Borneo Journal of Pharmacy
Vol 5 Issue 4 November 2022
Page 307 – 314

http://journal.umpr.ac.id/index.php/bjop/article/view/2822
DOI: https://doi.org/10.33084/bjop.v5i4.2822
e-ISSN: 2621-4814

Research Article

Antiproliferative Potency of God's Crown Fruit (*Phaleria macrocarpa*) Extract Against Breast Cancer Cell

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Keywords:
Antiproliferation
Breast cancer
Herb
MCF-7 cell

Abstract

Breast cancer is a sickness caused by abnormal cell growth in the breast. Mahkota Dewa fruit or god's crown fruit products (*Phaleria macrocarpa*) contain flavonoids, alkaloids, polyphenols, and tannins associated with active compounds. This work directs to influence the potency of P. macrocarpa fruit as an antiproliferative agent against breast cancer cells (MCF-7 cells). The antiproliferative potency of *P. macrocarpa* fruit was proved by extracting and fractionating *P. macrocarpa* fruit using maceration. The cytotoxicity of extracts and fractions was determined using Brine Shrimp Lethality Test (BSLT). The antiproliferative potency against MCF-7 cells was tested using the hemacytometer approach. This work demonstrates the crude ethanol extract, *n*-hexane fraction, ethyl acetate fraction, and water fraction. The LC_{50} values in crude ethanol extract, *n*-hexane fraction, ethyl acetate fraction, and water fraction were 13.72 ppm, 147.55 ppm, 405.81 ppm, and 149. 07 ppm severally. *Phaleria macrocarpa* fruit has shown antiproliferation potency against MCF-7 cells. The maximum part of crude ethanol extract antiproliferative potency (56 ppm) effectively suppressed MCF-7 cell growth by 70. 9% while doxorubicin (100 ppm) by 46. 92%. This work confirms that crude ethanol extract of *P. macrocarpa* fruit interacts synergistically as an antiproliferative compound against MCF-7 cells.

Received: October 15th, 2021
1st Revised: June 12th, 2022
2nd Revised: August 14th, 2022
Accepted: September 15th, 2022
Published: November 30th, 2022

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INTRODUCTION

The body cell's outgrowth grade causes cancer and surpasses pattern limits extent. The growth of cancer cells inclines to avoid apoptosis and is competent to replicate uncontrollably. Moreover, these cells directly metastasize or attack over-the-counter cells roughly and spread to tissues and other body organs[1,2]. Cancer usually is referred to as a virulent tumor or neoplasm[3]. Cancer buoy adopts men and women in all lifetimes. There were 12.7 million cancer cases worldwide which caused 7. 6 million death[4].

Breast cancer is one of the seconds near common classes of cancer after cervical cancer. Breast cancer is more common in women aged 40–49 years, followed by women aged 50-59[5]. When it was established, the average patient had highly-developed breast cancer in stage III[6]. MCF-7 cells are one of the breast cancer cells in the breast tissue of Caucasian women who is 69 years old and has an O blood type[7].

The god's crown (*Phaleria macrocarpa*) is a plant often used by Indonesian human beings as a medicinal herb. This herb has compounds that effectiveness in cancer treatment[8-10]. *Phaleria macrocarpa* fruit holds alkaloids, saponins, and polyphenols[11]. It has various pharmacological actions such as anti-tumor, anti-inflammatory, antihyperglycemic, antioxidants, and antifungal[12-14].

How to cite: Hasim, Mantik YA, Husnawati, Priosoeryanto BP, Puspita R. Antiproliferative Potency of God's Crown Fruit (Phaleria macrocarpa) Extract Against Breast Cancer Cell. Borneo J Pharm. 2022;5(4):307-14. doi:10.33084/bjop.v5i4.2822

Previous studies[15,16] have shown that *P. macrocarpa* fruit was rattling potency as an anti-tumor. Nevertheless, there is no known antiproliferation intensity of *P. macrocarpa* fruit extract and fraction for MCF-7 cells. This cognate influences the antiproliferation potency of *P. macrocarpa* fruit compared to doxorubicin, a commercial cancer drug.

