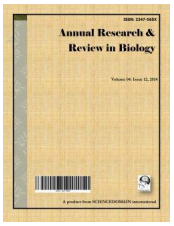

Annual Research & Review in Biology

18(5): 1-8, 2017; Article no.ARRB.34656
ISSN: 2347-565X, NLM ID: 101632869

Effectiveness of Ethyl Acetate Extract of Endophytic Fungi in Soursop Leaves towards the Growth of Mammary Tumor Induced by 7,12-dimethylbenz(α)anthracene in Female Rats

C. I. Asyura[1], A. E. Z. Hasan[1*], Hasim[1], H. Julistiono[2], Husnawati[1], N. Bermawie[3] and E. I. Riyanti[4]

[1]Department of Biochemistry, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Jl. Lingkar Akademik, Kampus IPB Darmaga, Bogor, Indonesia.
[2] Division of Microbiology, Indonesian Institute of Sciences, Indonesia.
[3]Research Institute of Medicinal and Aromatic Plants, Agricultural Research Agency, Bogor, Indonesia.
[4]Center for Biotechnology and Genetic Resources of Agriculture, Agricultural Research Agency, Bogor, Indonesia.

Authors' contributions

All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2017/34656
Editor(s):
(1) Wafaa Mohamed Shukry, Departement of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Shimaa R. Hamed, National Research Centre, Egypt.
(2) Emmanuel M. Papamichael, University of Ioannina, Ioannina, Greece.
(3) Ayodele Akinterinwa, Modibbo Adama University of Technology, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/21555

Original Research Article

Received 4th June 2017
Accepted 7th August 2017
Published 25th October 2017

ABSTRACT

Aims: This research aims to investigate the effectiveness of ethyl acetate extract of endophytic fungi isolated Sir G_5 from soursop leaf (*Annona muricata* L.) *in vivo* breast tumor growth using Sprague-dawley (SD) female strains induced by 7,12-dimethylbenz(α)anthracene (DMBA).
Study Design: This study was an *in vivo* experiment using a completely randomized design method (RAL).
Place and Duration of Study: This research was conducted from July 2016 to February 2017 in Microbiology Laboratory of Biology Research Center, Indonesian Institute of Sciences (LIPI),

**Corresponding author: E-mail: pakzainalhasan@gmail.com;*

Cibinong-Bogor, and Research Laboratory of Biochemistry Department of Bogor Agricultural University.

Methodology: Twenty-four female rats were divided into six groups; normal group (administered per os (p.o) with aquadest 2 ml for twenty two weeks), negative control (-) DMBA (administered intraperitoneal (i.p.) with 20 mg/kg body weight (bw) of 7,12-dimethylbenz(α)anthracene), positive control (+) Doxo (administered i.p with 20 mg/kg bw of 7,12-dimethylbenz(α)anthracene and p.o. with 2 µg/20 0 g bw of Doxorubicin), DI (administered i.p. with 20 mg/kg bw of 7,12-dimethylbenz(α)anthracene and p.o. with extract of 20 mg/kg bw isolate Sir G_5), DII (administered i.p. with 20 mg/kg bw of 7,12- dimethylbenz(α) anthracene and p.o. with 40 mg/kg bw extract of isolate Sir G_5) and DIII (administered i.p. with 20 mg/kg b.w.of 7,12- dimethylbenz(α)anthracene and with 120 mg/kg bw of extract of isolate Sir G_5).

Results: The result showed that body weight rats was not significantly different at each group ($P>0.05$). The quantity and volume of tumor samples from isolat Sir G_5 extract treatment groups was significantly lower ($P<0.05$) than negative control DMBA and positive control doxo.

Conclusion: Overall, it can be concluded that the ethyl acetate extract of endophytic fungi isolated Sir G_5 from soursop leaf (*Annona muricata* L.) can inhibit the growth of rat breast tumor particulary on the treatment dose DI (20 mg/Kg bw).

Keywords: Soursop leaves; endophytic fungi; breast tumor; cancer; rat.

1. INTRODUCTION

Cancer or malignant tumor is a disease characterized by the abnormal and uncontrolled of cell growth. WHO (2015) stated that the second leading cause of death in the world after cardiovascular disease among non-infectious diseases is cancer [1]. Currently, cancer is the cause of the major global health issues that should be taken seriously. Based on GLOBOCAN data, the International Agency for Research on Cancer (IARC), it is known that in 2012 there are 14.1 million new cases of cancer and 8.2 million cancer deaths worldwide [2].

