Effect of Phaleria Macrocarpa Supplementation on Apoptosis and Tumor Growth of C3H Mice With Breast Cancer Under Treatment With Adriamycin–Cyclophosphamide

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Effect of Phaleria Macrocarpa Supplementation on Apoptosis and Tumor Growth of C3H Mice With Breast Cancer Under Treatment With Adriamycin–Cyclophosphamide

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The objective of this study was to know the response of supplementation of Phaleria macrocarpa (PM) to adriamycin-cyclophosphamide (AC) in the treatment of C3H mice with breast cancer. Twenty-four C3H mice, who were successfully inoculated with breast cancer cells, were randomly allocated into 4 groups: without treatment, treated with AC, treated with AC + PM 0.07 mg/d, and treated with AC + PM 0.14 mg/d. The tumor size was measured using millimeter calipers before and 12 weeks after treatment. The tumor, liver, and kidneys were removed and prepared for pathologic examination using imunohistochemistry staining, and the apoptotic index was counted using the terminal deoxynucleotidyl transferase dUTP nick end labeling method. AC reduce the tumor growth significantly ($P < 0.001$), whereas supplementation of PM, which significantly reduced the tumor growth compared with AC only, was at the 0.14 mg/d dose ($P = 0.007$). AC increase the apoptotic index significantly ($P < 0.001$), and supplementation with PM showed that the higher dose increased the apoptotic index. The correlation between the apoptotic index and the diameter of tumor was significantly negative ($r = -0.884$; $P = 0.020$). The apoptotic index of the liver and kidney increased significantly in the AC group ($P < 0.001$ and $P = 0.002$, respectively); supplementation with PM decreased significantly the high apoptotic index caused by AC. We conclude that PM supplementation has a synergic effect to AC treatment in reducing the tumor growth, by increasing apoptosis, and protects the liver and kidney from damage caused by AC.

Key words: Breast cancer – Phaleria macrocarpa – Adriamycine – Cyclophosphamide – Tumor size – Apoptotic index

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Breast cancer treatment should be based on the clinical stage of disease. Surgery and radiation used to treat local breast cancer; chemotherapy is used accordingly for distant metastases. Adriamycin is the recommended first line chemotherapy with a response rate of 22% to 40%.[1,2] The response to treatment is influenced by the histologic degree of the tumor.[3] To improve the treatment efficacy, a combination of adjuvant and hormonal therapy may be used.[4] Preoperative chemotherapy using a combination of adriamycin and cyclophosphamide (AC) to reduce the tumor size, is now the standard management for local advanced breast cancer.[5]

At present, alternative treatment has become the trend worldwide; in 2005 in the United States 91% of 2386 respondents suggest that Congress and the Food and Drug Administration facilitate and initiate research regarding alternative medicine.[6] In Indonesia, very often patients with cancer use alternative herbal medicine as the sole treatment or supplementation to chemotherapy without permission from their doctor. Phaleria macrocarpa is one herbal medicine that is widely used to treat patients with cancer and is being marketed as an "anti-cancer" herbal medicine.[7] The fruit extract of Phaleria macrocarpa consists of alkaloid, terpenoid, saponin, and an active compound of polyphenol such as gallic acid (GA: 3,4,5-trihydroxybenzoic acid). *In vitro* experiments using esophagus cancer cells (TE-1) showed that gallic acid increased the proapoptotic protein Bax, decreased the antiapoptotic protein Bcl-2 and Xiap, and was not dangerous to normal cells. Phaleria macrocarpa is specifically active on mitotic cells.[8] Natural polyphenols may stimulate interferon-γ production in immunocytes which is very important in activating macrophage, cytotoxic T lymphocytes, and natural killer cells.[9,10] Ethanol extract of Phaleria macrocarpa increases perforin expression, increases cytotoxic T lymphocytes and natural killer cell production, increases the apoptotic index, and suppresses the tumor growth without vital organ involvement in animal experiments.[11,12]

This research is intended to determine the effect of Phaleria macrocarpa supplementation on C3H mice inoculated with breast cancer cells, under treatment with a combination of AC, regarding the apoptotic index, tumor growth, and its side effects on the liver and kidney.

