RJC

RASĀYAN *J. Chem.*
Vol. 13 | No. 3 |1992-1998| July - September | 2020
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

A STUDY OF ANTICANCER ACTIVITY FROM THE FRACTIONS OF RODENT TUBER SUPERIOR MUTANT EXTRACT (*Typhonium flagelliforme)* BY PRESTOBLUE ASSAY METHOD

N.F. Sianipar[1, 2,*], Y. E. Hadisaputri[3], K. Assidqi[1, 2], P. Simanjuntak[4] and R. Purnamaningsih[5]

[1]Food Technology Department, Faculty of Engineering, Bina Nusantara University,11480 Jakarta, Indonesia
[2]Research Interest Group Food Biotechnology, Bina Nusantara University, 11480 Jakarta, Indonesia
[3]Pharmacy Faculty, Padjajaran University, 45363 Sumedang, Indonesia
[4]Biotechnology Research Center - Indonesian Institute of Sciences, 16911 Cibinong, Indonesia
[5]Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (BB-Biogen), 16111 Bogor, Indonesia
*E-mail: nsianipar@binus.edu

ABSTRACT

Typhonium flagelliforme plant is an Indonesian herbal plant known as rodent tuber. Rodent tuber superior mutants have produced to increase the bioactive compound and more potential as anticancer than the mother plant. The purpose of this study was to determine the anticancer activity of ethanol extract, ethyl acetate fraction, and water fraction from the ethanol extract of rodent tuber superior mutant against MCF-7 breast cancer cells. Anticancer activity of ethanol extract, ethyl acetate fraction, and water fraction was carried out using the PrestoBlue assay method with multilevel concentration. The inhibitory concentration (IC_{50}) values of ethanol extract, ethyl acetate fraction, and water fraction were respectively obtained for 1.60, 1.09, and -572.14 µg mL^{-1}. Based on the results of the IC_{50} value, the water fraction does not have an anticancer activity or inhibits cancer cell proliferation of MCF-7. Rodent tuber superior mutant extract was tested to normal cells CV1 has an IC_{50} value of -81.72 µg mL^{-1} from these results there was no inhibition of growth of normal cells CV1. The cytotoxicity in ethyl acetate fraction is stronger than ethanol extract. Thus, the ethyl acetate fraction can be developed as promising agents as an anticancer treatment.

Keywords: *Typhonium flagelliforme*, Superior mutant, Cytotoxicity, MCF-7 Cell Line

INTRODUCTION

Rodent tuber (*Typhonium flagelliforme)* is an Indonesian herbal plant belongs to Araceae family. This plant has potential as an anticancer activity in which the raw material can be developed as anticancer drugs. According to the previous studies, rodent tuber plants have been carried out through *in vitro* biotechnology (somaclonal variation) in combination with gamma-ray irradiation to produce mutant plants. These mutant plants have been detected to have genetic changes in the first generation (MV1).[1] The second-generation (MV2) and third-generation (MV3) mutants have genetic changes through RAPD molecular markers compared to their mother plant[2] and the fourth generation mutant clones (MV4) are selected to produce stable mutants, namely, superior rodent tuber mutants.[2,3]

The mutation results obtained by new superior mutant plants that have high anticancer active ingredients compared to the mother plant.[3] The superior mutant clone, which has high anticancer content, is superior rodent tuber mutant.[4] GC-MS analysis showed that the leaf of MV4 mutant clones contained five types of anticancer compounds whose quantity was higher than the mother plant.[5] Anticancer bioactive compounds in tubers are hexadecanoic acid ethyl ester, octadecanoic acid, squalene, eicosane, octacosane.[4] The five

CrossMark

types of anticancer compounds on leaves such as hexadecanoic acid ethyl ester, hexadecanoic acid methyl ester, squalene, eicosane and octacosane.[5]

