ISSN: 3032-3134 (ONLINE)
InaJBCS | Indonesian Journal of Biomedicine and Clinical Sciences

# Chemopreventive effect of dayak onion [*Eleutherine bulbosa,* Mill. (Urb)] against 7,12-dimethylbenz [α] anthracene (DMBA)-induced breast cancer in rats: study on cancer antigen 15-3 (CA 15-3)

**Deo Gratias Efrem[1]*, Muhammad In'am Ilmiawan[2], Sari Eka Pratiwi[2]**

[1]Faculty of Medicine, Tanjungpura University, [2]Departement of Biology and Pathobiology, Faculty of Medicine, Tanjungpura University, West Kalimantan

https://doi.org/10.22146/inajbcs.v56i3.12245

Submitted: 2024-02-18
Accepted : 2024-04-29

## ABSTRACT

Dayak onion [*Eleutherine bulbosa,* Mill. (Urb)] is herbal plant believed to have anticancer effects. It contains triterpenoids, flavonoids, anthraquinones, and naphthoquinones which have antioxidants and anticancer activities. This study aimed to investigate the effect of ethanolic extract of dayak onion bulb (EEDO) on serum cancer antigen 15-3 (CA 15-3) levels in rats induced with 7,12-dimethylbenz [α] anthracene (DMBA). Thirty female Sprague Dawley rats were randomly divided into six groups, namely Normal Group, Positive Control Group (tamoxifen), Negative Control Group (dimethyl sulfoxide/DMSO) 5%), Treatment Group I (EEDO 180 mg/kgBW), Treatment Group II (EEDO 360 mg/kgBW) and Treatment Group III (EEDO 720 mg/kgBW). All groups, except the normal group, were induced with DMBA 20 mg/kg body weight. Serum CA 15-3 levels were determined using enzyme-linked immunosorbent assay (ELISA) method. The results showed significantly lower ($p < 0.05$) CA 15-3 levels in the Treatment Groups compared to the Negative Control Group. The most significant reduction in serum CA 15-3 level was observed in the Treatment Group I receiving EEDO at a dose of 180 mg/kgBW. In conclusion, the EEDO possesses a chemopreventive effect on DMBA-induced breast cancer in rats.

## ABSTRAK

Bawang dayak [*Eleutherine bulbosa,* Mill. (Urb)] adalah tanaman herbal yang diyakini memiliki efek antikanker. Tanaman ini mengandung triterpenoid, flavonoid, antrakuinon, dan naftokuinon yang memiliki aktivitas antioksidan dan antikanker. Penelitian ini bertujuan mengkaji efek ekstrak etanol dari umbi bawang dayak (EEDO) terhadap kadar antigen kanker 15-3 (CA 15-3) dalam serum pada tikus yang diinduksi dengan 7,12-dimetilbenzen [α] antrasen (DMBA). Tiga puluh tikus Sprague Dawley betina, dibagi menjadi 6 kelompok secara acak, yaitu Kelompok Normal, Kelompok Kontrol Positif (tamoksifen), Kelompok Kontrol Negatif (dimetil sulfoksida/DMSO 5%), Kelompok Perlakuan I (EEDO 180 mg/kgBB), Kelompok Perlakuan II (EEDO 360 mg/kgBB), dan Kelompok Perlakuan III (EEDO 720 mg/kgBB). Semua kelompok kecuali Kelompok Normal diinduksi dengan DMBA 20 mg/kgBB. Kadar CA 15-3 serum diukur menggunakan metode *enzyme-linked immunosorbent assay* (ELISA). Hasil penelitian menunjukkan bahwa terdapat penurunan signifikan ($p < 0.05$) pada kadar CA 15-3 Kelompok Perlakuan dibandingkan dengan Kelompok Kontrol Negatif. Penurunan kadar CA 15-3 serum yang paling signifikan terlihat pada Kelompok Perlakuan yang menerima EEDO dengan dosis 180 mg/kgBB. Dapat disimpulkan bahwa EEDO memiliki efek kemopreventif terhadap kanker payudara pada tikus yang diinduksi DMBA.