Methods

The experiments using C3H mice were done in the biotechnology and pathology laboratory of Faculty of Medicine, Diponegoro University Semarang and

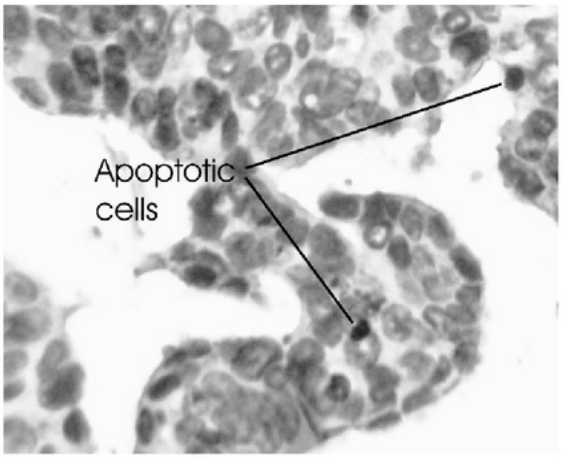

Fig. 1 Pathologic specimen from inoculated breast cancer was processed for imunohistochemistry staining, to measure the apoptotis index using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. The histologic morphology of apoptotic cells is loss of cell surface structures, cell shrinkage and shape change, condensation of cytoplasm and nuclei, nuclear envelope changes, apoptotic body formation, and nuclear cleavage. The anti-bromodeoxyuridine triphosphate (BrdU) biotin monoclonal antibody-labeled cleavage sites are detected by reaction with horse radish peroxidase (HRP)-conjugated streptavidin and visualized by 3,3'-Diamino Benzidine (DAB) showing brown dark color cells.

Gajah Mada University Yogyakarta, Indonesia, between August and November 2009. C3H mice were 8 weeks of age and weighed 20 to 30 g. After acclimatization for 1 week, healthy mice were selected to get 0.2 mL of breast cancer cell sauce, inoculated subcutaneously in the axilla region. Breast cancer sauce was made from maceration of breast cancer tumor of mice and proved microscopically before inoculated. After inoculation mice were caged individually and fed standard food. Successfully inoculated mice (monitored after 4 days) were then randomly allocated to 4 groups: G1, controls; G2, given AC; G3, given AC and Phaleria macrocarpa (PM) 0.07 mg/d; G4, AC and PM 0.14 mg/d. Based on the World Health Organization sample size for animal experiments, the minimum number of mice of each group should be 5. However, anticipating dropouts, we used 6 mice in each group. Ethanol extraction of Phaleria macrocarpa fruit, using the Soxhlet extraction method at a concentration of 0.2 mg/mL using 10% 3,4,5-trihydroxybenzoic acid, was done in the Chemical laboratory of the Department of Chemistry, Faculty of Mathematic and Natural Science of Diponegoro University Semarang.