MATERIALS AND METHODS

Materials

The materials used in the study were *P. macrocarpa* fruit obtained from Lulut Citeureup Village, West Java and has been determined at Biofarmaka Tropika Study Center, IPB University, Indonesia with specimen numberBMK0210092016. This study uses MCF-7 cells. The herb's solvents were 96% ethanol, *n*-hexane, ethyl acetate, and distilled water. Other materials were NaCl, dimethyl sulfoxide (DMSO), *Artemia salina* shrimp larvae, seawater, Dulbecco's Modified Eagle's Medium (DMEM), fungizone, gentamicin, doxorubicin, Fetal Bovine Serum (FBS), and trypan blue.

Methods

Fruit preparation

Phaleria macrocarpa fruit Simplicia preparation was conducted according to the modified method of Ramadhan *et al*[17]. The mature *P. macrocarpa* fruit was marked with 4-6 cm red skin. It was washed, cut into small, thin layers, and separated from the seeds. The sample was heated in an oven at 40-50°C for five days. The sample that had been dried was mashed using a blender to obtain simplicia.

The water content of simplicia was determined in the following way: The empty porcelain was weighed and heated at 105°C for 15 minutes in the oven. Then it cooled using a desiccator and weighed again. After that, 3 g of simplicia was put into the porcelain and reheated in the oven at 105°C for three hours. After that, the porcelain was removed from the oven and cooled in a desiccator. The porcelain was weighed again using an analytical balance. The porcelain was again put into the oven at 105°C until a constant weight was obtained. The sample simplicia contains 6.82% of water content.

Extraction of simplicia by ethanol 96% maceration was conducted according to the modified method of Ramadhan *et al*[17]. The maceration method was done with 60 g of simplicia in 500 mL of the solvent. The simplicia was macerated for three days. The obtained macerate was then filtered using a vacuum filter; the remaining filter was extracted using the same solvent for three days. The first and second filtrate was then evaporated using a rotatory evaporator at a temperature of 45°C with a speed of 75 rpm.

Fractionation of simplicia by liquid-liquid extraction was conducted according to the modified method of Rinayanti *et al*[18]. Fractionation was carried out multilevel approach using *n*-hexane, ethyl acetate, and distilled water. About 5 g of crude ethanol extract was dissolved with *n*-hexane and distilled water and homogenized with a sonicator. The mixture was put into a separating funnel and homogenized by shaking. After settling for 30 minutes, the mixture formed the n-hexane layer above and distilled water at the bottom. Then the two layers were separated, and the water fraction was fractionated again with n-hexane for up to three replications. After that, the fractionation was repeated with 50 mL of ethyl acetate solvent three times. The last fraction was the water fraction with the same volume repeated three times. Each fraction was concentrated with a rotatory evaporator.

Cytotoxicity test

The sample's cytotoxicity test of extract and fractions by Brine Shrimp Lethality Test (BSLT) was conducted according to Hasim *et al*[19]. The incubation of *A. salina* larvae was performed in the following way: 0.2 g *A. salina* eggs were put into the seawater for 48 hours at room temperature (25°C) with light and aeration illuminated until the eggs hatched. The test solution was a crude ethanol extract and *P. macrocarpa* fractions. The 0.25 g extracts and fractions were dissolved in 15 mL of seawater using a magnetic stirrer. After being homogeneous, the solution was put into a measuring flask and added to seawater to get a stock solution concentration of 25,000 ppm. Each stock solution was then diluted to 100, 200, 400, 600, 800,

and 1,000 ppm. For the cytotoxicity test of fractions and extracts, 2 mL of seawater was put into a microplate well containing 10 *A. salina* larvae per well. About 0.5 mL of test solution was added to each well. After 24 hours of incubation, the number of larvae mortality was calculated and analyzed using Minitab 17 program to obtain the LC_{50} value.