***Keywords***:
7,12;
dimethylbenz [α]
anthracene;
breast cancer; ca 15-3;
*Eleutherine bulbosa,* Mill.
(Urb);
rat

*corresponding author: efremtan@gmail.com

## INTRODUCTION

Cancer is the second leading cause of death in the world. Based on data from The International Agency for Research on Cancer (IARC)'s Global Cancer Statistics (GLOBOCAN), in 2018 there were an estimated 18.1 million new cancer cases and 9.6 million cancer deaths. In women, breast cancer is the most prevalent type, accounting 24.2% of the 8.6 million new cases and the highest mortality rate, comprising 15.0% of 4.2 million deaths.[1]

One of the tumor markers in breast cancer is cancer antigen 15-3 (CA 15-3), which is a part of the mucin glycoprotein 1 (MUC1) found in the glandular and luminal epithelial cells of the mammary gland. In malignant conditions, MUC1 in the epithelium will multiply and secrete CA 15-3 in large quantities.[2] This condition causes an increase in serum CA 15-3 levels. In comparison to normal condition, serum CA 15-3 level can be used as a cancer marker. Cancer cells will produce CA 15-3 which can be found in serum or cancer cells tissue.[3] The CA 15-3 levels have been shown to be an independent parameter for breast cancer prognosis and are used to assess chemoprevention and anticancer treatments.[4,5]

It has been reported that DMBA (7,12 dimethylbenz(α)antracene) exposure can cause breast cancer in rats.[6] Metabolic by products from DMBA can damage DNA structure through binding of purines to DNA or depurination, resulting in DNA mutations, inhibition of apoptosis, and causing malignancy in mammary tissue.[6,7] The DMBA induction leads to estrogen receptor positive (ER+) breast cancer.[8]

Tamoxifen was the first FDA-approved chemopreventive agent, which reduces the risk of ER+ breast cancer. A systematic review by The United States Preventive Services Task Force (USPSTF) estimated that compared with placebo, tamoxifen reduced the incidence of invasive breast cancer by 7 events (95% CI) per 1,000 women over 5 years.[9,10]

Dayak onion (*Eleutherine bulbosa*) is a medicinal plant belonging to the Iridaceae family. This plant originates from South America and grows at an altitude of 600 – 2000 meters above sea level. Currently, dayak onion has been cultivated and spread to Indonesia (Kalimantan), Thailand, South China, and South Africa.[11] This plant has been used empirically by people in Southeast Asia as a traditional medicine for hypertension, diabetes, cholesterol, dysentery, anti-inflammatory, laxative, accelerate wound healing, and antifertility.[12] According to previous studies, dayak onion is also believed to have anticancer, antiosteoporosis, antibacterial, antiviral, and cytotoxic effects.[11,13-15] Based on previous studies, it is known that dayak onion has several active compounds such as naphthoquinones, anthraquinones, naphthalene, alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids, and steroids.[11,13,16,17]

Naphtoquinones and their derivatives such as eleutherine, eleutherol, eleutherinone, and elecanacine, exhibit biological activity as antimicrobial, antiviral, anti-inflammatory, antipyretic, antifungal, and also have cytotoxic effect against colon cancer and breast cancer. *In silico* studies by Lubis *et al.*[13] found that the active compound eleutherinol from dayak onion has the highest affinity among other compound to bind with human estrogen receptor alpha and has anticancer potential.[10] Based on previous *in vitro* studies by Fitri *et al.*[18] the n-hexane, ethyl acetate, and ethanolic extract from dayak onion had a cytotoxic effect on WiDr colon cancer cells and T47D breast cancer cell.[16] However, all previous studies lacked *in vivo* experiments, leading to limitations in evaluating the effects of dayak onion on more complex living organism than single cell line. The *in vivo* experiment provides a better understanding of how the dayak onion affects the

tumorogenesis dan chemoprevention in more complex living organism. The purpose of this present study was to evaluate the *in vivo* chemopreventive effect of ethanolic extract of dayak onion in rats induced with DMBA.

## MATERIAL AND METHODS

### Animals

Thirty female Sprague dawley rats aged 5 wk at initiation of experiment were used. All animal-use procedures were ethically reviewed and approved by Ethical Clearance Committee of the Faculty of Medicine, University of Tanjungpura prior to experiment in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed under a 12 h light/dark cycle with free access to food and water. All efforts were made to minimize suffering and the number of animals used.

