PROCEEDING

ISBN : 978-602-9030-08-2

HALALSTECH+ 2012

INTERNATIONAL CONFERENCE ON HALAL SCIENCE & TECHNOLOGY (CURRENT ISSUES ON FOOD, PHARMACEUTICAL & HEALTH PRODUCTS)

4 – 6 JULY 2012 DENPASAR, BALI, INDONESIA

SANUR PARADISE Plaza HOTEL & SUITES

Hosted by :
Faculty of Pharmacy, University of Jember

JOINTLY ORGANISED BY :

UNIVERSITAS JEMBER

UNIVERSITI KEBANGSAAN MALAYSIA
National University of Malaysia

UNIVERSITI SAINS ISLAM MALAYSIA

Jember University Press.

Published by Faculty of Pharmacy & Jember University Press, University of Jember, Indonesia

Proceeding of International Conference on Halal Science & Technology 2012 – **HALALSTECH+ 2012**

ISBN: 978-602-9030-08-2

The editing of this proceeding has been carried out by **B. Kuswandi** with assisted by the Scientific Committee of **HALALSTECH+ 2012.**

Preface

The **HALALSTECH+ 2012,** `International Conference On Halal Science & Technology: Current issues on Food, Pharmaceutical & Health Products 2012' took place in Sanur Paradise Plasa Hotel, Denpasar Bali Indonesia on 4-6 July 2012. This conference has been hosted by the Faculty of Pharmacy, University of Jember (UNEJ), Indonesia, in collaboration with the Faculty of Science & Technology Universiti Kebangsaan Malaysia (UKM), and the Faculty of Science & Technology, Universiti Sains Islam Malalaysia (USIM).

This proceeding contains papers that have been presented at the **HALALSTECH+ 2012** as plenary lectures, keynote, oral and poster presentations. About 100 participants attended the conference, with 11 plenary lectures, 1 keynote lectures and 22 oral and 14 poster presentations. The proceeding of **HALALSTECH+ 2012** has been published in electronic form as *.pdf file for simple and easy publication and to avoid heavy book of proceeding. We hope that this publication can be easily read, handled and transferred to other form. Furthermore, this paperless proceeding can be fruitful for all participants of the conference.

My sincerely thanks go to all the members of Scientific Committee for their valuable help in the review of the submitted papers, and also to the authors for their collaborative attitude. A special mention must go to our organizing committee, who has put in a terrific amount of effort not only in general conference matter but also in the assembly of the papers for this proceeding. Finally, I congratulate the authors of all papers for producing the new and novel idea in areas of food, pharmaceutical and health products related to halal issues as well as other related fields.

.

Jember, July 2012

B. Kuswandi
Editor
HALALSTECH+ 2012 Proceeding

Scientific Committee

1. Prof. Dr. B. Kuswandi (University of Jember, Indonesia)
2. Prof. Dr. Aminah Abdullah (Universiti Kebangsaan Malaysia)
3. Prof. Dr. Lee Yook Heng (Universiti Kebangsaan Malaysia)
4. Prof. Dr. Musa Ahmad (Universiti Sains Islam Malaysia)
5. Assoc. Prof. Dr. Mdy. Alina Abdul Rohim (Universiti Sains Islam Malaysia)

Table of Content

Page

I Preface i

III Scientific Committee iii

II Content iv

Raw Materials From Animal Sources And Concerns Of Halal Meat Products Among Muslim Consumers In Asia
Babji, A.S.[1], Sahilah, A. M. [1,2] and Azhana , H.[1]* 1-5

The Immunoassay Tools for Detection of Pork and Its Derivatives in Pharmaceutical, Food and Cosmetic Products
Yuri Lebedin 6-13

The Effect of Partial Replacement of Wheat Flour with Dehydrated Oyster Mushroom (*Pleurotus sajor-caju*) on Nutritional Composition and Acceptability of Butter Biscuit
W. I. Wan Rosli and M. S. Aishah 14-19

Approach for Controlling non Halal Substance in Food Processing
*Tri Wahono** 20-25

Importance of fruits for human health: Quranic perspectives
Eqbal M. A. Dauqan and Aminah Abdullah 26-32