Antiproliferation activity test

The antiproliferation activity test of extract and fractions was conducted according to Hasim *et al*[19]. For media preparation, 45 mL DMEM media, 5 mL FBS, 10 µL fungizone, and gentamicin were put into 50 mL Eppendorf tubes. The mixture was then homogenized. For sample preparation, the variation concentration of each sample was based on the LC_{50} value as a median value. The two concentrations above and below the median value were taken. Distilled water was sterilized by using an autoclave to be used as a solvent. The crude ethanol extracts were 0.001 g and dissolved with 10 mL of sterile distilled water to obtain a stock solution of crude ethanol extract with a concentration of 100 ppm. The crude ethanol extract stock solution was then diluted with sterile distilled water to get an ethanol extract test solution with concentrations of 3.5, 7, 14, 28, and 56 ppm.

The water fraction of 0.01 g was dissolved with 10 mL of sterile distilled water to obtain the water fraction stock solution with a concentration of 1000 ppm. The water fraction stock solution was then diluted with sterile distilled water until a water fraction test solution was obtained with 37.5, 75, 150, 300, and 600 ppm concentrations. The ethyl acetate fraction of 0.02 g was first dissolved with 10 µL DMSO, then dissolved with 10 mL of sterile distilled water so that a stock solution of ethyl acetate fraction was obtained with a concentration of 2,000 ppm. The ethyl acetate fraction stock solution was then diluted with sterile distilled water to get a test solution of ethyl acetate fraction with 100, 200, 400, 600, 800, and 1,600 ppm. The *n*-hexane fraction of 0.01 gram dissolved first with a DMSO of 10 µl. Then it was dissolved with 10 ml of sterile distilled water so that a stock solution of *n*-hexane fraction was obtained with a concentration of 1000 ppm. The stock solution of the *n*-hexane fraction was diluted with sterile distilled water until the n-hexane fraction test solution was obtained with 37.5, 75, 150, 300, and 600 ppm.

For MCF-7 cell calculation, microplate cultures that had been incubated were removed from the incubator. Trypan blue was pipetted 20 µL into an empty microplate. MCF-7 cells were homogenized, pipetted 80 µl into a microplate containing trypan blue, then put into a microplate reader. Calculation of the number of cells was accomplished using a light microscope with 100x magnification. After the cells were counted, an analysis was done with the SPSS program.

RESULTS AND DISCUSSION

The yield of maceration sample simplicia and fractionation of crude ethanol extract is presented in **Table I**. The yields of crude ethanol extract were relatively similar to Syukri & Saepudin[20] results, which macerated *P. macrocarpa* fruit fresh using 70% ethanol, which was 12.52%. Differences in the concentration of the solvent used can cause the difference.

Table I. The yield of *P. macrocarpa* fruit extract and fractions

Sample	Yield (%)
Crude ethanol extract	10.89
n-hexane fraction	3.04
Ethyl acetate fraction	11.84
Water fraction	54.24

A study by Lukmandaru & Gazidy[21] showed that the crude ethanol extract of *P. macrocarpa* fruit contains 43.91 µg/mg of flavonoids, while Handayani *et al*.[22] showed that the methanol extract of *P. macrocarpa* fruit contains flavonoids of 1.26 µg/mg. This research uses 96% ethanol solvent to get the optimum amount of flavonoids. According to Arifianti *et al*.[23], 96% ethanol is an ideal solvent for compounds with low molecular weight, such as flavonoids and saponins. 96% ethanol solvent was also chosen because it could extract more flavonoids and evaporate the solvent faster. Ethanol is a polar solvent and can attract large quantities of active compounds such as flavonoids, tannins, polyphenols, terpenoids, steroids, and alkaloids[24], while ethanol extract of *P. macrocarpa* fruit contains alkaloids, tannins, phenols, saponins, and glycosides[11,13].