### Ethanolic extract preparation

Eleutherine *bulbosa* was obtained from Sintang Regency, West Kalimantan Province, Indonesia. It was dried, powdered, and macerated with ethanol 96% then processed using rotary evaporator to get ethanolic extract of *E. bulbosa* bulb (EEDO). The ethanolic extract of dayak onion was dissolved in 5% DMSO according to each group's dosage before being administered to the rats. The DMBA (Tokyo Chemical Industry, Japan) was dissolved in corn oil with a final concentration of 4 mg/mL. Tamoxifen (Kalbe Farma, Indonesia) was dissolved in 5% DMSO with a final concentration of 0.2 mg/mL.

### Treatment

After acclimatization for 7 d, 30 rats were randomly divided into 6 groups with each group consist of 5 rats. The groups were as follows: (1) Normal Control Group: only got standard food and water, (2) Negative Control Group: DMBA induction and DMSO 5%, (3) Positive Control Group: DMBA induction and tamoxifen 2 mg/kgBW/d, (4) Treatment Group I: DMBA induction and EEDO 180 mg/kgBW/d, (5) Treatment Group II: DMBA induction and EEDO 360 mg/kgBW/d, and (6) Treatment Group III: DMBA induction and EEDO 720 mg/kgBW/d.

The DMBA was administered intragastrically at a dose 20 mg/kgBW. The induction process was carried out twice weekly for 5 wk. The induction was evaluated by palpation on the chest and abdomen area; the induction was considered successful if a tumor nodule was palpable. Tamoxifen was administered intragastrically every day for 70 d in the Positive Control group (+). The EEDO was administered intragastrically every day for 70 d in Group I, Group II and Group III.

### Examination of CA 15-3

After 70 d, all rats were anesthetized using chloroform and euthanized, then blood was drawn through the aorta. The blood was placed in a serum separator tube immediately after being taken from the aorta and stored at room temperature. The blood was allowed to clot for 30 min, then centrifuged for 20 min at 2000 rpm at 8° C. The separated serum sample (supernatant) was aliquoted into 1.5 mL microtube and stored in a freezer at -20° C. After 7 d, the serum was thawed at room temperature and immediately underwent the ELISA procedure to measure the CA 15-3 level. The CA 15-3 level was measured using the appropriate ELISA Kit (Bioenzy, Indonesia, CAT No. BZ-08189311-EB).

### Statistical analysis

Data are expressed as mean ± standard deviation (SD) and were

analysed by one-way analysis of variance (one-way Anova). Means between treatment groups were compared using the Least Significant Difference test. All statistical analysis were conducted using SPSS software (version 23, IBM Corp, Armonk, New York). A p value <0.05 was considered statistically significant.

## RESULTS

Induction of breast tumors in test animals using DMBA was evaluated by palpating the thoracic and abdominal regions where the breast glands are located. Palpation was performed every week to determine the presence or absence of tumor nodules. Tumor nodules were first palpated in the 8th wk after induction. At the end of the treatment, all rats in all groups had tumor nodules confirmed by palpation.

After measurement with ELISA method, the optical density values of each sample were obtained. Subsequently, we calculated the CA 15-3 level in each sample using the formula from the standard curve. The CA 15-3 serum level are shown in FIGURE 1.