2D Infrared Characteristic Spectra Pattern of Type B Porcine and Bovine Bone Gelatine in Low Scan
Himmatul Barroroh[1] and Abdul Hakim[1] 33-38

Effectiveness Citric Acid In Milkfish Bone (Chanos-Chanos Forskal) Gelatin Production
Akyunul Jannah[1], A.Ghanaim Fasya[2] and Dewi Fatimah[3] 39-45

Development of Paper Based Biosensor for Formaldehyde Detection in Food Samples
M. Amrun Hidayat[1], Lindawati Setyaningrum[2] and Bambang Kuswandi[2] 46-51

Page

Setting up a *Halal* Analysis Laboratory, *Syariah* Services and Trainings from USIM's Experiences
A. R. Alina[1], A. Siti Mashitoh[1] and K. M. W. Syamsul[1] *52-59*

Indonesian Halal Agro-industrial Stakeholder Collaboration Model
Dwi Purnomo *60-70*

Kinetic Modeling of Immobilized *Candida rugosa* Lipase Catalyzed Esterification Of Butyl Oleate
S. S. Othman[1,], N. A. Mohd Amin[1], S. Mat Radzi[1] and M. B. A. Rahman[2]* *71-77*

Effect of Vanadyl Sulphate on The liver of Diabetic Mice
Diana Holidah *78-85*

Construction and Overexpression of cyp71av1 Gene in *Echerichia coli*
Evi Umayah Ulfa[1] and Tresna Lestari[2] *86-93*

The Halal Traceability System of a Bakery and Confectionary Manufacturer
A. R. Alina[1], S. Zurina[2], Syahidawati[2], M. Nurul Aini[1], H. Hasnizam[3], M. A. Mohd Fauzi[4], A. Z. Yunus Abd. Aziz[3], M. Z. Siti Zamanira[2], W. M. N. Wan Siti Khadijah[2] and A, Siti Mashitoh[1] *94-99*

Synthesis of Conjugated Linoleic Acid (CLA) from Catfish Oil and Its Nuclear Magnetic Resonance Properties
Z. Noorul Jannah[1], B. Abdul Salam[1], and S. Mamot[1] *100-105*

Product Recall Management for Halal Product
S. Zurina[1], S. Syahidawati[1], M. Nurul Aini[2], A. R. Alina[2], H. Hasnizam[3], M. A. Mohd Fauzi[4], A. Z. Yunus Abd. Aziz[3], M. Z. Siti Zamanira[1] *106-109*

The Islamic Approach of Negotiating Interests in Halal Certification to Create Halal Job Opportunities among *Zakat* Recipients
A. R. Norhayati Rafida[1], A. R. Alina[2], H. H. Hafiz[3], B. Saifol[4], and A. Siti Mashitoh[2] *110-115*

Preliminary Research on the Perception towards Halal Awareness and the its Correlation with Halal Certification among Muslims
A. R. Norhayati Rafida[1], A. R.Alina [2], and A. Siti Mashitoh[2] *116-121*

Page

In Vivo Antimalarial Activity of Terpenoid-Rich Fraction of Ethanolic Extract of *Tithonia diversifolia* (Hemsley) A. Gray Leaves
Nuri[1], Wiwien Sugih Utami[2] and Yunita Armiyanti[2] *122-125*

Development of a Halal Certification Module and Training Programme for the Pharmaceutical Industry
N. A. Khairun Nain[1], A. B. Nazefah[1], C. I. Che Ilina[1], W. S. Wan Noraini[1], A. R. Alina[2], M. S. Norhaizam[3], A. A. Siti Hanizah[2], and A. K. Imtinan[2] *126-129*

Analysis of Pork Adulteration in Beef Burgers Using Real-Time Polymerase Chain Reacton (PCR)
Zilhadia[1], Dienar Fitri[1] and Yanis Musdja[1] *130-136*

Test the Antimicrobial Activity of Garlic Against Staphylococcus aureus in In-Vitro Diffusion Method
Yuliati, MBiomed and dr. Annisa Nurul Kirana *135-139*

A Simple and Rapid Test Kit Based on Potassium Dichromate for Determination of Alcohol in Liquid Cosmetics
Lestyo Wulandari, Reni Rahmania and Bambang Kuswandi *140-144*

