DOI Number: 10.5958/0976-5506.2018.00641.1

Parasitic Tea *Scurrula atropurpurea* (Blume) Danser Active Compound Potencies Towards Inhibition of DNA Methylation in Cancer: An *In Silico* Study

Ni Luh Putu Eka Sudiwati[1], Tatit Nurseta[2], Mulyohadi Ali[3], Aulani'am Aulani'am[4], Heru Santoso Wahito Nugroho[5]

[1] Department of Nursing, State Health Polytechnic of Malang; Indonesia, [2] Department of Obstetri and Gynecology, Saiful Anwar Hospital, Malang, Indonesia / Faculty of Medicine, Brawijaya University, Malang, Indonesia, [3]Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, Indonesia, [4] Laboratory of Organic Chemistry, Mathematic and Natural Sciences, Brawijaya University, Malang, Indonesia, [5] Health Polytechnic of Ministry of Health at Surabaya, Indonesia

ABSTRACT

DNA methylation is metil group (CH_3) adding towards DNA. This mechanism will affected cells function and arise difference gene expression. Tumor suppresor gene hipermethylation, was silencing gene expression, induced cancer progressivity, and interfere the therapy. Recent studies have been reported that some compound from plants could act as an inhibitor on DNA methylation. Since methylation is inhibiting, the silenced tumor suppressor gene caused by hipermethylation, will be re-active. Previous study was succesfully identify flavonoid from *Scurrula atropurpurea* (Blume) Danser (SAD) that collected from Lawang city, Jawa Timur. Fractionation of a compound have been carried out with a solvent n-heksan, chloroform, and ethanol. Identification of a compound through investigating liquid chromatography mass specthrometry mass (LCMS). The outcome showed that flavonoid compound in SAD were included flavanon, dihidroflavonol, flavon, flavonol, katekin, and Epigallocathecin-3-O-gallate (EGCG). The study was an advanced study, has an objectives to identify the SAD potency towards methylation inhibition, and it was insilico using Autodock Vina on software PyRx 0.8. The result shown that an active compound isolated from SAD have potency to inhibit DNA methylation, in which EGCG as the strongest candidate with binding affinity was -10.4 Kcal./mol. Result of this study can be used to tested a potential active compound of SAD as anticancer both in vitro and in vivo.

Keywords: *DNA methylation, Scurrula atropurpurea (Blume) Danser*

INTRODUCTION

Epigenetic modification have an important role on cancer progresivity and therapy. DNA methylation is one of epigenetic modification which have an effect towards cells funnction, via gen expression changing [(1), (2), (3)]. In cancer, some epigenetic modification such as methylation towards some important tumor supressor genes is due on cancer proression, and caused gene silencing [(4)]. Some recent studies showed that EGCG could act as an *DNA methyltransferase* (DNMT) inhibitor via direct interaction, and induce demethylation and reactivation of silenced tumor supressor genes caused by methylation [(5), (6)]. The research objective was to identify the SAD potency as a DNA methylation inhibitor in silico experiment.

Corresponding author :
Heru Santoso Wahito Nugroho
Health Polytechnic of Ministry of Health at Surabaya, Address: Midwifery Campus of Magetan, S. Parman Street No. 1, Magetan District, Jawa Timur Province, Indonesia, Email: heruswn@gmail.com

MATERIAL AND METHOD

On the previous study, we already able to identify *Scurrula atropurpurea* (Blume) Danser (SAD)

flavonoids from Lawang Jawa Timur. Those flavonoids were : flavanon, dihidroflavonol, flavon, flavonol, cathecin, dan EGCG [7]. The potency of those compound was test using PASS method. The method was using *Structure Actvity Relation (SAR)* approach, that be able to predict the compound activity based on the functional group similarity with drugs active group that been known before [8].