Rodent tuber is useful in treating lung and breast cancer[6], liver[7], leukemia[8], intestine, prostate gland, and cervix[9]. The mutant plant of rodent tuber KB 6-1-3-4 and KB 6-9-5 were extracted with ethanol have high activity to kill breast cancer cells.[10] The rodent tuber mutant plant extract has been known to encourage apoptosis in some cancer cells *in vitro*. Rodent tuber with ethanol fraction has been proven to be effective in inhibiting the growth of T47D breast cancer cells[11,] and rodent tuber with DCM fraction can inhibit MCF-7 breast cancer cells[12]. Several studies of rodent tuber plants have been proved can inhibit cell proliferation human T4-lymphoblastoid cancer[8,13,] and NCI-H23 cell culture of non-small cell lung carcinoma.[14]

Cancer cell death (cell apoptosis) can be induced with bioactive compounds produced by plants. According to Sdiri et al.[15] stated that *Cynomorium coccineum* plant extract with ethanol fraction could inhibit proliferation, invasion, migration, and colony formation in cancer cells. Curcuminoids compounds from plants play an active role in various chronic diseases such as colon cancer, lung cancer, breast cancer.[16] Bioactive compounds produced by *Euphorbia hirta* can induce cell apoptosis in MCF-7 breast cancer cells after 24 h.[17] Walnut seed extract shows a strong antiproliferative effect on human liver HepG2 and Caco-2 colon cancer cells.[18] Bioactive compounds resulting from ethyl acetate fraction from seaweed have high anticancer activity against HCT-116 cells.[19]

The methodology of anticancer activity can be carried out by the antiproliferative test of extracts against the growth of cancer cells using the clonogenic method.[20] Antiproliferation test extract can also be performed using the PrestoBlue assay method with stratified concentrations in *Pipturus arborescens*.[21] The potential of rodent tuber mutant plants to be used by cancer sufferers, it is necessary to prepare medicinal raw materials from a superior mutant that are clinically tested. Therefore, it must be known that active fraction acts as an anticancer and an optimal dose (effectively killing cancer cells but does not negatively impact normal cells). The active fraction to identify compounds that act as anticancer. The initial step of the research was carried out through the anticancer activity test method in a biological test contained in the extract and the results of the partitioning of the superior mutant extract against breast cancer cells. This study aims to determine the antiproliferation ability of ethanol extract, ethyl acetate, and water fractions from the ethanol extract of rodent tuber superior mutant plants against MCF-7 breast cancer cells.

EXPERIMENITAL

Plant Material

Rodent tuber superior mutant has been irradiated by gamma-ray to produce *in vitro* mutagenesis and obtained rodent tuber superior mutant plants. The rodent tuber superior mutants were collected from Sianipar & Purnamaningsih's collection. The superior mutant was acclimatized and maintained at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Ministry of Agriculture, Bogor, Indonesia. The rodent tuber superior mutant was harvested and dried to extract all substances.

Preparation of Ethanolic Extract and the Fractions of Rodent Tuber Superior Mutant

The tubers were washed and dried at a constant temperature of 50ºC. The tuber powder was macerated in ethanol for two days at room temperature. The process was repeated three times. The extract was filtered from the residue by filtration through Whatman No. 1 filter paper. The solvent was removed under reduced pressure at 40ºC using a rotary evaporator (Rotavapor® R-300, Buchi) until a thick paste extract is obtained. The ethyl acetate and water-soluble fraction were yielded from the ethanol extract. The ethyl acetate soluble fraction was filtered over Whatman No.1 paper as filtrate. The ethyl acetate soluble fraction was collected then evaporated by a rotary evaporator at 60°C. Afterward, it was evaporated on the water bath at 60°C until it became viscous. The water layer was freeze-dried to give a water fraction.

In vitro Anticancer Activity and Data Analysis

Anticancer activity against breast cancer cells (MCF-7) was performed by the PrestoBlue assay method. MCF-7 cells (breast cancer cells in humans) were obtained from ATCC (American Type Cell Cancer), a collection from the Division of Biology Activities at the Central Laboratory of Padjajaran University,