A preliminary test (BSLT) was conducted to determine the cytotoxicity of *P. macrocarpa* extract and fractions declared as LC_{50}, the minimum concentration required to kill 50% of the population. The extract or fraction allegedly has cytotoxic potential if the LC_{50} value is less than 1000 ppm. The crude ethanol extract has the lowest LC_{50} value (13.72 ppm), so it is considered to have the most toxic effect. The *n*-hexane fraction, ethyl acetate, and water fractions have LC_{50} values of 147.55 ppm, 405.81 ppm, and 149.07 ppm (**Figure 1**). Compounds with LC_{50} values less than 1,000 ppm are considered to have the potential to be anti-cancer. The categories of cytotoxicity were determined based on LC_{50}: moderate toxicity is LC_{50} > 100-1000 ppm, high toxicity is LC_{50} > 30-100 ppm, and very high toxicity is LC_{50} < 30 ppm. The crude ethanol extract has very high toxic activity, while *n*-hexane, ethyl acetate, and water fractions have moderate toxicity.

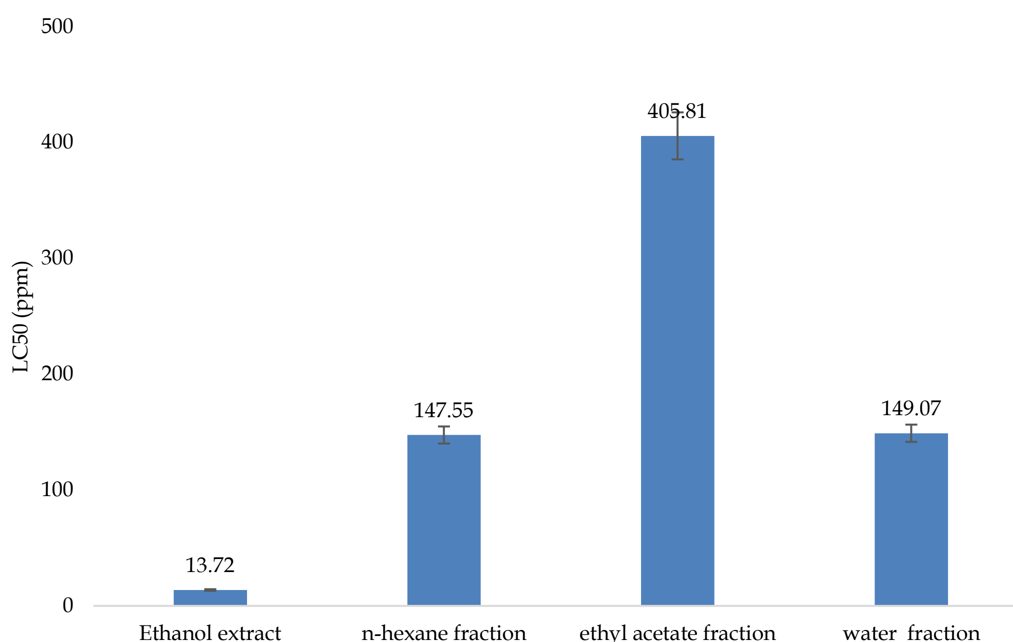

Figure 1. The LC_{50} value of *P. macrocarpa* fruit extract and fractions.

The extract, rather than the fraction, indicated the lowest LC_{50} value through BSLT. It showed that there was a synergistic effect of several chemical compounds. The synergistic effect means an active compound combination is more remarkable than a single compound[25]. Further research on the impact of synergy can be carried out using the network target-based identification of multicomponent synergy (NIMS) method.

The BSLT correlates with cytotoxic activity in some human solid tumors and pesticide activity and led to the discovery of a new class of natural pesticides and active anti-tumor agents. The cytotoxic activity of the drug can affect and disrupt the fundamental mechanisms associated with cell growth, mitotic activity, differentiation, and function[26]. The results of the cytotoxic activity for this extract may be due to one of these mechanisms. Toxicity is a relative measure of the degree of toxicity between chemicals and other chemicals in organisms, similar to toxins (molecules) that cause damage when entering the body and the location of susceptible organs[27]. The degree of the cytotoxic extract against *A. salina* can be related to the content of metabolite compounds in the extract, i.e., the flavonoid compound and several other identified compounds. Based on this, the toxicity effect can be caused by the synergistic effect of the extracted compounds so that the cytotoxic activity becomes higher or lower.