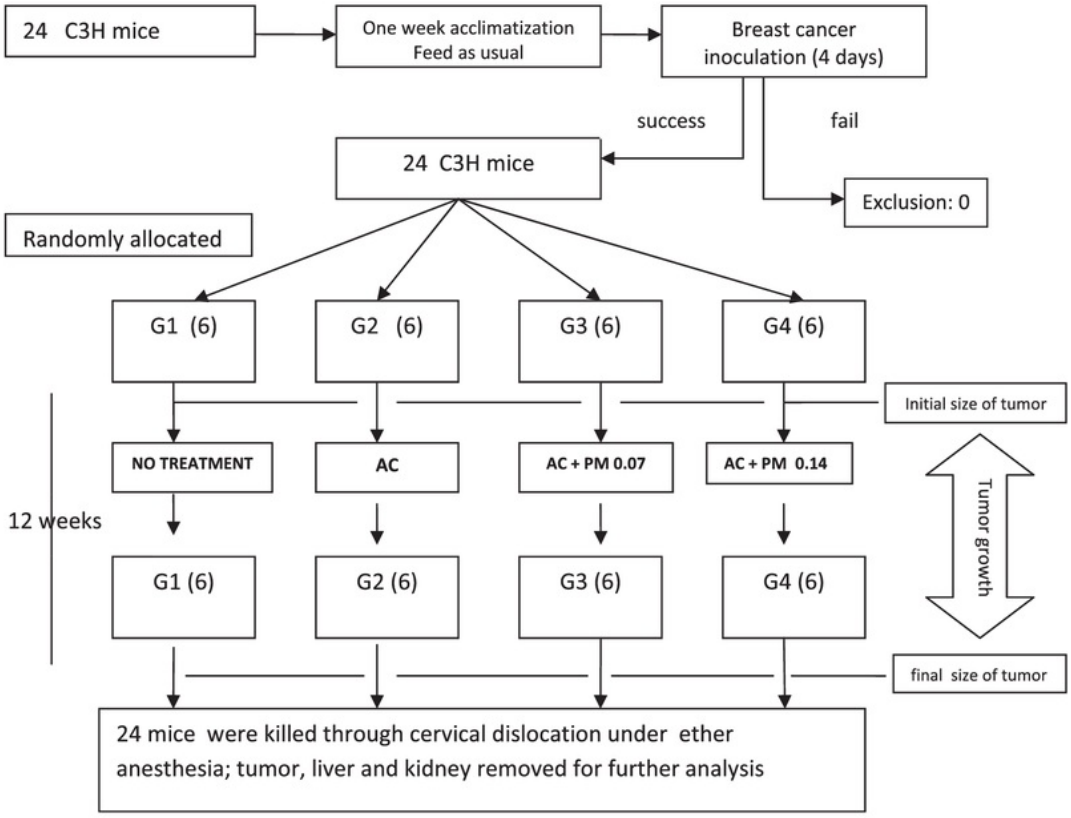

Fig. 2 Consolidated report of trial.

Adriamycin (0.013 mg) and cyclophosphamide (0.0156 mg) were injected intravenously every 3 weeks for 4 cycles. The diameter of the tumor was measured on the day before treatment was initiated using millimeter caliper and again immediately at the end of treatment (12 weeks). Under ether anesthesia, the mice were killed by cervical dislocation and the tumor, liver, and kidneys were removed for pathologic examination. The histologic specimen was processed for immunohistochemistry staining and the apoptosis index was measured using the terminal deoxynucleotide transferase dUTP nick end labeling method (Fig. 1). Measuring the apotosis index was done by two pathologists with clinical agreement of more than 95%. The distribution of data, using the Shapiro-Wilk test, were normal distribution. The mean difference among group was tested by analysis of variance (ANOVA) and between group using pooled *t* test and considered statistically significant if $P < 0.05$. SPSS version 10.0 (Clinical Epidemiology Unit, Faculty of Medicine, Diponegoro University) for Windows was used to analyze the data.

Results

Research was done on 24 C3H mice, 8 weeks old with body weight 20 to 30 g, randomly allocated to 4 groups of treatment. All mice were still alive at the end of the research project, therefore each treatment group was comparable except for the type of treatment received. A consolidated report of the trial is presented on Fig. 2.

On follow-up the tumor size increased: 1.98 ± 0.15 cm, 1.03 ± 0.37 cm, 0.72 ± 0.23 cm, and 0.5 ± 0.18 cm for groups G1, G2, G3, and G4, respectively; the difference among groups were statistically significant (ANOVA, $P < 0.001$). Treatment with AC significantly reduced the tumor growth compared with controls ($P < 0.001$), supplementation with Phaleria macrocarpa, especially at dose 0.14 mg/d, reduced the tumor growth significantly compared with the AC-only group ($P < 0.007$) (Fig. 3). The mean of apoptotic index were 8.96% ± 1.33%, 35.167% ± 1.33%, 36.64% ± 1.66%, and 42.05% ± 2.47%, for groups G1, G2, G3, and G4,