Docking Studies on Flavonoid Anticancer Agents With DNA Methyl Transferase
Ayik R Puspaningtyas, Dwi Koko Pratoko *145-151*

Docking Studies on Flavonoid Anticancer Agents With DNA Methyl Transferase

Ayik R Puspaningtyas, Dwi Koko Pratoko
Department of Pharmaceutical Chemistry, Faculty of Pharmacy
University of Jember - Indonesia
Jl. Kalimantan 1 No. 2 Kampus Tegal Boto Jember 68121
Corresponding author : aixrose_pee@yahoo.co.id

Abstract

Flavonoid is a group of polyphenolic compounds that widely found in fruits and vegetables. Based on early research, some flavonoid had higher antioxidant effect than the others. Those were kaempferol, galangin, quercetin, robinetin, fisetin, kaempferide, 3-hydroxy-flavone, morin, naringin, coumestrol and daidzein. In some studies, antioxidant drugs are able to act as anticancer drugs by inhibiting reactive oxygen species (ROS) in cancer cells.

Molecular docking of some flavonoid compound against DNA-methyltransferase receptor using Molegro Virtual Docker (MVD) has been done to compare binding energy with native ligand. The results showed that naringin had better bond with DNA-methyltransferase receptor than other flavonoid compounds but not native ligand. *Moldock score* was -157,031 and *Rerank score* was -109,658, means that the energy was lower and binding was more stable. Naringin compounds performed hydrogen bonds with amino acids Gln 301, Phe 18, Asp 16, Cys 76, Arg 165, Glu 119 and Glu 40 of DNA-methyl transferase receptor.

Key words: flavonoid compounds, anticancer, DNA-methyl transferase, Molegro Virtual Docker

1. INTRODUCTION

Flavonoids is a group of polyphenolic compounds, can widely be found in fruits and vegetables. Flavonoids are a class of plant secondary metabolites. Numerous positive health effects of flavonoids have been described. They have been reported to exhibited anticancer, antiviral, antiinflammatory effects and to reduce the risk of cardiovascular diseases [1,2].

Flavonoids are most commonly known of their antioxidant activity in vitro. At high experimental concentrations that would not exist in vivo, the antioxidant abilities of flavonoids in vitro may be stronger than those of ascorbic acid and alpha tocopherol, depending on concentrations tested [3]. These antioxidant activities are generally associated with reported pharmacological effect.

The number of flavonoid derivatives is more than 4000 and their antioxidant properties are very different. Antioxidant and antiradical activities of some flavonoids have been reported. Some flavonoid had higher antioxidant effect than the others. Those were *kaempferol, galangin, quercetin, robinetin, fisetin, kaempferide, 3-hydroxy-flavone, morin, naringin, coumestrol and daidzein* [4]. These antioxidant activities associated with anticancer mechanism by inhibiting reactive oxygen species (ROS) in cancer cells.

The DNA methyltransferase (DNA MTase) is a type of transferase enzyme

that transfer of a methyl group to DNA. DNA methylation serves a wide variety of biological functions. DNA methyltransferase use a reactive methyl group bound to sulfur in S-adenosyl methionine (SAM) as the methyl donor. DNA methylation may also be linked to cancer development, as a methylation of tumor supressor genes promotes tumorigenesis and metastasis [5].

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). Docking is a method which predicts the preferred orientation of one molecule (ligand) to a protein receptor when bound to each other to form a stable complex. The focus of molecular docking is to computationally simulate the molecular regognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized [6].

According to the above review, to estimate the most stable binding between flavonoids compound and DNA MTase receptor that associated with anticancer activity, molecular docking approach can be done.