Breast cancer is the most common malignant tumor and the leading cause of cancer death among women in the world [3]. Breast cancer is a process of abnormal and uncontrolled growth of breast cells from breast tissue, usually in epithelial cells in the ducts (lining of the milk ducts) or lobules as a result of mutations in genes responsible for regulating normal cell growth and keeping the cells in order stay healthy [4]. Pathological Based Registration reported that breast cancer has the first rank in Indonesia with relative frequency of 18.6% and incidence rate of 12 / 100,000 women and this number has tendency for increasing from year to year [5,6].

Common treatments for breast tumors includes surgery, radiation therapy, drugs prescription (which are cyclophosphamide, methotrexate, 5-fluorouracil, liposomal doxorubicin and herceptin), hormonal therapy and biological therapy [7,8]. Various obstacles for cancer treatments including such as expensive, it's side effects, the length of treatment and very low cure rate. This condition triggers the need for an alternative treatment of cancer with high effectivity and a minimal side effects. The use of natural ingredients (herbs) is one effort undertaken to develop the preparation of herbal medicine.

Natural materials contain potential bioactive compounds. These bioactive compounds can be obtained from plants and also from several other sources of animals, microbes and marine biota [9]. It is reported that endophytic microbes known as a source for bioactive compounds and potential for further development into drugs [10]. According to Tan and Zou, endophytic microbes can produce bioactive compounds that have a similar character with the host due to evolutionary genetic exchange [11]. Endophytic microbes commonly isolated from natural products are fungi compared to bacteria[12]. These endophytic fungi uses nutrients from the host for their growth, but it does not pathogenic for their host [13]. Several endophytic fungi species that had been successfully isolated from various plant tissues are able to produce secondary metabolites that can be used for medicine and agriculture products [14]. It is known that soursop leaf commonly used as traditional medicine for various diseases such as cancer [15]. Total of 212 bioactive compounds found in soursop plants have been reported. Major bioactive compounds found in soursop is acetogenin, followed by alkaloids, phenols and other compounds such as flavonoids, terpenoids, siclopeptide, flavonols triglikocida, phytosterols

that have anticancer and antioxidants effects [16-19]. Soursop leave contains very high concencentration of alkaloids followed by acetogenin compounds, both have cytotoxic properties against cancer cells [20,21].

Cytotoxic activity of bioactive compound from crude extract of ethyl acetate of endophytes fungi in soursop leaves showed that endophytes in Soursop leaf Sir G_5 isolate originated from Garut has the most active activity toward breast cancer cells MCF-7 with IC_{50} of 19.20 µg/mL. Based on the result of GC-MS analysis of endophytic fungi in soursop leaf isolates G_5 contains active compounds belongs to alkaloid group of piperidinone, piperidine, hexadecanenitle which have property as anticancer compounds [22]. Therefore further research is needed on the effectiveness of ethyl acetate extract from endophytes fungi in soursop leaf isolate Sir G_5 toward the *in vivo* growth of breast tumor using female white rat strain Sprague-dawley (SD) induced by 7.12-dimethylbenz(α)anthracene carcinogen (DMBA).

2. MATERIALS AND METHODS

2.1 Cultivation of Endophytic Fungi from Soursop Leaf Isolate Sir G_5 (Minarni Modification)

Isolate of endophytic fungi from sousop leaves of Sir G_5 genus Phomopsis sp was obtained from the previous study [22]. Sir G_5 endophytic fungi isolate from tilted agar was grown into MGYPA medium then incubated for 7 days at room temperature. Liquid culture was done in 2 L of MGYP media, and incubated at room temperature and shaken at 120 rpm for 21 days.

2.2 Extraction of Endophytic Fungi Active Compounds in Soursop Leaf Isolate Sir G_5

Isolates of Sir G_5 endophytic fungi that have been cultivated for 21 days in MGYPB media, then extracted by Maceration method using ethyl acetate to extract the bioactive compound. The extract was then evaporated using a rotary vacuum evaporator, and then concentrated using nitrogen gas and stored in the refrigerator.