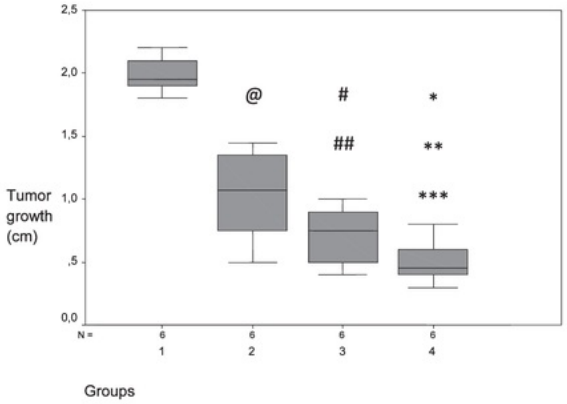

Fig. 3 Box plot of tumor growth of group 1 (control), group 2 (AC), group 3 (PM 0.07 mg/d and AC), group 4 (PM 0.14 mg/d and AC). There were significantly differences among the groups (ANOVA, $P < 0.001$). The difference between groups: *compared to group 1, $P < 0.001$; **compared to group 2, $P = 0.007$; ***compared to group 3, $P = 0.856$; #compared to group 1, $P < 0.001$; ##compared to group 2, $P = 0.2254$; @compared to group 1, $P < 0.001$.

respectively. The differences were statistically significant (ANOVA, $P < 0.001$). AC supplementation significantly improved the apoptotic index ($P < 0.001$); supplementation with Phaleria macrocarpa significantly improved the apoptotic index at both doses, 0.07 and 0.014 mg/d (Fig. 4). There was a significant negative correlation between the index apoptotic and tumor growth ($r = -0.884$; $P = 0.020$) (Fig. 5). The apoptotic index of the liver was 9.03% ± 0.55% in group 1, 12.7% ± 0.52% in group 2, 11.35% ± 0.46% in group 3, and 11.05% ± 0.48% in group 4. These differences were statistically significant (ANOVA, $P < 0.05$). Compared with the control group, AC supplementation increased the apoptotic index of the liver significantly ($P < 0.001$), whereas PM supplementation to AC treatment decreased the high apoptotic index caused by AC significantly (AC versus AC and PM 0.07 mg/d: $P = 0.001$; AC versus AC and PM 0.14 mg/d: $P < 0.001$), although it never reached the control level (Fig. 6).

The apoptotic index of kidney was 2.93% ± 0.23% in group 1, 3.7% ± 0.4% in group 2, 3.17% ± 0.4% in group 3, and 2.78% ± 0.34% in group 4. The difference among the groups was statistically significant (ANOVA, $P < 0.05$). In comparison with the control group, AC supplementation increased the apoptotic index of the kidney significantly ($P = 0.002$). Supplementation of PM to AC treatment significantly reduced the high index of apoptotic caused by AC (AC versus AC and PM 0.07 mg/d: $P = 0.044$; AC versus AC and PM 0.14 mg/d: $P = 0.002$); it even reached the control group level (Fig. 7).

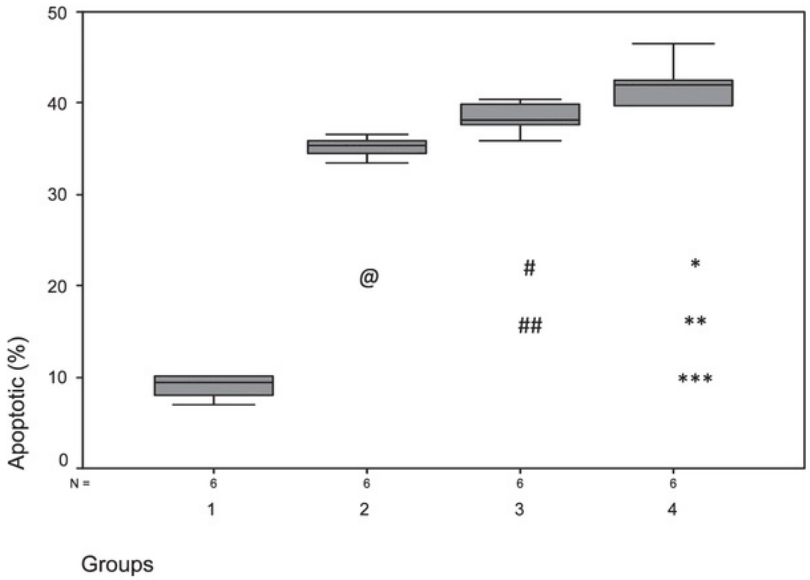

Fig. 4 Box plot of apoptotic index (%) of group 1 (control), group 2 (AC), group 3 (PM 0.07 mg/d and AC), group 4 (PM 0.14 mg/d and AC). The differences among groups were statistically significant (ANOVA, $P < 0.001$). The difference between groups: *compared to group 1, $P < 0.001$; **compared to group 2, $P < 0.001$; ***compared to group 3, $P = 0.009$; #compared to group 1, $P < 0.001$; ##compared to group 2, $P = 0.025$; @compared to group 1, $P < 0.001$.