Indonesia. The media was used at Roswell Park Memorial Institute Medium (RPMI) liquid culture media. Cisplatin was used as a positive control. Cell culture using 96 well plates. The cell used must reach a confluent of at least 70%. The cell should be inserted into 96 well plates must have a final cell density of 17,000 cells / well. Cell culture that has entered into 96 well plates, then incubated for 24 hr at 37°C and 5% CO_2 gas. The cell cultures that had been incubated on 96 well plates, using micropipettes were transferred 100 µL each of the treatment samples, ethanol extract, ethyl acetate, and water fractions and cisplatin from the microtube into each well that contained cells. After all treatment samples were entered into 96 well plates, then re-incubated for 24 hr. The cell viability measurement in each treatment sample, it is necessary added "PrestoBlue ™ Cell Viability Reagent" (10 µL reagent for 90 µL media), then was added 100 µL of the mixture of the solution into each good microplate and then incubated for 1-2 hr until noticeable changes in color (when entering live cells, PrestoBlue® reagents will be reduced from blue resazurin compounds without intrinsic fluorescent values, to red and highly fluorescent resorufin compounds. Conversion values are proportional to the number of cells that are metabolically active and can, therefore, be measured quantitative, to measure absorbance, absorbance spectrum used for resazurin and resorufin). Furthermore, absorbance was measured at a wavelength of 570/600 nm using a multimode reader. The percentage of cell viability equals (absorbance of treated cells/absorbance of untreated cells) × 100%. The morphological features were captured using by microscope Invitrogen the EVOS XL Core Imaging System.

RESULTS AND DISCUSSION

The ethanol extract, ethyl acetate, and water fraction in rodent tuber superior mutants were tested for anticancer activity against MCF-7 breast cancer cells. IC_{50} values of the sample are calculated based on the regression equation of the percentage of cell viability versus the extract and fraction concentration. IC_{50} values of samples in ethanol extract and ethyl acetate fraction showed strong cytotoxic activity, as shown in Fig.-1 and 2.

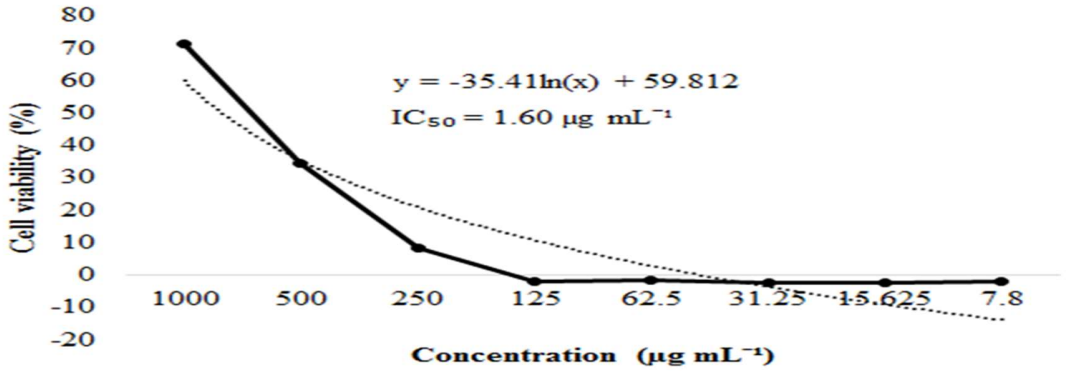

Fig.-1: The Inhibition of Cell Viability of Rodent Tuber Mutant Plant in Ethanolic Extract on MCF-7 Cell Line

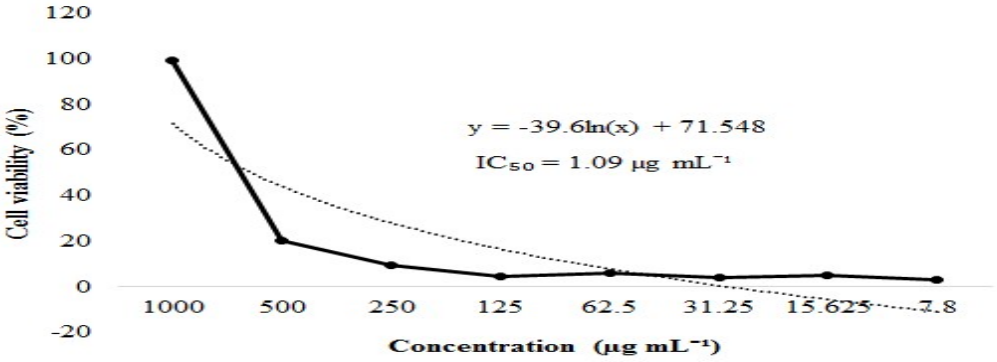

Fig.-2: The Inhibition of Cell Viability of Rodent Tuber Mutant Plant in Ethyl Acetate Fraction on MCF-7 Cell Line