2.3 Preparation of DMBA Solution [23]

The given DMBA dose was 20 mg/Kg b.w. (single doses) [24]. DMBA solution was made at volume of 30 mL for once administration with concentration 4 mg/Kg b.w. A total of 120 mg DMBA powder was added with 10 mL of pure olive oil and then dissolved using a sonicator until homogen. Further olive oil was added into the solution to make a volume of 30 mL.

2.4 Dissolution of Dry Ethyl Acetate Extracts of Endophyte Fungi in Soursop Leaf Isolate Sir G_5

The dry extract of ethyl acetate endophytic fungi of Sir G_5 soursop leaves obtained was made in 3 doses,i.e. 20 mg/Kg bw, 40 mg/Kg bw and 120 mg/Kg bw and then dissolved completely in 1% CMC.

2.5 Experimental Design

This experiment uses physically fit female rats Spaque –Dawley strain with characteristics such as: no stand fur, pure white color, clear red eyes, no mushy stool, normal behavior and active move, at age of 7 weeks and with initial body weight between 118-133 grams. A total of 24 female rats Sprague-Dawley strain aged were divided into 6 groups were randomly assigned as: Normal group (N) fed with aquadest; DMBA group as a negative control, rats induced with DMBA carcinogen compound at 20 mg/Kg b.w. and fed with aquadest; Doxo group as a positive control, rat DMBA was induced with carcinogenic compounds at a dose of 20 mg/kg b.w. and treated with commercial drug doxorubicin at a dose of 2 µg/200 g bw; DI group of carcinogenic compounds that rat was induced with a carsinogenic compounds at a dose of 20 mg/kg and then treated using ethyl acetate extracts of endophytic fungi in soursop leaves at a dose of 20 mg/kg; DII group of rat that was induced with DMBA carcinogenic compounds at a a dose of 20 mg/kg and then treated with ethyl acetate extract of endophytic fungi with a dose of 40mg/kg; DII group of rat that was induced with DMBA carcinogenic compounds at a dose of 20 mg/kg and then treated using ethyl acetate extract of endophytic fungi at a dose of 120 mg/kg.

Carcinogenic solution induction using DMBA was administered by injecting the intraperitoneal part of the lower cavity of the rat's abdomen and then examined for 45 days until a tumor lump appeared. Medication treatment using ethyl acetate extract of endophytic fungi isolate G_5 was done orally, by stringing using gastric sonde

after the tumor lump appeared, which is on the 49th day until the end of treatment that is day 168 (for 18 weeks). Body weight observation was conducted once a week starting at adaptaion period (before treatment) from – 21 day to 168 day (the last treatment).

2.6 Tumor Palpation

Palpation examination was done macroscopically. The observation consists of recording the number of tumors, measuring the diameter and the position of the tumor. Palpation examination was conducted daily, starts from one day after DMBA induction until the end of treatment. The data of tumor diameter growth was then calculated for the volume of cancer formed by using the formula [25]. Volume of tumor (v) = (π x L x W2)/ 6, where *L* and *W* are tumor diameter (L> W).

2.7 Statistical Analysis

The quantitative data from weight measurement, tumor count and tumor volume were statistically analyzed using the MINITAB version 16 program for Windows. Data were analyzed with nonparametric statistical analysis method, Kruskall Wallis test and followed by Mann Whitney's advanced test [26]. The data was categorized as different if $p < 0.05$ and not significantly different if $p > 0.05$.

3. RESULTS AND DISCUSSION

3.1 The Growth Weight of the Sprague Dawley Rats

An adaptation period of the animals was conducted for 21 days before treatment (-21 day). The weight of rat during the adaptation period increased (Fig. 1) and there was no significant difference between the treatment groups $p > 0.05$. The effect of carcinogen induction and extract administration on physiological decline and rat's body response were determined by body weight measurement. At day 0 rats were treated with carcinogenic solution (DMBA) to develope cancer, while the normal group was not treated with carcinogen. DI group experienced the weight loss on 21 day but not significantly different ($p > 0.05$) with the other treatment groups. On the 28 day, DII group also experienced weight loss but not significantly different ($p > 0.05$) with the other treatment groups. While the body weight of DMBA, Doxo and DIII groups were increased. On the other hand, carcinogenic induction did not have a significant effect ($p > 0.05$) to the weight for all groups [27].