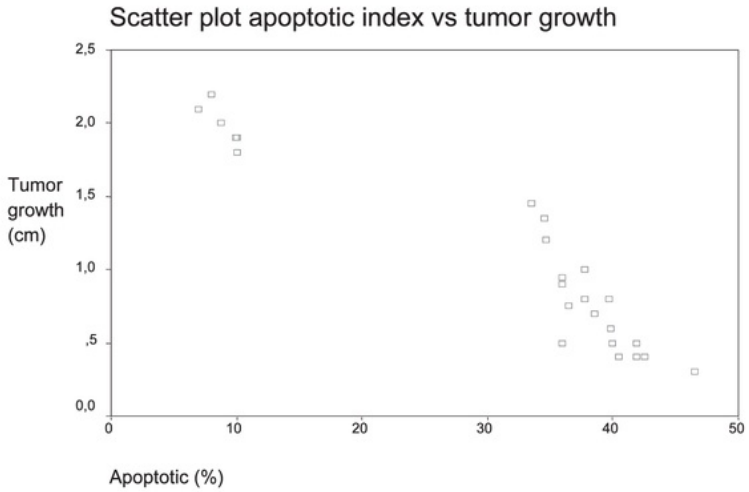

Fig. 5 Correlation between apoptotic index and tumor growth ($r = -0.884$; $P = 0.020$).

Discussion

There was tumor growth in all groups; however, the AC treatment significantly suppressed it and supplementation with Phaleria macrocarpa suppressed it even more. Supplementation with Phaleria macrocarpa suppressed tumor growth significantly more than AC-only treatment, and showed a dose–response relationship (the higher the dose of Phaleria, the more suppression of tumor growth). Cyclophosphamide suppresses tumor growth by DNA destruction, which prevents cancer cell mitosis,[13,14] whereas adriamycin increases apoptosis by increasing caspase production.[15,16] The role of Phaleria supplementation on tumor growth suppression was by increasing apoptosis.[10]

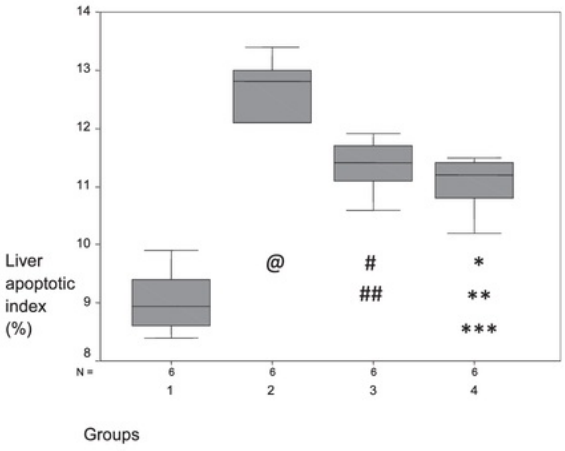

Fig. 6 Box plot of apoptotic index of the liver (%) of group 1 (control), group 2 (AC), group 3 (PM 0.07 mg/d and AC), group 4 (PM 0.14 mg/d and AC). There were significantly differences among groups (ANOVA, $P < 0.05$). The difference between groups: *compared to group 1, $P < 0.001$; **compared to group 2, $P < 0.001$; ***compared to group 3, $P = 0.299$; #compared to group 1, $P < 0.001$; ##compared to group 2, $P = 0.001$; @compared to group 1, $P < 0.001$.