2. METHODOLOGY

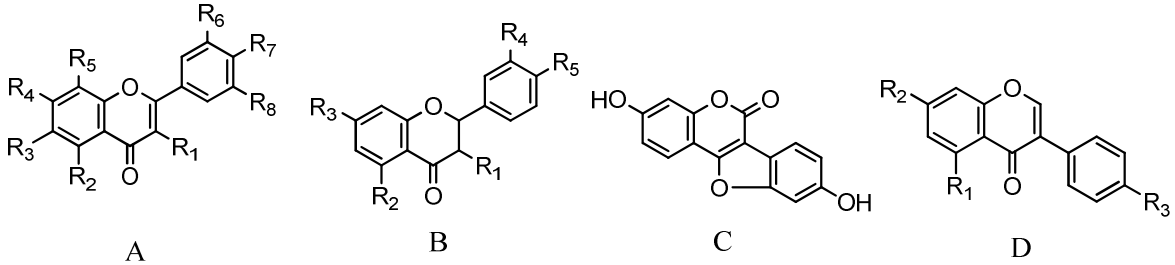
DNA Metyltransferase (DNA MTase) catalityc structural analysis

The 3D structures of DNA Methyltransferase (PDBID: 1HMY) with S-adenosyl methionine (SAM) was obtained from protein data bank (www.pdb.org). The ligand binding domain of DNA Methyltransferase consist of 327 residues with 15 α-helices and 16 strands β-sheet.

Flavonoid compounds and antioxidant activity

Based on early research, some flavonoid had higher antioxidant effect than the others. Those were kaempferol, galangin, quercetin, robinetin, fisetin, kaempferide, 3-hydroxy-flavone, morin, naringin, coumestrol and daidzein. The antioxidant activity data of 11 flavonoids has been taken from reference [4]. Their antioxidant activities have been characterized by the ability to inhibit heat-induced oxydation in β-carotene linoleic acid-model-system-carotene-linoleic [4]. Structures of flavonoids and antioxidant acitivity values are shown in Table 1.

Table 1. Structures and antioxidant activity of tested flavonoids



Compounds	R1	R2	R3	R4	R5	R6	R7	R8	Antioxidant activity (%)
Kaempferol (A)	OH	OH	OH	H	H	H	OH	H	65,3
Galangin (A)	OH	OH	OH	H	H	H	H	H	64,9
Quercetin (A)	OH	OH	OH	H	H	OH	OH	H	63,6
Robinetin (A)	OH	H	OH	H	H	OH	OH	OH	61,7
Fisetin (A)	OH	H	OH	H	H	OH	OH	H	61,6
Kaempferide (A)	OH	OH	OH	H	H	H	OMe	H	60,0
3-hydroxy-flavone (A)	OH	H	H	H	H	H	H	H	59,4
Morin (A)	OH	OH	OH	H	OH	H	OH	H	63,5
Naringin (B)	H	OH	O-Neo hesperidin	H	OH	-	-	-	47,4
Coumestrol (C)	H	OH	OH	-	-	-			38,7
Daidzein (D)	H	OH	OH	-	-				32,9

The 2D and 3D structures were represented using ChemBioDraw Ultra 11.0 and ChemBio3D Ultra 11.0 (trial version). Those software used to draw 2D, 3D structure and analyze of physicochemical properties.

Molecular Docking

*Molegro Virtual Docker (MVD) 5.*0 (www.molegro.com) is a docking programme, used to investigate the best ligand binding affinity with DNA MTase using a grid parameter file [6]. MVD is an integrated platform for predicting protein - ligand interactions. MVD handles all aspects of the docking process from preparation of the molecules to determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligands. The identification of ligand binding modes is done by iteratively evaluating a number of candidate solutions (ligand conformations) and estimating the energy of their interactions with the macromolecule. The highest scoring solutions are returned for further analysis.

MVD requires a three-dimensional structure of both protein and ligand (usually derived from X-ray/NMR experiments or homology modeling). MVD supports the following file formats, Protein Data Bank (pdb), Sybyl Mol2 (mol2) and MDL (sdf/mol/mdl). Potential binding sites (cavities or active sites) can be identified using the built-in cavity detection algorithm. MVD performs flexible ligand docking, so the optimal geometry of the ligand will be determined during the docking.

Docking parameter that used to find best binding affinity among flavonoid molecules,

- Score : MolDock Score [GRID]
- Grid resolution : 0,30 Å
- Algorithm : MolDock SE
- Number of run algorithm : 10
- Max iterations : 1500
- Max population size : 50
- Energy treshold pose generation : 100

3. RESULTS AND DISCUSSION

Among all the structural hits obtained from the PDB, 1HMY i.e DNA Methyltransferase (DNA MTase) with S-adenosylmethionin (SAM) was taken as a role model for the generation of the best inhibitor of cancer development.