Medication was conducted on 42 day. The DI, DII and DIII groups were given endophytic fungi extract while the doxo group was given commercial drug, doxorubicin. As shown in Fig. 1, the body weight increment in rat generally fluctuated, starting from the beginning of treatment, 42 day until 98 day. On 105 dat DII groups started losing weight but did not different significantly with DI, DIII, DMBA and Doxo groups (Table 1). While the DI group had stable weight from the beginning of treatment (day 42nd) and increased on the day 112. DIII group experienced weight lost on days 140 but was not significant ($p > 0.05$). Medication treatment with endophytic fungi extract did not have any significant effect on the weight loss.

3.2 Number and Volumes of Tumors in Treatment Group

Tumor is a swelling or a lump caused by a continuously abnormal cell division to form a lump. In this study, the highest total tumors were found in the DMBA treatment group of 4 small tumors which was obtained during surgery with a 31.07 cm^3, followed by a doxo group with 3 larger tumors than the DMBA group with a volume of 38.83 cm^3, the group The DIII had a total of 3 tumors with a volume of 30.43 cm^3, while the DI group had a total of 2 small tumors of 4.87 cm^3 (Fig. 2) and the DII group had a total of 2 tumors with a volume of 25.96 cm^3 (Fig. 3).

The experimental group of Doxo, DI, DII and DIII had a lower total tumor than the DMBA group with a significant difference ($p < 0.05$) against the negative control. The doxo group has a lower total and volume than the DMBA, DI, DII, and DIII groups, since the doxorubicin drug can suppress the breast tumor and has been used in the treatment of various cancers treatment widely [28]. The use of doxorubicin in this study was orally administered at a dose of 20 mg/kg bw and the total dose did not exceed 550 mg/Kg bw, so the less effective of drug absorption was seen from the large tumor volume compared to the DMBA group as a negative control. It is suspected that doxorubicin should be administered intravenously as it turned inactive when absorbed through digestive tract (applied orally) [29].

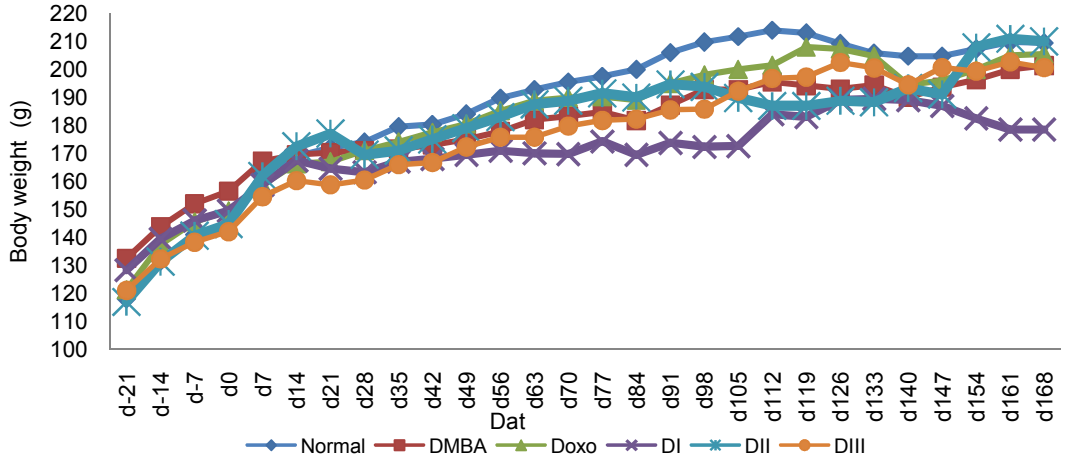

Fig. 1. The development of experimental animal body weight in each group before and after treatment

Table 1. Body weight of rat during treatment (post-carcinogen induction and medication)

Group	Weight (Median±STDEV)			
	Post carcinogenic induction		Medication with extract	
	21 dat	28 dat	105 dat	140 dat
Normal	116.00±10.80[a]	174.50±14.40[a]	213.50±17,30[a]	202.00± 12.60[a]
DMBA	127.50±17.30[a]	170.5±14.60[a]	194.00±11.10[a]	204.00±9.50[a]
Doxo	114.50±10.50[a]	165.50±12.60[a]	202.50±14.50[a]	194.50±9.40[a]
DI	130.50±14.30[a]	156.50±8.8[a]	175.00±4.70[a]	189.00±7.0[a]
DII	118.00±9.80[a]	174.00±14.40[a]	189.00 ±10.8[a]	195.50±10.3[a]
DIII	119.50±12.50[a]	164.00±10.3[a]	200,50±11.9[a]	193.00±9.10[a]