IC_{50} values in ethanol extracts of rodent tuber superior mutant were 1.60 µg mL^{-1}, whereas for the IC_{50} value in the ethyl acetate fraction was 1.09 µg mL^{-1}. In this study, the water fraction did not show any anticancer activity against MCF-7 breast cancer cells, where IC_{50} value was obtained for -572.14 µg mL^{-1} (Fig.-3). Ethyl acetate fraction has better cytotoxic activity compared to ethanol extract and water fraction. This is due to the possibility of compounds that are semi-polar, can dissolve more in ethyl acetate.[22]

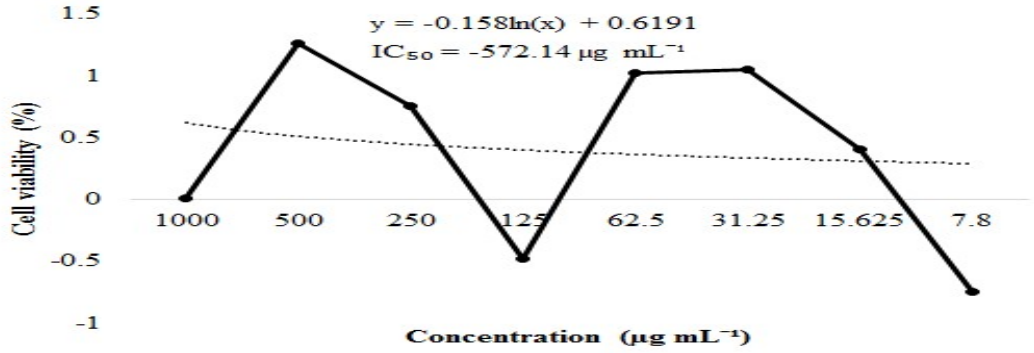

Fig.-3: The Inhibition of Cell Viability of Rodent Tuber Mutant Plant in Water Fraction on MCF-7 Cell Line

The cytotoxic activity of ethanolic extracts of rodent tuber superior mutant against normal cells or healthy cells had an IC_{50} value of -81.72 µg mL^{-1} (Fig.-4). This indicates that the ethanolic extract of rodent tuber superior mutant does not inhibit cell proliferation and does not have toxicity to normal cells. According to the American National Cancer Institute, IC_{50} values are said to be very cytotoxic if <20 µg mL^{-1}, cytotoxic is strong if IC_{50} is between 100-500 µg mL^{-1}.[23,24] The effect of death that occurs on MCF-7 cells is probably caused by the content of secondary metabolites, the major compounds found in ethanol extracts of the rodent tuber superior mutant through GC-MS analysis such as hexadecanoic acid and squalene.[4,25] Hexadaconoic acid is a palmitic acid that has a cytotoxic effect on MOLT-4 leukemia cancer cells, where the compound interacts with DNA topoisomerase I to induce apoptosis *in vitro* and shows antitumor activity *in vivo*.[17,26]

The difference in the cytotoxic level of ethanol extract, ethyl acetate, and water fraction can be caused by differences in the content and concentration of active compounds contained in the extract or fraction.[17,27,28] Bioactive compounds from various natural products can cause MCF-7 cell apotosis.[29,30] Amurensin and cosmosiin flavonoid bioactive compounds from *Trigonella foenum graecum* extract function to inhibit MCF-7 cell proliferation[31], ethyl acetate fraction *Sargussum siliquosum* has a high activity against inhibition of MCF-7 cell growth.[19] According to Ishaqat et al.[32] revealed that *Elaeagus angustifolia* extract has antiproliferation activity against MCF-7 cells. The ethyl acetate fraction of *Centaurea fenzlii* inhibits the growth of MCF-7 cancer cells.[33]