The extract and fractions of *P. macrocarpa* were tested for antiproliferation activity against MCF-7 cells. Variation concentration of each extract and fraction was made based on LC_{50} values. Negative controls containing only culture media and MCF-7 cells were considered to have a proliferation activity of 100% and an antiproliferation activity of 0%. Positive control (doxorubicin) has an antiproliferation activity of 46.92%. Doxorubicin is a therapeutic agent commonly used to treat breast cancer in humans. However, treatment using doxorubicin increases the side effects of hepatocytotoxicity and

cardiotoxicity for sufferers[28]. Percentage of antiproliferation activity of crude ethanol extract > water fraction > ethyl acetate fraction > *n*-hexane fraction (**Figure 2**). The antiproliferation activity results are proportional to the cytotoxicity test results: the more polar metabolites in the fraction, the more antiproliferation potential of MCF-7 cells.

Crude ethanol extract 3.5-56 ppm has antiproliferation activity (50.72-70.90%) greater than 100 ppm doxorubicin (46.92%). The lowest concentration of crude ethanol extract (3.5 ppm) can inhibit MCF-7 cell proliferation by more than 50%. Crude ethanol extract is the most influential in inhibiting MCF-7 cell proliferation compared to other fractions. It shows the excellent antiproliferation potential of ethanol extract. Factors influencing the efficiency of crude ethanol extract as an antiproliferative agent are crude ethanol extract contains complex polar metabolite compounds compared to yield fractions. Furthermore, crude ethanol extract has synergistic activity among phytochemical compounds[29,30].