[a] *The same letter shows no significant difference at the 95% level (p> 0.05)*

Fig. 2. (a) Size of tumor present in DMBA group; (b) the smallest tumor size in the DI group

The volume of tumor on the day of necropsy (Fig. 3) shown that the DI group had the smallest average of tumor volume compared to the DMBA group. Based on the statistical analysis of the tumor volume indicates that there is a significant difference in the treatment group ($P < 0.05$), the results of a further test of Mann-Whitney'test showed that there is a significant difference between normal and DI groups against DMBA group ($P < 0.05$). Administration of endophytic fungi extract at a dose of 20 mg/kg (DI) is more effective compared to two other doses of 40 mg/kg (DII) and 120 mg/kg (DIII) (Fig. 3). This is presumably due to the bioactive compounds in the Sir G5 endophytic fungi which functions as an anticancer belongs to alkaloid derivative such as piperidone, piperidine and hexadecanenitrile [30]. The active compounds in extracts of endophytic are able to suppress the oncogenesis development, then alkaloids is also able to modulate the signaling pathway key which involves in cell proliferation, stimulating

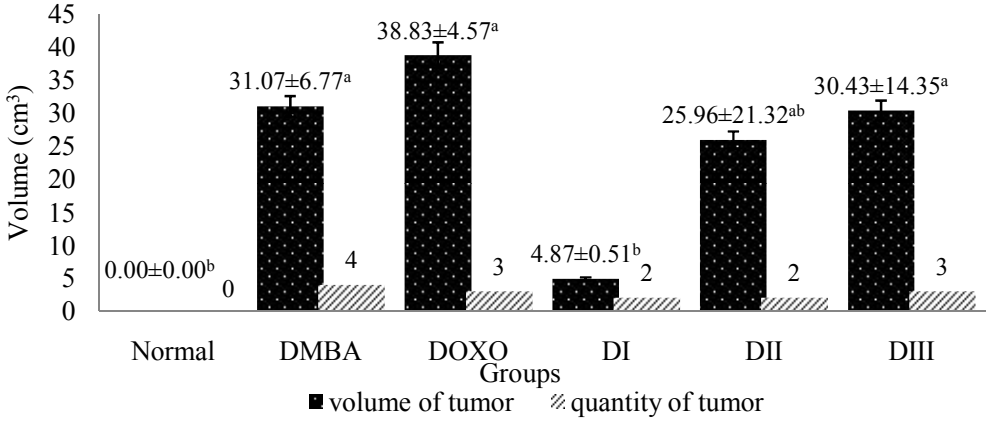

Fig. 3. Number and volume of tumor in each treatment group on day of necropsy; [a]The samesuperscript letter showed no significant difference at the 5% test level; n = 3

oncogenes DNA damage, and induces apoptosis. Therefore, these active compounds may act as an anticancer agent [31]. Furthermore, piperidine which is one of the active compounds in extracts of endophytic can induce apoptosis and increase the percentage of cells in G2/M phase of the 4TI cell and induces K562 cell to differentiate into macrophages [32,33,34].

It is known that the alkaloids, also have a function as anticancer and antioxidant. However an excessive antioxidants in the body causes toxic effect. This is consistent with the results of Chen et al, study of p53 inactivation in rat (tumor suppressor genes) revealed that the group of rats which were given low-dose antioxidant (vitamin E) treatment may decrease carcinogenesis, while reversing result when the rat given high doses [35,36]. Furthermore, the results study of Marban (2010) found that the basic mechanisms of cancer cell development in the body caused by high antioxidant doses resulted in excessive inhibition of oxidative signals (both in cell cultures and in vivo) [37]. Therefore, groups of rats treated with doses of 40 mg/Kg bw and 120 mg/Kg bw produced larger tumor volume each week. Based on the results above, it can be concluded that the administration of soursop leaves endophytic extract in the DI group with a dose of 20 mg/Kg bw more effective than DII and DIII. This is proven by the small volume of tumors.