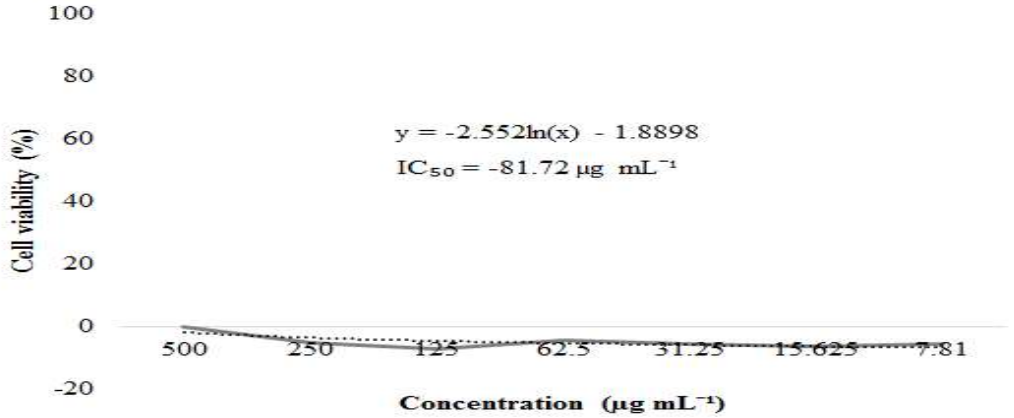

Fig.-4: The Inhibition of Cell Viability of Rodent Tuber Superior Mutant Plant in Ethanolic Extract on CV1 Cell Line (Normal Cell)

Several studies *in vitro* and *in vivo* showed the role of tumor inhibitors for squalene. A mechanism is proposed for squalene tumor inhibitory activity based on the strong inhibitory activity known from the catalytic activity of β-hydroxy-β-methylgutaryl CoA reductase *in vivo*, thereby reducing the availability of farnesyl pyrophosphate for prenylation of oncogenes, which relocates oncogenes to cell membranes and is necessary for transduction function cell signaling.[34] According to Warleta et al.[35] it has been reported that squalene can protect the oxidative DNA damage activity in normal breast cells, in this case, the possibility of squalene has potential as a compound for the prevention of human breast cancer. Squalene has been shown to inhibit the carcinogenesis of various cancer cell lines, such as colon cancer, breast cancer.[36,37,38]