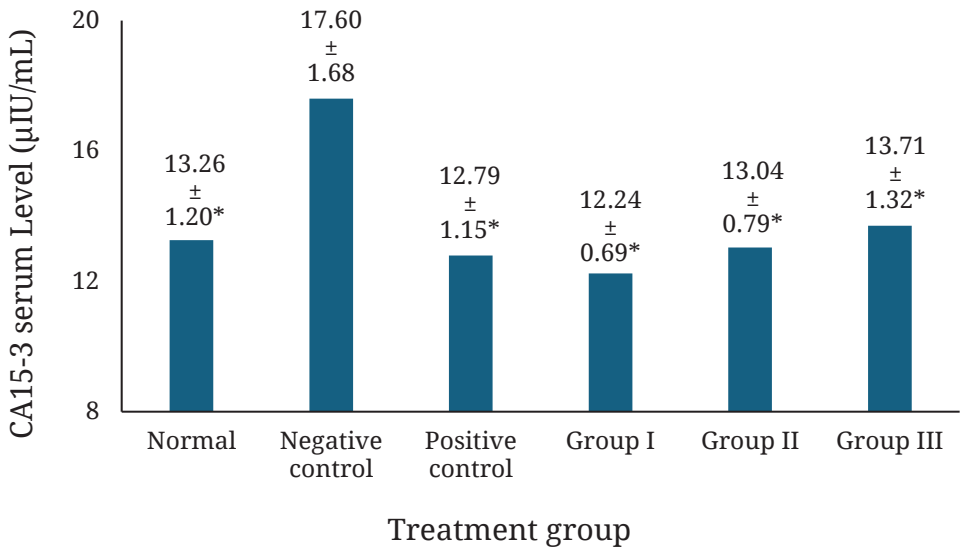


FIGURE 1. Effect of EEDO on CA 15-3 serum level in DMBA-induced breast cancer in rats. The graph shows the mean CA 15-3 serum level (µIU/mL) across different experimental groups. Data are expressed as mean ± SD. *p< 0.05 versus Negative Control group. The groups are as follows: Normal (received standard food and water), Negative Control (DMBA induction and 5% DMSO), Positive Control (DMBA induction and tamoxifen 2 mg/kg body weight/day), Group I (DMBA induction and EEDO 180 mg/kg body weight/day), Group II (DMBA induction and EEDO 360 mg/kg body weight/day), and Group III (DMBA induction and EEDO 720 mg/kg body weight/day). CA 15-3: cancer antigen 15-3; EEDO: ethanolic extract of dayak onion; DMBA: 7,12-dimethylbenz[α]anthracene; SD: standard deviation.

This study showed that the average of serum CA 15-3 level in Normal Group, Negative Control Group, Positive Control Group, Treatment Group I, Treatment Group II, and Treatment Group III were 13.26±1.20 µIU/mL, 17.60±1.68 µIU/mL, 12.79±1.15 µIU /mL, 12.24±0.72 µIU/mL, 14.04±0.69 µIU/mL, and 13.71±1.32 µUI/mL, respectively (FIGURE 1). A significantly different in the serum CA 15-3 level between the Normal Group, Positive Control Group, Treatment Group I, II and III compared to the Negative Control Group was observed ($p<0.05$). However, there was no significance difference between the Normal Group, Positive Control Group, Treatment Group I, II and III (LSD test and HSD test; $p>0.05$). Therefore, the most effective dose of EEDO for chemoprevention on DMBA induced breast cancer in rats cannot be concluded in this study.

## DISCUSSION

7,12 Dimethylbenz(α)antracene is a potential carcinogen compound. This compound is an inactive procarcinogen (proximate carcinogen) in the body. It undergoes changes to become a primary carcinogen or an ultimate carcinogen. The ultimate carcinogen is the final metabolite of DMBA, which damages the DNA structure through the formation of radical cations and epoxide dihydrodiol. Epoxide dihydrodiol alters the DNA structure by covalently binding to the exocyclic amino groups of DNA purines and forming a stable DNA adduct, while the radical cation binds to N7 or C8 positions, resulting in unstable DNA adducts due to the loss of purines in DNA or depurination.[19-21]

7,12 Dimethylbenz(α)antracene undergoes activation in mammary gland epithelial cells and produces active metabolites in the form of DNA adducts, which are complexes formed by spesific DNA sections that are covalently bound to DMBA mutagen compounds.[21] Reactive oxygen species (ROS) are also products of DMBA metabolism and are formed during DMBA metabolic activation. Reactive oxygen spesies cause DNA adducts to bind to guanine bases in DNA, causing oxidative damage to the structure and function of DNA, proteins and lipids. This oxidative damage results in DNA to mutation. Cellular DNA can return to normal if the DNA repair mechanism takes place normally, if this mechanism does not function normally then the mutated cells will grow and develop into tumors.[20,21]

There is a difference in serum CA 15-3 levels between the Normal Group and the Negative Control Group, where the serum CA 15-3 level in the Negative Group was higher than the Normal Group. This was one of the pieces of evidence for the success of breast tumor induction in the negative control group.[22] According to Lee *et al.*[22] serum CA 15-3 levels in patients with breast tumors tend to increase compared to normal conditions, this increase tends to be even higher when the tumor becomes invasive and metastasizes to other tissues.

The difference in serum CA 15-3 levels between the Negative Control Group and Treatment Groups I, II, and III demonstrated that there is an effect of the administration of the EEDO on the serum CA 15-3 levels in rats with breast tumors. Spesifically, the EEDO was effective in reducing serum CA 15-3 levels in these rats. The most effective dose in reducing serum CA 15-3 levels in this study was the dose in the Treatment Group I (180 mg/kgBW).