4. CONCLUSION

Administration of ethyl acetate extract of endophytic fungi in Sir G_5 isolate soursop leaves can inhibit the growth of breast tumor of Sprague Dawley female white rat that is induced with 7.12-DMBA in effective dose of DI (20 mg/Kg bw) indicated by a lower number and volume of breast tumor than the group DMBA (negative control) and doxorubicin (positive control).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

The authors are grateful to the Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture of the Republic of Indonesia, to supporting financial research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. [WHO] World Health Organization. Cancer, Key facts; 2015.
Available:http://www.who.int/mediacentre/factsheets/fs297/en/
(Accessed 7 June 2016)
2. [IARC] International Agency for Research on Cancer. GLOBOCAN 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012. Diakses melalui; 2012.
Available:http://globocan.iacr.fr/Pages/fact sheets_population.aspx
(pada tanggal 20 Oktober 2016)

3. Arroyo-Acevedo J, Chavez-Asmat RJ, Anampa-Guzman A, Donaires R, Raez-Gonzales J. Protective effect of *Piper aduncum* capsule on DMBA-induced breast cancer in rats. Breast Cancer: Basic Clinical Res. 2015;9:41-48.
4. Kirubha ASP, Anburjan M, Venkataraman B, Akila R, Sharath D, Raj Baldev. Evaluation of mammary cancer in 7,12-dimethylbenz(a)antracene- induced wistar rats by asymmetrical temperature distribution analysis using thermography: A comparison with serum CEA levels and histopathology. J Biomedic and Biotech. 2012;2012:1-11.
5. Satuma, Fatmawati H. Sel punca kanker payudara dan upaya pengendaliannya. Malang: Fakultas Kedokteran Universitas Brawijaya; 2009.
6. Kemenkes. Panduan Pelakasanaan Kanker Payudara. Jakarta, Indonesian; 2016.
7. [TCH] Tim Cancer Helps. Stop Kanker (Kanker Bukan Lagi Vonis Mati) Panduan Deteksi Dini dan Pengobatan Menyeluruh Berbagai Jenis Kanker. Jakarta: Agro Media Pustaka; 2010.
8. Baba AL, Câtoi C. Comparative oncology. The Publishing House of the Romanian Academy; 2007.
9. Strobel GA, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbial Mol Biol Rev. 2003; 67:491-502.
10. Strobel GA. Endophytes as source of bioactive products. Microb Infect. 2003; 5:535-544.
11. Tan RX, Zou WX. Endophyte: A rich source of fungtional metabolite. Nat Prod. 2001;18:448-459.
12. Prasetyoputri dan Atmosukarto. Mikroba endofit: Sumber molekul acuan baru yang berpotensi. Bio Trends. 2006;1(2):13-15.
13. Deacon JW. Fungal biologi 4th edition. Australia: Blackwell Publishing; 2006.
14. Strobel GA, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbial Mol Biol Rev. 2003; 67:491-502.
15. Gavamukulya Y, Abou-Elella F, Wamunyokoli F, AE-Shemy H. Phytochemical screening, anti-oxidantactivity and in vitro anticancer potential of ethanolic and water leaves extracts of *Annona muricata* (Graviola). Asian Pac J Trop Med. 7. 2014;(Suppl 1):S355-S363.
16. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. *Annona muricata* (Annonaceae): A review of its traditional uses, isolated acetogenins and biological activities. Int J Mol Sci. 2015;16:15625-15658.
17. Coria-Tellez AV, Montalvo-Gonzalez E, Yahia EM, Obledo-Vazquez EV. *Annona muricata:* A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab J Chem. 2016; 01(004):1-32.
18. Retnani V. Pengaruh suplementasi ekstrak daun *Annona muricata* terhadap kejadian displasia epitel kelenjar payudara tikus sprague dawley yang diinduksi 7,12-dimetilbenz(a)antrasena (DMBA) [skripsi]. Semarang: Fakultas Kedokteran Universitas Diponegoro. Indonesian; 2011.
19. Adewole S, Ojewole J. Protective effects of *Annona muricata* linn (annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med. 2009; 6:30–41.
20. Coria-Tellez AV, Montalvo-Gonzalez E, Yahia EM, Obledo-Vazquez EV. *Annona muricata:* A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arab J Chem. 2016; 01(004):1-32.
21. Erlinger TP, Muntner P, Helzlsouer KJ. WBC count and the risk of cancer mortality in a National sample of U.S. adults: Results from the second National health and nutrition examination survey mortaly study. Canc Epidemiol Biomark Prev. 2004;13(6):1052-1056.
22. Minarni. Aktivitas antikanker ekstrak etil asetat kapang endofit daun sirsak (*Annona mucirata* L) [tesis]. Bogor: Institut Pertanian Bogor. Indonesian; 2016.
23. Barroso AC, Muranaka ENK, Mori LJ, Pelizin CHT, Iriya K, Giocondo G, et al. Induction of experimental mammary carcinogenesis in rats with 7,12-dimethylbenz(α)anthracene. Rev Hospital Clinicas. 2004;59(5):257-261.
24. Arroyo-Acevedo J, Chavez-Asmat RJ, Anampa-Guzman A, Donaires R, Raez-Gonzales J. Protective effect of *Piper aduncum* capsule on DMBA-induced breast cancer in rats. Breast Cancer: Basic Clinical Res. 2015;9:41-48.