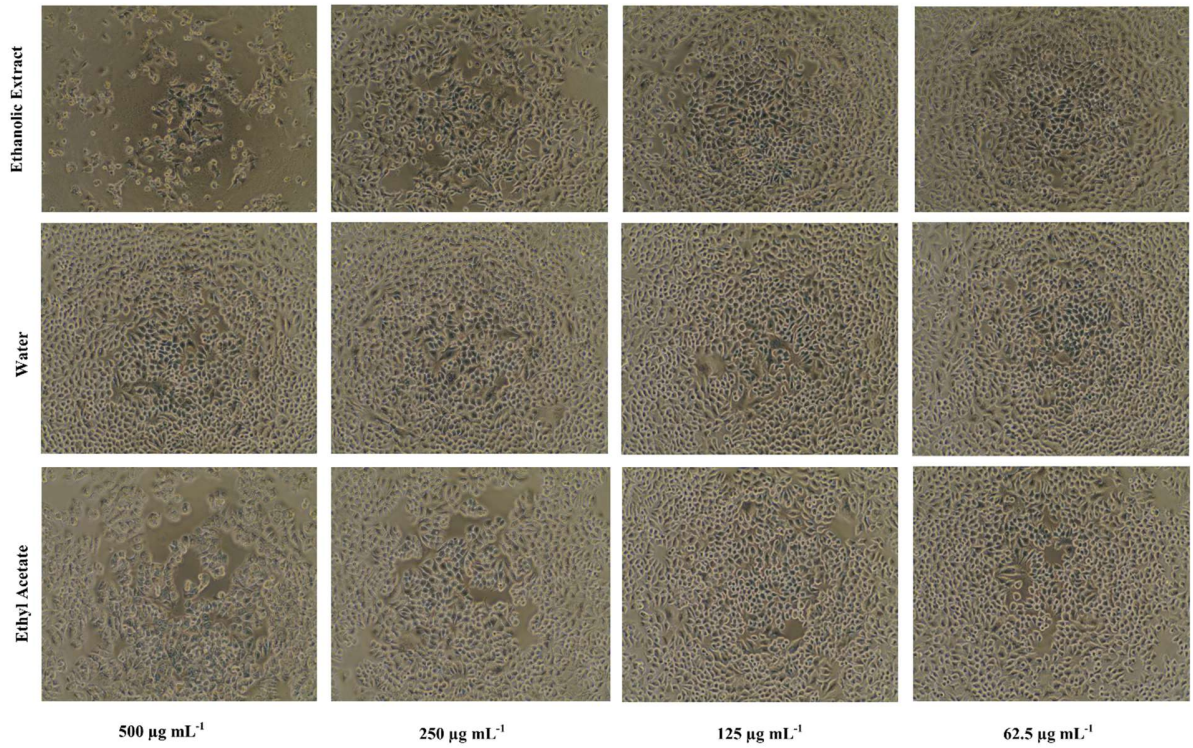

Fig.-5: The Effect of Ethyl Acetate and Water Fraction of Rodent Tuber Mutant Plant on the Morphology of MCF-7 Cell. The Cells were incubated for 24 h in the different of concentrations 500, 250, 125, 62.5 µg mL^{-1}. The magnification is 200x.

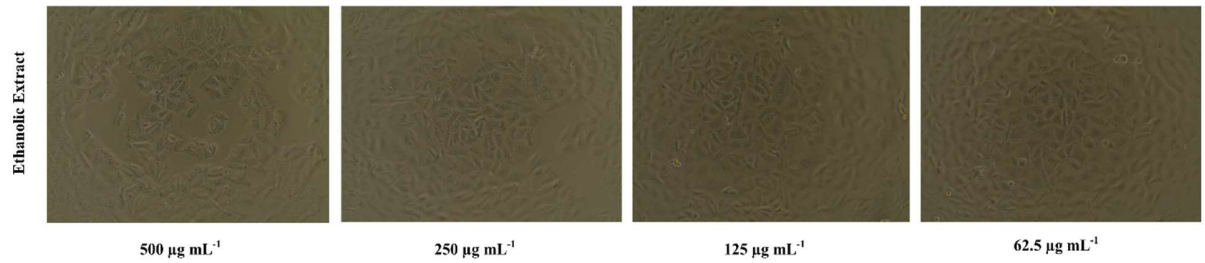

Fig.-6: The effect of ethanolic extract of rodent tuber mutant plant on the morphology of CV1 cell (normal cell). The cells were incubated for 24 h in the different of concentrations 500, 250, 125, 62.5 µg mL^{-1}. The magnification is 200x.

The morphology of MCF-7 cells in the ethyl acetate fraction shows anticancer activity against breast cancer cell lines by inducing apoptosis, cells shrinking (Fig.-5). As seen in Fig.-6, the results showed that ethanol extract in superior mutant taro mice against CV1 normal cell proliferation growth did not inhibit. Thus, the ethanol extract of rodent tuber superior mutant not toxic to healthy cells. This proves that the ethyl acetate

fraction of a superior mutant can be a potential anticancer agent by affecting cancer chemotherapy, which can be very important for human health. This is a great opportunity for the pharmaceutical research area in the development of effective drugs to overcome cancer problems.

CONCLUSION

The results of this study indicate that superior mutant extracts had IC_{50} 1.60 µg mL^{-1}. IC_{50} values the ethyl acetate, and water fractions have 1.09 and -572.14 µg mL^{-1}. Based on the results of IC_{50} values, the water fraction does not inhibit cancer cell proliferation and does not cause the death of MCF-7 cancer cells. The antiproliferation activity of cells in the ethyl acetate fraction was higher than the ethanol extract. The rodent tuber superior mutant extract was tested to normal cells CV1 had an IC_{50} value of -81.72 µg mL^{-1}. Rodent tuber superior mutant did not cause apoptosis in normal cell CV1 *in vitro*.

ACKNOWLEDGEMENT

This work was fully funded by a grant from the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia.