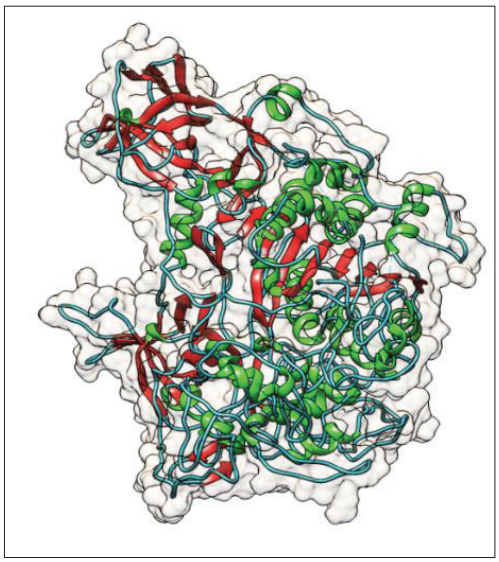

Figure 1. The 3D Structure of Human DNMT 1

DNMT sample were collected from Protein Data Bank (rcsb.org) with ID 3SWR (Figure 1) [9]. The model was DNMT protein from Human that linked to inhibitor. DNMT activity was depend on it active functional site. DNMT active site was on amino acid 1226. The co-factor for this enzyme was zink (Zn) and *zink finger* position (*DNA binding domain*) was the amino acid 646-692. Natural flavonoid data (EGCG, catechin, dihidroflavonol, flavone, flavonol, flavanon), were collected from PUBCHEM NCBI. The potency of each compound as DNMT inhibitor were analyze to find the afinity of the coumpound with active site oof DNMT. The Molecular Docking analysis was using Autodock Vina from PyRx 0.8 program. The *docking* was done at active site of DNMT.

We were inhibition mechanism to examine the molecular interaction, and we can find which active group function from the compound that will linked to amno acid site. The molecular interaction analyze and its visualization were use Ligand Scout V.2.0 [10], [11].

FINDINGS

The SAD flavonoids potency towards DNA inhibiting methylation were presented on Table 1.

Table 1. Docking flavonoid on SAD with DNMT

Active Compound	Protein Target	*Binding Affinity* (Kcal / mol)	Link Site
EGCG	DNMT	-10.4	Glu1266A, Asn1578A dan Gly1223A
Catechin	DNMT	-8.4	Asn1267A dan Glu1168A
Dihidroflavonol	DNMT	-8.2	Met1169A, Ile1167A, Phe1145A, Leu1247A dan Glu1168A
Flavon	DNMT	-8.0	Met 696A, Val1268A dan Asn1267A
Flavanon	DNMT	-7.8	Trp1170A, Asn1578A, Ala699A dan Val1268A
Flavonol	DNMT	-7.7	Met1169A, Ile1167A, Leu1247 dan Phe1145A

Table 1 stated that on every SAD compound will link on DNMT active site, with different afinity. EGCG Afinity towards DNMT was the highest, with smallest bindiing affinity value. When a compound and another were linking, and have a small *binding affinity*, and this meanss the link will be strong. This mechanisme will give strong link and higher inhibitor effect towards methylation. Contrary, highest *binding affinity* value gives weak inhbitor towards DNA methylation. The docking of ECCG on SAD towards DNMT is shown on Figure 2.