25. Wu W, Bi C, Credille KM, Manro JR, Peek VL, Donoho GP, Yan L,Wijsman JA,Yan SB, Walgren RA. Inhibition of tumor growth and metastasis in non-small cell lung cancer by LY2801653, an inhibitor of several oncokinases, including MET. Clin Cancer Res. 2013;19(20):5699-5710.
26. Mello MLS, Benedicto CV, Jose R, Wolgang P, Ulrich S. Image analysis of The AgNOR response in ras-transformed human breast epithelial cells. Acta Histochem. 2008;110:210-216.
27. Malita S. Aktivitas kemopreventif ekstrak temu ireng (*Curcuma aeruginosa* Roxb.) terhadap histopatologi tumor payudara tikus putih yang diinduksi 7,12-dimetilbenz(α)antrasena [tesis]. Bogor: Institut Pertanian Bogor, Indonesian; 2016.
28. Carlson RW. Continius intravenous infusion chemotherapy. Di dalam: Perry MC editor. The Chemotherapy Source Book Fourt Edition. Philadelphia (USA): Lippincott Williams & Wilkins; 2008.
29. Doroshow JH. Topoisomerase II inhibitors: Anthracylines. In: Longo DL, Chabner BA, editors.Cancer chemotherapy and biotherapy: principles and practice. Philadelphia: Lippincott Wiliams & Wilkins; 2010.
30. Hasan AEZ, Mangunwidjaja D, Sunarti TC, Suparno O, Setiyono A. Investigating the antioxidant and anticytotoxic activities of propolis collected from five regions of Indonesia and their abilities to induce apoptosis. Emir. J. Food Agric. 2014; 26(5):390-398.
31. Habli Z, Toumieh G, Fatfat M, Rahal ON, Gali-Mihtasib H. Emerging cytotoxic alkaloid in the battle against cancer: overview of molecular mechanisms. Molecules. 2017;22(250):1-22.
32. Lai LH, Fu QH, Liu Y. Piperine suppresses tumor growth and metastatis *in vitro* and *in vivo* in a 4T1 murine breast cancer model. Acta Pharmacologica Sinica. 2012;33(4): 523-530.
33. Song QF, Qu YC, Zheng HB, Zhang GH, Lin HG, Yang JL. Differentiation of erythroleukimia K562 cells induced by piperine. Ai Zheng. 2008;27(6):571-574.
34. Hasan AEZ, Mangunwidjaja D, Sunarti TC, Suparno O, Setiyono A. Antibreastcancer activity of nanopropolis Indonesia on induced mammary gland tumor by DMBA in virgin sprague-dawley rats. Biotropia. 2016;23(1):35-41.
35. Chen CS, Squire JA, Well PG. Reduced tumorigenesis in p53 knockout mice exposed in utero to low-dose vitamin E. Cancer. 2009;115:1563-1575.
36. Chen CS, Wells PG. Enhanced tumorigenesis in p53 knockout mice exposed in utero to high-dose vitamin E. Carcinogenesis. 2006;27:1358-1368.
37. Marban E, Li TS. Physiological level of reactive oxygen species are required to maintain genomic stability in stem cells. Stem Cells. 2010;28(7):1178-1185.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21555