Based on several previous studies, the EEDO has anticancer activities.[13,18,23,24] Dayak onion bulbs contain active compounds such as naphthoquinones, anthraquinones, and naphthalene.[11,13,16,17] Eleutherinol which is one of the derivatives of naphthoquinones, is an active compound known to have the most potent anticancer activity among other active compounds in dayak onion.[13,18]

One of the known effects of dayak onion extract is the suppression of mutant p53 expression. Mutant p53 is a p53 protein that undergoes a missense mutation, resulting in the p53 protein losing its tumor suppression ability and promote tumorigenesis.[25,26]

The results of this study are consistent with *in vitro* studies by Lubis *et al.*[13] which showed a suppressive effect of the EEDO on the expression of mutant p53 protein in T47D cancer cells.[18] According to Fitri *et al.*[18] the anticancer mechanism of dayak onion is achieved by inhibiting the cell cycle at the G0-G1 phase, causing the cell to be trapped in the G0 or G1 phase. This mechanism occurs through the process of inhibiting the expression of cyclin D, so that cyclin D cannot bind to CDK4 and CDK6. As a result, the phosphate group on CDK is unable to phosphorylate Retinoblastoma protein (Rb). Unphosphorylated Rb protein will bind to the E2F transcription factor and inhibit the transcription of cyclin E and cyclin A, which are needed to enter the S phase of cell replication, causing the cell will be stuck in the G1 phase.[18]

The termination of the cell cycle in the G0-G1 phase provides an opportunity for the repair process to occur in the mutated DNA, or if the mutated DNA cannot be repaired, the cell will undergo apoptosis to prevent further spreading. This apoptosis process is trigerred by the inhibition of BCL2, which is an anti-apoptotic protein.[18] The decreased rate of cell proliferation and the cessation of the cell cycle result in the inhibition of the growth and development of mammary tumor cells, preventing the metastasis process from occuring.

Cancer antigen 15-3 protein is the epitope part of Mucin 1 (MUC1). MUC1 is a transmembrane glycoprotein that is found on the surface of epithelial cells of mammary glands, pancreas, prostate, testes, liver, lungs, and kidneys.[27] In normal condition, MUC1 plays a role in cell hydration, defense against microorganisms and degenerative enzymes, as well as inhibiting interactions between cells. When the cells become malignant, MUC1 levels increase above normal, and MUC1 becomes underglycosylated and overexpressed. The location of MUC1 and growth factor receptors also become irregular due to changes in cell shape.[2,28]

Due to its extracellular location, CA 15-3 can be released and go along with the bloodstream. The amount of CA 15-3 is directly proportional to the amount of MUC1. If the levels of MUC1 increases, the levels of CA 15-3 in the blood will also increase.[2,29]

MUCI 1 and CA 15-3 are known to be predictors of prognosis in patients with breast tumors, with elevated MUC1 levels being associated with poor prognosis. MUC1 is likely to play a role in tumorigenesis, progression and metastasis in tumors. The association of MUC1 with EGFRs, β-catenin, and NF-κb can also affect tumor progression and invasiveness.[30] Overexpression of MUC1 has been shown to increase angiogenesis and chemoresistance in breast tumors.[29,31,32] Therefore, a low level of MUC1 is a factor associated with a positive prognosis in breast tumor patients.

Increased tumorigenesis activity and the number of tumor cells in breast tissue lead to increased MUC1, which in turn further worsens the condition of the tumor.[2,28] Conversely, a decrease in the amount of MUC1 increases the possibility of a good prognosis for patients with breast tumors. Inhibition of cyclin D by the EEDO can lead to a decrease in the number of tumor cells, resulting in a reduction in the amount of MUC1 in tumor cells.[2,28] The decrease in the number of MUC1 can be predicted from the decreased CA 15-3 protein levels in the serum, which are lower than in the Negative Control Group that did not receive the EEDO.

This study has some limitations. The

EEDO use in this study is not an isolated pure bioactive compound. In addition, the most effective dose of the EEDO for breast cancer chemoprevention could not be concluded, yet. Therefore, we recommend isolating bioactive compounds from dayak onion and investigating the most effective dose of dayak onion for chemoprevention.