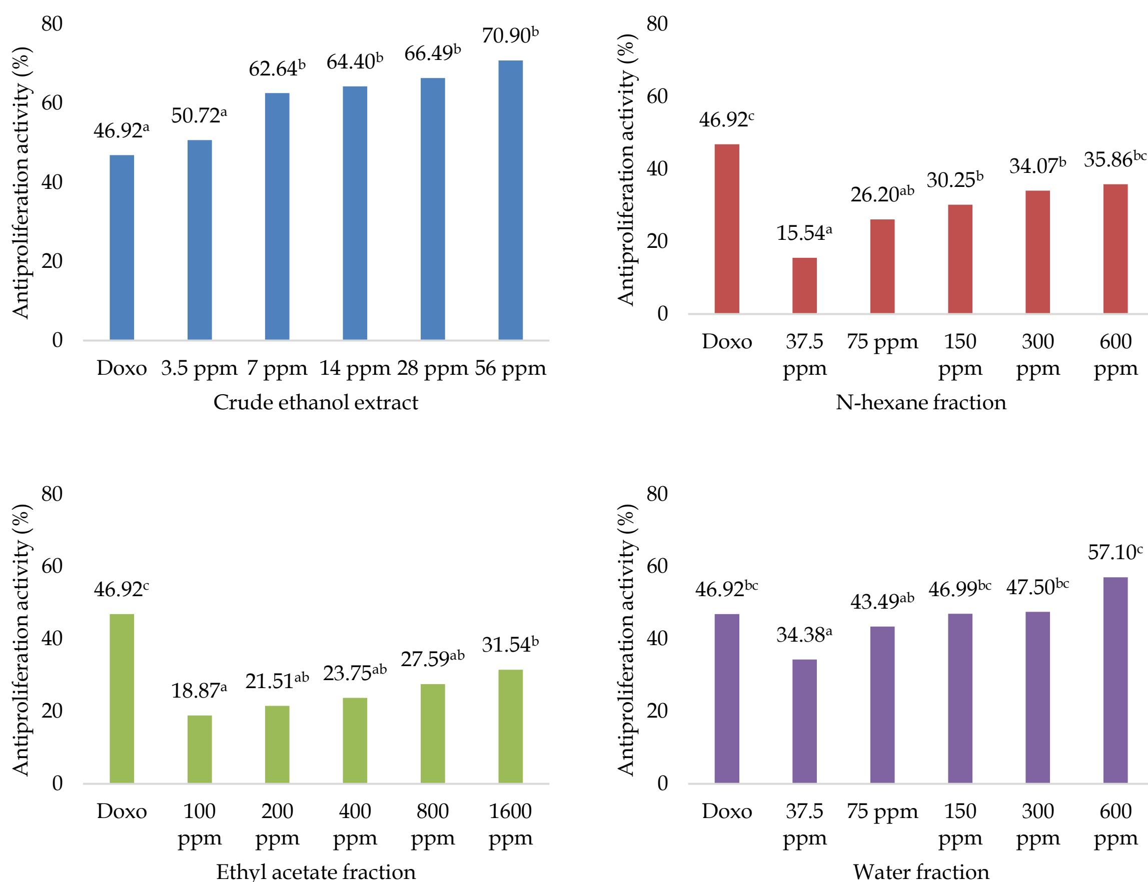

Figure 2. Antiproliferation activity of *P. macrocarpa* fruit extract and fractions. The different letters indicate significant differences in antiproliferation activities ($p < 0.05$), while the same letters indicate not significant ($p > 0.05$).

Antiproliferation activity of water fraction generally can decrease MCF-7 cell proliferation. The greater the water fraction concentration used, the greater its antiproliferation activity. Water fraction 150-600 ppm has antiproliferation activity (46.99-57.10%) greater than 100 ppm doxorubicin (46.92%). The highest concentration of water fraction (600 ppm) can inhibit MCF-7 cell proliferation by more than 50%.

Similarly, antiproliferation of ethyl acetate fraction can generally decrease MCF-7 cell proliferation. The greater the ethyl acetate fraction concentration used, the greater its antiproliferation activity. The ethyl acetate fraction 100-1600 ppm has antiproliferation activity (18.87-31.54%) smaller than 100 ppm doxorubicin (46.92%). However, the highest concentration of ethyl acetate fraction (1600 ppm) has not been able to inhibit MCF-7 cell proliferation by more than 50%.

Antiproliferation of *n*-hexane fraction can generally decrease MCF-7 cell proliferation. The fraction of *n*-hexane 37.5-600 ppm has antiproliferation activity (15.54-35.86%) smaller than 100 ppm doxorubicin (46.92%). It shows that the *n*-hexane fraction has a different potential than doxorubicin. The greater the *n*-hexane concentration used, the greater its antiproliferation activity. However, the highest concentration of *n*-hexane fraction (600 ppm) has not inhibited MCF-7 cell proliferation by more than 50%.

The water fraction contains the highest flavonoids—however, the result shows that the water fraction antiproliferation activity is not better than crude ethanol extract. A compound can be a good medicine if it does not affect other normal cells. Based on Astutiningsih *et al.*[31], who used normal human peripheral mononuclear cells, the crude ethanol extract of *P. macrocarpa* fruit was not toxic to normal cells. The crude ethanol extract of *P. macrocarpa* is selective against cancer cells, causing death but has minor cytotoxicity to normal cells. Cancer therapy is declared good if chemotherapy drugs identify specific differences between normal and cancer cells[32].

The difference in antiproliferation activity in extracts and fractions is synergizing. The synergistic effect on the active ingredient is a condition where the action of the active ingredients is greater than the single active ingredient. The act of a secondary metabolite is not only determined based on its level[33]. According to Gengaihi *et al.*[34], it is also caused by the synergistic and antagonistic effects between the components.

CONCLUSION

The cytotoxicity test result was in line with the antiproliferation activity test result. The ethanol extract of *P. macrocarpa* has better MCF-7 cell antiproliferation activity than its fractions and doxorubicin. The crude ethanol extract of *P. macrocarpa* can be used as an antiproliferative agent for MCF-7 breast cancer cells.

ACKNOWLEDGMENT

The authors are thankful to the Faculty of Sciences and Faculty of Veterinary Medicine, IPB University, Indonesia, for all laboratory facilities and for providing cancer-derived cell lines.

AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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