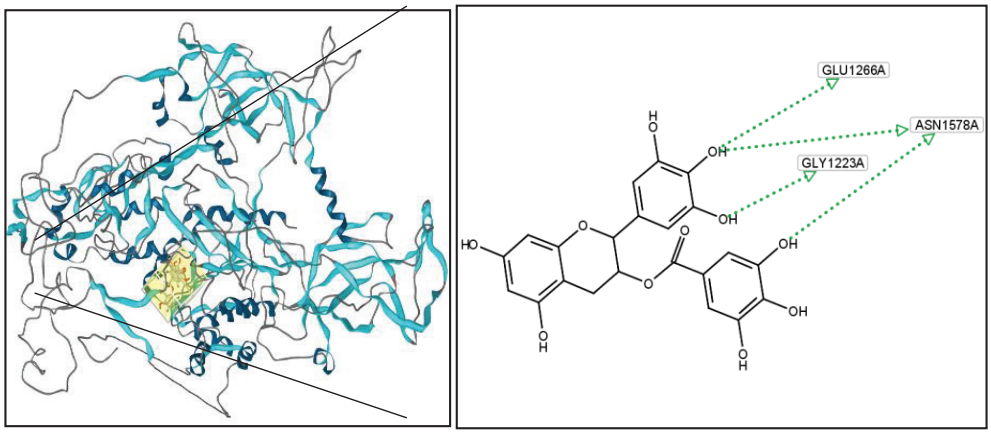

Figure 2. Binding of EGCG on DNMT active site, with afinity -10.4 Kcal / mol via hidrogen bond with Glu1266A, Asn1578A and Gly1223A residu on DNMT

Based on table 1 and figure 2 we known that ECCG have potency as a demethylation agent and flavonol ave the lowest demethylation potency among other SAD flavonoid. EGCG predicted have strongest inhibitor DNMT potency, becaus its capable to bond the actove site of DNMT using 3 hidrogent bonds.

DISCUSSION

SAD Favonoid compound that isolated from Lawang Jawa Timur can bond with active site of DNMT, and have a potency as an inhibotor DNA methylation. Insilico study was shown that every SAD flavonoid compound, could form a bond with DNMT active site via certain and diferent amino acids. EGCG was a compound that shown the highest afinity towards DNMT active site, followed by : katekin, dihidroflavonol and flavon, with *binding affinity* value respectively were : -10.4 Kcal / mol, -8.4 Kcal / mol, -8.2 Kcal / mol, dan -8.0 Kcal / mol. The smallest binding affinity value means that he compound have stronger inhibition effect potency towards DNMT action.

Previous studies have beeen shown that flavanol compound, cathechin and EGCG were potencial demethylation agent. EGCG will inhibit DNMT via blocking the cytosin to link in the DNMT active site. EGCG eill form a hidrogen bond with DNMT active site, and methylation will be inhibit. This will reactivate the genes that silenced by methylation. The othet study also reported that cathechin and EGCG on green tea and apple give an demethylation effect 5-aza-2dC like. 5-aza-2dC is an synthetic DNMT 1 inhibitor, already clinially tested, but still give a toxic effect [4].

Previous study have shown that etanol fraction of SAD Lawang content of EGCG, flavon and flavonol. Chloroform fraction, were content : cathecin, dihidroflavonol and flavanon. From recent study, we found that those comppound have potencies as an *p53 expression enhancer* and strong proliferation inhibitor agent compare to n-hexan fraction [7] . The anti-cancer effect assumed from interaction from chloroform fraction compound in the chlorodorm and n-hexan towards DNMT inhibition activity, and inhibition apoptosis and proliferation indirct and directly. The epigenetic modification like methylation and demethylation will be able to influenc the tumor progresion, metastasis, and resistency towards chemotherapy [1], [2], [12], [13], [14], [15] . Some studies have reported tha some genes were silenced becaus og the methylation on its promotor [16], [17] . The active compound of SAD Lawang *in silico* have been proved inhibit DNA methyltion, and have opportunity as a natural demethylation agent, that potencial as a drug for cancer in the future.

CONCLUSION

This in silico study have been prove that flavonoid compound in the SAD Lawang owing a potency towards inhibition DNA methylation, with EGCG as the strongest candidate. Result of this study can be used to explore potencies active compound from SAD as anticancer agent in future both in vitro and in vitro.

ADDITIONAL INFORMATIONS

Conflict of Interest Statement

All members of the team of research and article writing stated that there is no conflict of interest related to all research activities or publications of this research article.

Source of Funding : All funds used to finance all research activities and publications of research articles come from the research team and the authors of the article.

Ethical Clearance : To ensure that there is no ethical violation as a result of the implementation of this research, before the research has been conducted ethical feasibility testing. Ethical approval recommendation for this study taken from Ethics Committee of State Health Polytecnic of Malang, Indonesia with number " Reg. No. : 009 / KEPK – Polkesma / 2015 ".