REFERENCES

1. N. F. Sianipar, Ariandana, W. Maarisit, *Procedia Chemistry*, **14**, 285(2015), **DOI:** 10.1016/j.proche.2015.03.040
2. N. F. Sianipar, R. Purnamaningsih, D. L. Gumanti, V. M. Rosaria, *Pertanika Journal of Tropical Agricultural Science,* **40**, 185(2017).
3. N. F. Sianipar, R. Purnamaningsih, Chelen, *Jurnal Teknologi*, **78**, 35(2016), **DOI:**10.11113/jt.v78.8635
4. N. F. Sianipar, R. Purnamaningsih, *Pertanika Journal of Tropical Agricultural Science*, **41**, 305(2018).
5. N. F. Sianipar, R. Purnamaningsih, I. Darwati, D. Laurent, *Jurnal Teknologi,* **78**, 1(2016), **DOI:** 10.11113/jt.v78.9883
6. C. S. Lai, R. H. Mas, N. K. Nair, S. M. Mansor, V. Navaratnam, *Journal of Ethnopharmacology,* **127**, 486(2010), **DOI:**10.1016/j.jep.2009.10.009
7. C. S. Lai, R. H. Mas, N. K. Nair, M. I. Majid, S. M. Mansor, V. Navaratnam, *Journal of Ethnopharmacology*, **118**, 14(2008), **DOI:**10.1016/j.jep.2008.02.034
8. S. Mohan, A. Abdul, S. Abdelwahab, A. Al-Zubairi, M. Sukari, R. Abdullah, …& S. Syam, *Journal of Ethnopharmacology,* **131**, 592(2010), **DOI:**10.1016/j.jep.2010.07.043
9. D. S. H. Hoesen, *Berita Biologi*, **8**, 413(2007), **DOI:**10.14203/beritabiologi.v8i5.1905
10. N. F. Sianipar, K. Assidqi, R. Purnamaningsih, T. Herlina, *Asian Journal of Pharmaceutical and Clinical Research*, **12**, 185(2019).
11. A. Nurrochmad, E. Lukitaningsih, E. Meiyanto, *International Journal of Phytomedicine*, **2**, 138(2011).
12. Putra, Tjahjono, Winarto, *Journal of the Indonesian Medical Association*, **62**, 10(2012).
13. S. Mohan, A. Bustamam, S. Ibrahim, A. S. Al-Zubairi, M. Aspollah, *Journal of Pharmacology and Toxicology,* **3**, 449(2008), **DOI:**10.3923/jpt.2008.449.456
14. C. S. Lai, R. H. Mas, N. K. Nair, S. M. Mansor, V. Navaratnam, *Journal of Ethnopharmacology,* **127**, 486(2010), **DOI:**10.1016/j.jep.2009.10.009
15. M. Sdiri, X. Li, W. W. Du, S. El-Bok, Y. Xie, M. Ben-Atia, B. B. Yang, *Cancers*, **10**, 2(2018), **DOI:** 10.3390/cancers10100354
16. A. Amalraj, A. Pius, S. Gopi, S. Gopi, *Journal of Traditional and Complementary Medicine*, **7**, 205 (2017), **DOI:**10.1016/j.jtcme.2016.05.005
17. Y. P. Kwan, T. Saito, D. Ibrahim, F. M. S. Al-Hassan, C. E. Oon, Y. Chen, S. L. Jothy, J. R. Kanwar and S. Sasidharan, *Pharmaceutical Biology*, **54**, 1223(2016), **DOI:**10.3109/13880209.2015.1064451
18. J. Yang, R. H. Liu, L. Halim, *LWT-Food Science and Technology*, **42**, 1(2009), **DOI:** 10.1016/j.lwt.2008.07.007
19. G. O. A. Tantengco, A. C. Limbo, E. M. N. Montaño, D. S. Jacinto, *International Journal of Biosciences*, **7**, 207(2015).
20. H. Masuda, C. Alev, H. Akimaru, R. Ito, T. Shizuno, M. Kobori, M. Horii, T. Ishihara, K. Isobe, M. Isozaki, J. Itoh, Y. Itoh, Y. Okada, B. A. S. McIntyre, S. Kato, T. Asahara, *Circulation Research*, **109**, 20(2011), **DOI:**10.1161/CIRCRESAHA.110.231837
21. M. M. De Los Reyes, G. G. Oyong, Jr. V. D. Ebajo, S. Ng. V. Antonio, C. Shen, C. Y. Ragasa, *Journal of Applied Pharmaceutical Science*, **5**, 023(2015), **DOI:**10.7324/JAPS.2015.501104
22. M. Da'i, K. A. Meilinasary, A. Suhendi, S. Haryanti, *Indonesian Journal of Cancer Chemoprevention*, **10**, 95(2019), **DOI:**10.14499/indonesianjcanchemoprev10iss2pp95-100
23. E.S. Abdel-Hameed, A. Salih, S. A. Bazaid, M. M. Shohayeb, M. M. El-Sayed, E. A. El-Wakil, *European Journal of Medicinal Plants*, **2**, 93(2012), **DOI:**10.9734/EJMP/2012/1040
24. J. Boik, Natural Compounds in Cancer Therapy, Oregon Medical Press, Minnesota, USA, p. 31,97 (2001).
25. O. Atolani, C. B., Adeosun, A.P. Oluyori, J. Olota. *Rasayan Journal Chemistry,* **12,** 1052(2019), **DOI:**10.31788/RJC.2019.1235122