## CONCLUSION

In conclusion, the EEDO possesses a chemopreventive effect on DMBA-induced breast cancer in rats. Further study to investigate the most effective dose of the EEDO is recommended.

## ACKNOWLEDGMENT

We would like to express our gratitude to the staff at the Faculty of Medicine Tanjungpura University, West Kalimantan for valuable assistances during the study.

## REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6):394-424. https://doi.org/10.3322/caac.21492
2. Nath S, Mukherjee P. Muc1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med 2014; 20(6):332-42. https://doi.org/10.1016/j.molmed.2014.02.007
3. Li X, Dai D, Chen B, Tang H, Xie X, Wei W. Determination of the prognostic value of preoperative CA15-3 and CEA in predicting the prognosis of young patients with breast cancer. Oncol Lett 2018; 16(4):4679-88. https://doi.org/10.3892/ol.2018.9160
4. Di Gioia D, Dresse M, Mayr D, Nagel D, Heinemann V, Stieber P. Serum HER2 in combination with CA 15-3 as a parameter for prognosis in patients with early breast cancer. Clin Chim Acta 2015; 440:16-22. https://doi.org/10.1016/j.cca.2014.11.001
5. Nandhakumar R, Salini K, Niranjali Devaraj S. Morin augments anticarcinogenic and antiproliferative efficacy against 7,12-dimethylbenz(a)-anthracene induced experimental mammary carcinogenesis. Mol Cell Biochem 2012; 364(1-2):79-92. https://doi.org/10.1007/s11010-011-1207-5
6. Kwon YJ, Ye DJ, Baek HS, Chun YJ. 7,12-Dimethylbenz[α]anthracene increases cell proliferation and invasion through induction of WNT/β-catenin signaling and EMT process. Environ Toxicol 2018; 33(7):729-42. https://doi.org/10.1002/tox.22560
7. Nassan MA, Soliman MM, Ismail SA, El-Shazly S. Effect of *Taraxacum officinale* extract on PI3K/Akt pathway in DMBA-induced breast cancer in Albino rats. Biosci Rep 2018; 38(6):BSR20180334. https://doi.org/10.1042/BSR20180334
8. Alvarado A, Lopes AC, Faustino-Rocha AI, Cabrita AMS, Ferreira R, Oliveira PA, *et al*. Prognostic factors in MNU and DMBA-induced mammary tumors in female rats. Pathol Res Pract 2017; 213(5):441-6. https://doi.org/10.1016/j.prp.2017.02.014
9. Gu KJ, Li G. An overview of cancer prevention: chemoprevention and immunoprevention. J Cancer Prev 2020; 25(3):127-35. https://doi.org/10.15430/JCP.2020.25.3.127
10. Kodama M, Kodama T. The nature of tamoxifen action in the control of female breast cancer. In Vivo 2001; 15(4):319-25.
11. Insanu M, Kusmardiyani S, Hartati R. Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr. Procedia Chem 2014; 13:221-8.