REFERENCES

1. Esteller M. Molecular Origins of Cancer: Epigenetics in Cancer. The New England Journal of Medicine. 2008; 358 (11): 1148 - 1159.
2. Sharma S, K. Kelly T, A. Jones P. Epigenetics in Cancer. Carcinogenesis. 2010; 31 (1): 27 - 36.
3. Taberlay P C, Jones, P A. DNA Methylation and Cancer. Epigenetics and Disease, Progress in Drug Research. 2011; 67: 1 - 6.
4. Dueñas - González A, Lizano M, Candelaria M, Cetina L, Arce C, Cervera E. Epigenetics of Cervical Cancer. An Overview and Therapeutic Perspectives. Molecular Cancer. 2005; 4 (38): 1 - 24.
5. Li Y, Tollefsbol T O. Impact on DNA Methylation in Cancer Prevention and Therapy by Bioactive Dietary Components. Curr Med Chem. 2010; 17 (20): 2141 - 2151.
6. Meeran S M, Ahmed A, Tollefsbol T O. Epigenetic Targets of Bioactive Dietary Components for Cancer Prevention and Therapy. Clin Epigenet. 2010; 1: 101 - 116.
7. Sudiwati N L P E, Nurseta T, Aulanni'am, Ali M. In-vitro and In-silico Anticancer Activity of Parasitic Tea Plant Scurrula atropurtupea (Blume) Danser Against Cervical Cancer. International Journal of Pharm Tech Research. 2015; 8 (7): 12 - 18.
8. Goel R K, Singh D, Lagunin A, Poroikov V. PASS-assisted Exploration of New Therapeutic Potential of Natural Products. Med. Chem. Res. 2011; 20: 1509e14
9. RSCB PDB. Biological Macromolecular Structures Enabling Breakthroughs in Research and Education [Internet]. Protein Data Bank. 2011 [cited. 2017 January 1]. Available from: http://www.rcsb.org/pdb/results/results.do?tabtoshow=Current&qrid=2748BE7D
10. Pettersen E F, Goddard T D, Huang C C, Couch G S, Greenblatt D M, Meng E C, Ferrin TE. UCSF Chimera Visualization System for Exploratory Research and Analysis. J Comput Chem. 2004; 25 (13): 1605 - 1612.
11. Trott O, Olson A J. AutoDock Vina: Improving The Speed and Accuracy of Docking with A New Scoring Function, Efficient Optimization and Multithreading. Journal of Computational Chemistry. 2010; 31: 455 - 461
12. Banerjee H N, M. Verma. Epigenetic mechanisms in cancer. Biomarkers Med. 2009; 3 (4): 397 - 410.
13. Lopez J, Percharde M, Coley H, Webb A, Crook T. The Context and Potential of Epigenetics in Oncology. British Journal of Cancer. 2009; 100: 571 - 577.
14. Kresno S B. Basic Science of Oncology (Ilmu Dasar Onkologi) (2 ed.). Jakarta: Badan Penerbit Fakultas Kedokteran Universitas Indonesia; 2011.
15. Sulewska A, Nikliñska W, Kozowski M, Minarowski L, Naumnik W, Nikliński J. DNA Methylation in States of Cell Physiology and Pathology. Folia Histochemica et Cytobiologica. 2007; 45 (3): 149 - 158.
16. Murphy N, Ring M, Heffron C C B B, King B, Killalea A G, Hughes C. $p16^{INK4A}$, CDC6, and MCM5: Predictive Biomarkers in Cervical Preinvasive Neoplasia and Cervical Cancer. J Clin Pathol. 2005; 58: 525 - 534.
17. Spathis A, Aga E, Alepaki M, Chranioti A, Meristoudis C, Panayiotides I. Promoter Methylation of p16 ^{INK4A,} hMLH1, and MGMT in Liquid-Based Cervical Cytology Samples Compared with Clinicopathological Findings and HPV Presence. Infectious Diseases in Obstetrics and Gynecology. 2011; 1 - 5.