26. H. Harada, U. Yamashita, H. Kurihara, E. Fukushi, J. Kawabata, & Y. Kamei, *Anticancer Research,* **22**, 2587(2002).
27. M. Liu, Y. Lin, X. Chena, C. Liaoa, W. Poo, *Experimental and Toxicologic Pathology*, **65**, 77(2013), **DOI:**10.1016/j.etp.2012.11.004
28. G. M. Saibu, D. R. Katerere, D. J. G. Rees, M. Meyer, *Journal of Ethnopharmacology*, **164**, 203(2015), **DOI:**10.1016/j.jep.2015.01.040
29. A. S. Thangiah. *Rasayan Journal Chemistry*, **12**, 300(2019), **DOI:**10.31788/RJC.2019.1215042
30. P. C. Situmorang, S. Ilyas, S. Hutahean, Rosidah, R. D. Manurung. *Rasayan Journal Chemistry,* **13**, 780(2020), **DOI:**10.31788/RJC.2020.1325621
31. S. I. Ahmed, M. Q. Hayat, S. Zahid, M. Tahir, Q. Mansoor, M. Ismail, K. Keck, R. Bates, *Tropical Journal of Pharmaceutical Research*, **16**, 1391(2017), **DOI:**10.4314/tjpr.v16i6.25
32. A. A. Ishaqat, R. Abu-Dahab, H. M. Hammad, M. Al-Zihlif, I. F. Abaza1, Z. D. Nassar, F. U. Afifi, *Natural Products Chemistry & Research*, **6**, 3(2018), **DOI:**10.4172/2329-6836.1000319
33. U. Yirtici, F. Göger, M. Sarimahmut, A. Ergene, *Turkish Journal of Biology*, **41**, 370(2017), **DOI:**10.3906/biy-1609-74
34. L. Newmark, K. Yang, N. Kurihara, K. Fan, L. H. Augenlicht, M. Lipkin. *Carcinogenesis*, **30**, 88(2009), **DOI:**10.1093/carcin/bgn229
35. F. Warleta, M. Campos, Y. Allouche, C. Sánchez-Quesada, J. Ruiz-Mora, G. Beltrán, J. J. Gaforio, *Food and Chemical Toxicology*, **48**, 1092(2010), **DOI:**10.1016/j.fct.2010.01.031
36. D. N. Brown, I. Caffa, G. Cirmena, D. Piras, A. Garuti, M. Gallo, S. Alberti, A. Nencioni, A. Ballestrero, G. Zoppoli, *Scientific Reports*, **6**, 1(2016), **DOI:**10.1038/srep19435
37. V. Rao, H. L. Newmark, & B. S. Reddy, *Carcinogenesis,* **19**, 287(1998), **DOI:**10.1093/carcin/19.2.287
38. M. M. De Los Reyes, G. G. Oyong, C. Antonio Shen, C. Y. Ragasa, *International Journal of Pharmacognosy and Phytochemical Research*, **8**, 668(2016).

[RJC-5703/2020]