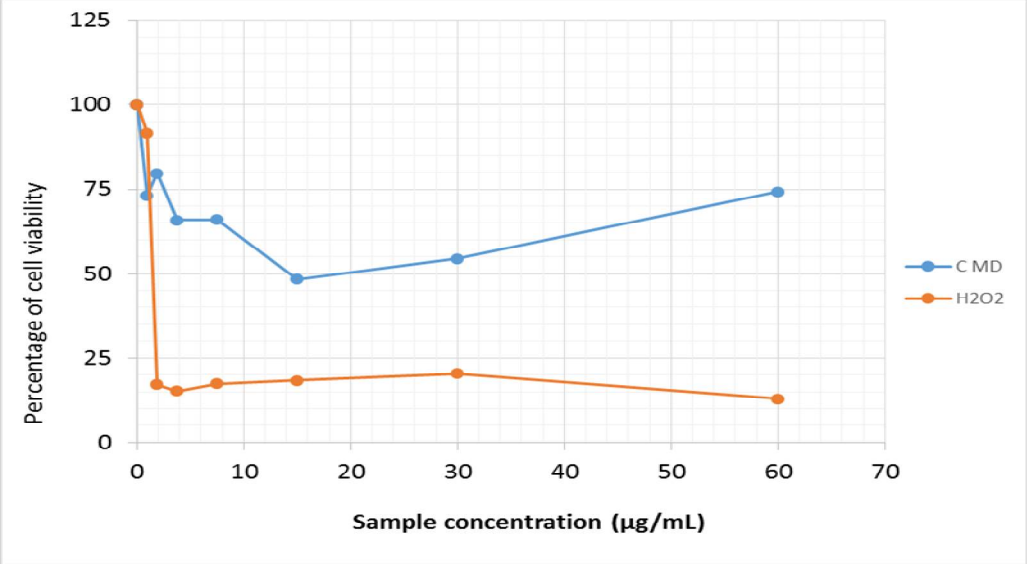

Figure 1. Cytotoxicity Property of *P. macrocarpa* leaves methanol extracts against MCF-7 cells compare to the control H_2O_2

µg/mL) on the cell morphology to confirm this activity. In this study, *P. macrocarpa*. leave methanol extracts showed high cytotoxicity property against MCF- 7 cell lines, but with the antagonistic weak correlation. This antagonistic effect might be resulted from the interaction among chemical constituents in *P. macrocarpa*. leave extracts, as reported by Golden *et al*. (2009), where anti-carcinogenic and antitumor effects of individual compound could be blocked in presence the other compounds. Therefore, the characteristics of each chemical compound should be identified to determine their cytotoxicity against breast cancer (MCF7) cell lines.

Previous study reported that *P. macrocarpa.* leave contain abundant of various compounds, such as alkaloid, flavonoid, polifenol, and tannin. Similarly, two new phenolic compounds, vis., *4',6-dihydroxy,4-methoxybenzophenone-2-O-β-D-gentiobioside and 4',6-dihydroxy,4methoxybenzophenone-2-O-β-D-glucoside* had been isolated from *P. macrocarpa*. leave methanol extract by Andriani *et al*. (2014) and showed antioxidant activity by 66 and 69 %, whose could be correlated to its bioactivities property especially against breast cancer cell line (MCF7). Anticancer properties of phenolic and flavonoids compounds had been widely reported (Yao *et al*., 2011; Fresco *et al*., 2010). Moreover, a linear correlation between phenolics or flavonoids content and antioxidant activity was reported by Ghasemzadeh *et al*. (2010) and various biological activities at nontoxic concentrations in organisms had offered an promising anticancer agents (Ren *et al*. , 2003).

CONCLUSION

The study showed that methanol *P. macrocarpa*. leave extracts had a toxicity activity against breas cancer MCF7 cell lines. Presence of phenolic antioxidant compounds in the extracts might have an important role in reducing cell number. Further study is needed to explore mechanism pathway of the extract in combating the breast cancer and, in turn, to provide more affordable treatment against breast cancer.

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