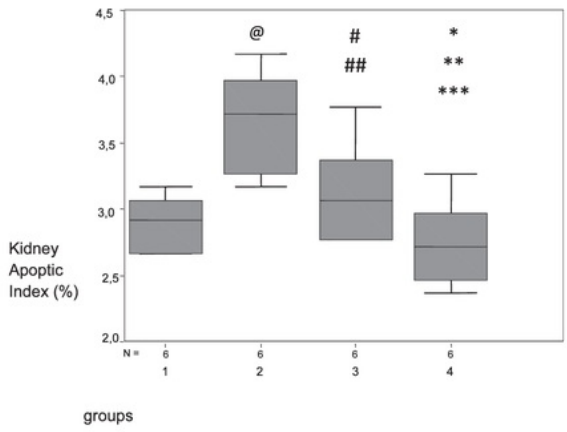

Fig. 7 Box plot of apoptotic index of kidney (%) of group 1 (control), group 2 (AC), group 3 (PM 0.07 mg/d and AC), group 4 (PM 0.14 mg/d and AC). There were significantly differences among groups (ANOVA, $P < 0.05$). The difference between groups: *compared to group 1, $P = 0.392$; **compared to group 2, $P = 0.002$; ***compared to group 3, $P = 0.107$; #compared to group 1, $P = 0.244$; ##compared to group 2, $P = 0.044$; @compared to group 1, $P = 0.002$.

The apoptotic index significantly increased after AC treatment in comparison with the control group. This is in accordance with previous studies that adriamycin increases apoptosis through increased caspase production.[15,16] Supplementation of Phaleria with AC treatment increases the apoptotic index significantly more than AC only and it showed a dose–response relationship (the higher the dose of Phaleria, the more suppression of tumor growth). This phenomenon is supported by the existence of a negative correlation between the tumor growth and apoptotic index ($r = -0.884$; $P = 0.020$) (the higher the apoptotic index increase, the least growth of the tumor). The increase of the apoptotic index with Phaleria supplementation is caused by increasing perforin[11] and granzyme[12] production. Together with granzymes, perforin produces a macromolecular complex associated with serglycin 38 and binds to the target cell surface through the mannose 6-phosphate receptor and enters the target cell as an endosome. The role of perforin is to disrupt endosomal trafficking, help granzyme to enter mitochondria, and to activate caspase leading to apoptosis.[17,18] AC treatment showed a significant increase in the apoptotic index in both the liver and kidneys. Adriamycin may increase the apoptotic index by increasing the caspase production.[15,16] Supplementation with Phaleria at both doses, 0.07 mg/d and 0.14 mg/d, reduced the increase of apoptotic index of the liver caused by AC significantly, but it never reached the control group level. The reduction effect of Phaleria supplementation on AC treatment with regard to apoptosis of the liver is not known, additional studies are needed to explain this phenomena. Also supplementation of Phaleria at both doses reduced the increase of the apoptotic index of the kidney caused by AC significantly and reached the control group level. The mechanism of reduction effect of Phaleria supplementation on AC treatment with regard to apoptosis of the kidney is not known—further study should be planned.

In conclusion, there is a synergic effect between Phaleria macrocarpa and AC in reducing tumor growth in C3H mice with breast cancer by increasing the apoptotic index, by different ways. Adriamycin increases the apoptosis by increasing caspase production, whereas Phaleria, by increasing perforin and granzyme production, eventually activates caspase. The contribution of apoptosis in reducting tumor growth was ~88% ($r = -0.884$) and the other factor that reduced tumor growth is DNA destruction caused by cyclophosphamide. Phaleria macrocarpa supplementation reduces liver and kidney cell damage caused by AC. Based on the results of this study it is prospective to study Phaleria macrocarpa supplementation to AC treatment with the purpose of downgrading stage III breast cancer preoperatively. Because Phaleria macrocarpa has already been used extensively and proved that it is safe, a phase III clinical study may be planned.