https://doi.org/10.1016/j.proche.2014.12.032
12. Paramita S, Nuryanto MK. Anti-inflammatory activity of bawang dayak (*Eleutherine bulbosa* (Mill. Urb.)) ethanol bulb extracts. J Vocat Health Stud 2019; 2(2):51-5. https://doi.10.20473/jvhs.V2.I2.2018.51-55
13. Lubis IA, Ichwan MF, Mustofa M, Satria D. Anticancer activity of *Eleutherine bulbosa* (Mill.) Urb. extract on WIDR cell line *in vitro*. Atlantis Press 2017. https://doi.org/10.2991/phico-17.2018.25
14. Bahtiar A, Annisa R. Effects of dayak onion bulbs (*Eleutherine bulbosa* (Mill.) Urb) on bone development of the hipoestrogen model rat. Pharmacog J 2018; 10(2):299-303. https://doi.org/10.5530/pj.2018.2.52
15. Jiang H, Man WJ, Hou AJ, Yang L, Xing XD, Yan ML, *et al*. The chemical constituents from the active fractions of *Eleutherine bulbosa* with their antimicrobial activity. Nat Prod Res 2020; 34(12):1743-49. https://doi.org/10.1080/14786419.2018.1530229
16. Daryono BS, Deisshinta W, Sudarsono. Identification of bawang sabrang (*Eleutherine americana* Merr. ex K. Heyne) in Indonesia based on chromosome characters. Indones J Pharm 2013; 24(1):22-9. https://doi.org/10.14499/indonesianjpharm24iss1pp22-29
17. Rani V. Isolation of a compound from the bulbs of *Eleutherine bulbosa* (Miller) Urban (Iridaceae). Asian J Pharm Clin Res 2018; 11(6):406. https://doi.org/10.22159/ajpcr.2018.v11i6.25059
18. Fitri Y, Rosidah, Suwarso E. Effects of inhibition cell cycle and apoptosis of sabrang onion extract (*Eleutherine bulbosa* (Mill.) Urb.) on breast cancer cells. Int J PharmTech Res 2014; 6(4):1392-96.
19. PubChem. *7,12-Dimethylbenz[a] Anthracene*. https://pubchem.ncbi.nlm.nih.gov/ compound/6001
20. Androutsopoulos VP, Tsatsakis AM, Spandidos DA. Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. BMC Cancer 2009; 9:187. https://doi.org/10.1186/1471-2407-9-187
21. Rajalakshmi TR, AravindhaBabu N, Shanmugam KT, Masthan KMK. DNA adducts-chemical addons. J Pharm Bioallied Sci 2015; 7(Suppl 1):S197-9. https://doi.org/10.4103/0975-7406.155901
22. Lee JS, Park S, Park JM, Cho JH, Kim SI, Park BW. Elevated levels of serum tumor markers CA 15-3 and CEA are prognostic factors for diagnosis of metastatic breast cancers. Breast Cancer Res Treat 2013; 141(3):477-84. https://doi.org/10.1007/s10549-013-2695-7
23. Sudarmawan IH. Pengaruh pemberian fraksi etanolik dan petroleum eter ekstrak umbi bawang dayak (*Eleutherine palmifolia* (L), Merr ) terhadap ekspresi p53 mutan galur sel kanker payudara T47D. [PhD Thesis]. Program Pendidikan Dokter Spesialis I Ilmu Bedah Fakultas Kedokteran, Universitas Sebelas Maret; 2009.
24. Putri ENA, Haryoto. Aktivitas antikanker ekstrak etanol umbi bawang dayak (*Eleutherine americana* Merr.) terhadap sel kanker payudara T47D. Presiding of The 7th University Research Colloqium 2018STIKES PKU Muhammadiyah Surakarta, 7:192-203.
25. Muller PAJ, Vousden KH. Mutant p53 in cancer: New functions and therapeutic opportunities. Cancer Cell 2014; 25(3):304-17. https://doi.org/10.1016/j.ccr.2014.01.021
26. Perri F, Pisconti S, Scarpati GDV. p53 mutations and cancer: a tight linkage. Ann Transl Med 2016; 4(24):522. https://doi.org/10.21037/atm.2016.12.40
27. Brayman M, Thathiah A, Carson DD. MUC1: a multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endocrinol

2004; 2:4.
https://doi.org/10.1186/1477-7827-2-4
28. Yang C, Murray JL, Ibrahim NK. MUC-1 and cancer immunotherapy. Immunology 2018; 225-40.
https://doi.org/10.1016/B978-0-12-809819-6.00015-0
29. Pillai K, Pourgholami MH, Chua TC, Morris DL. MUC1 as a potential target in anticancer therapies. Am J Clin Oncol 2015; 38(1):108-18.
https://doi.org/10.1097/COC.0b013e31828f5a07
30. Bernier AJ, Zhang J, Lillehoj E, Shaw ARE, Gunasekara N, Hugh JC. Non-cysteine linked MUC1 cytoplasmic dimers are required for Src recruitment and ICAM-1 binding induced cell invasion. Mol Cancer 2011; 10:93.
https://doi.org/10.1186/1476-4598-10-93
31. Jing X, Liang H, Hao C, Yang X, Cui X. Overexpression of MUC1 predicts poor prognosis in patients with breast cancer. Oncol Rep 2019; 41(2):801-10.
https://doi.org/10.3892/or.2018.6887
32. Apostolopoulos V, Stojanovska L, Gargosky SE. MUC1 (CD227): a multi-tasked molecule. Cell Mol Life Sci 2015; 72(23):4475-500.
https://doi.org/10.1007/s00018-015-2014-z