Acknowledgments

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References

1. Smith IE, A'Hern RP, Coombes GA, Howell A, Ebbs SR, Hickish TF *et al*. A novel continuous infusion 5-fluorouracil-based chemotherapy regimen compared with conventional chemotherapy in the neo-adjuvant treatment of early breast cancer: 5-year results of the TOPIC trial. *Ann Oncology* 2004;**15**:751–758
2. Beretta G. Breast cancer and gynecological malignancy. In: Beretta G, ed. *Cancer Treatment Medical Guide*. 10th ed. Milan: Farmitalia Carlo Erba-Erbamont, 1991:174–176
3. Takimoto CH, Mc.Leod HL, Boucher G. Pharmacokinetics, pharmacodynamics. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles & Practice of Oncology*. 7th ed. Philadelphia: Lippincott-Raven, 2005:317–331
4. NCCN clinical practice guidelines in oncology. Breast cancer V.2.2010. National comphrehensive cancer network, 2010; **1**:18–22, 38–40. Available at: www.nccn.org/professionals/physician_gls/PDF/breast.pdf. Accessed April 20, 2009
5. Chua S, Smith IE, Hern RPA, Coombes GA, Hickish TF, Robinson AC *et al*. Neoadjuvant vinorelbine/epirubicin (VE) versus standard adriamycin-cyclophosphamide (AC) in operable breast cancer: analysis of response and tolerability in a randomised phase III trial. *Ann Oncology* 2005;**16**(9):1435–41.
6. Medscape Instant pooling conducted. Feb 2005. Available at: http://www.medscape.com/px/instantpollservlet/result?PoIIID=1341. Accessed February 7, 2008
7. Sidomuncul PT. Aneka resep mahkota dewa. Nutrend Mahkota Dewa; smallcrab online 2007. Available at: http://www.smallcrab.com/kesehatan/67-aneka-resep-mahkota-dewa. Accessed January 17, 2009
8. Faried A, Kurnia D, Faried LS, Usman NT. Miyazaki KH. Anticancer effects of gallic acid isolated from Indonesian herbal medicine, *Phaleria macrocarpa* (Scheff.) Boerl, on human cancer cell lines. *Int J Oncol* 2007;**30**:605–613

9. Amit KT, Madhumita R, Bhattacharya RK. Natural products as inducers of apoptosis: implication for cancer therapy and prevention. *Curr Sci* 2001;**80**(11):1387–1396
10. Tazulakhova EB, Parshina OV, Guseva TS, Ershov FI. Russian experience in screening, analysis, and clinical application of novel interferon inducers. *J Interferon Cytokine Res* 2001;**21**(2): 65–73
11. Selamat B, Benny I, Djoko H, Dwi P, Riwanto I. Pengaruh ekstrak mahkota dewa (phaleria macrocarpa) terhadap skor ekspresi perforin CTL dan sel-NK serta indeks apoptotic pada adenokarsinoma mamma mencit C3H. *M Med Indones J* 2007;**42**(1):13–20
12. Selamat B, Benny I, Djoko H, Dwi P, Riwanto I. Pengaruh ekstrak mahkota dewa (phaleria macrocarpa) terhadap skor ekspresi perforin CTL dan sel-NK serta perkembangan massa tumor adenokarsinoma mamma mencit C3H. *M Med Indones J* 2007;**42**(3):37–40
13. Nguyen DH, Colvin ME, Fink WH. The molecular aspect of anticancer drug-DNA interactions and its implication to drug design. Galley proof 2007 Viplok Paper;**10**:402–16
14. Sann CL. Maleimide spacers as versatile linkers in the synthesis of bioconjugated of anthracyclines. Available at: http://www.rsc.org/delivery/_ArticleLinking/DisplayArticleForFree.cfm?doi=b600666n&JournalCode=NP. Accessed April 28, 2009
15. Ken-Ichi K, Masaru K, Hiroshi N, Saburou M. The role of the proteasome in apoptotic induced by anthracyclin anticancer agents. *Intern J Oncol* 2002;**20**(3):1205–1209
16. Ping DQ, David MS, Kenyon GD, Aslamuzzaman K. Interruption of tumor cell cycle progression through proteasome inhibition: implication for cancer therapy. *Progr Cell Cycle Res* 2003;**5**:441–446
17. Trapani JA, Smyth MY. Functional significance of the perforin/granzyme cell death pathway. *Immunology* 2002;**2**: 735–745
18. Veugelers K, Motyka B, Goping IS, Shostak I, Sawchuk T, Bleackley RC. Granule-mediated killing by granzyme B and perforin requires a mannose 6-phosphate receptor and is augmented by cell surface heparan sulfate. *Mol Biol Cell* 2006;**17**:623–633

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