Native ligand extracted and put in active site for binding to determine of ligand ability to reproduce of inhibitor orientation and position where was observed in cavity of crystal structure. Ligand energy has been minimized with MM2, put again in cavity (volume 217.6) and performed docking with receptor. The results included *MolDockScore Rerank Score* and *HBond* parameters as functions assessments. *MolDockScore* shows energy that used during docking process.

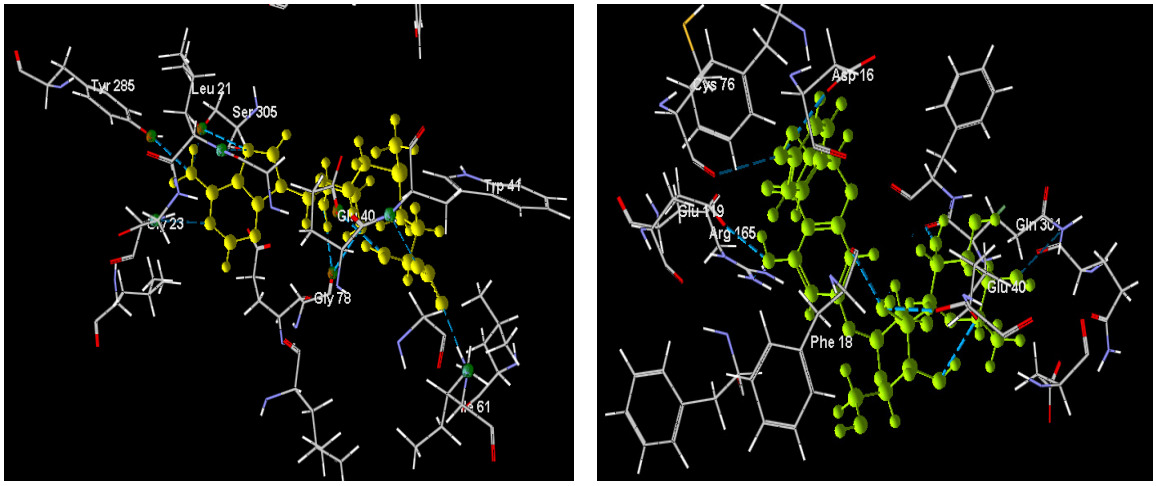
We have been analyzed active side residues of DNA MTase through molecules visualization, and obtained SAM that was supported eight hydrogen bonding interactions with Tyr 285, Gly 23, Gly 78, Leu 21, Ser 305, Glu 40, Trp 41 and Ile 61.

Cavity with volume of 217.6 in this study was used as place of flavonoid compounds as ligand in docking process. Flavonoid compunds that have been drawn and energy minimized by MM2, saved in Sybil2 (mol2) file. Finally docking procedure of flavonoid compunds was used as same as with native ligand. Based on analysis of docking of flavonoids with DNA MTase 1HMY receptors (see Table 2), it was found that naringin compound has docking energy lower than that of other flavonoid compounds, but not lower than native ligand where stability and harmonization in DNAMTase receptor binding compared with other flavonoid compounds. Energy in docking process connect with ligand binding affinity of receptor.

Table 2. Docking result of flavonoid compounds with DNA Methyltransferase (1HMY)

Flavonoid Compound	MolDock Score	Rerank Score	HBond
Native Ligand	*-157,906*	*-131,934*	*-14,1278*
Kaempferol	-123,548	-99,0448	-4,65066
Galangin	-103,300	-53,1705	-9,36522
Quercetin	-111,735	-80,8936	-11,9046
Robinetin	-122,348	-109,913	-13,1239
Fisetin	-114,754	-98,0172	-9,09377
Kaempferide	-112,95	-99,3147	-3,32405
3-hydroxyflavone	-97,6971	-84,7012	-1,77837
Morin	-121,537	-104,846	-8,42676
Naringin	*-157,031*	*-109,658*	*-8,21617*
Coumestrol	-123,571	-99,0955	-4,65014
Daidzein	-119,48	-100,43	-4,34122

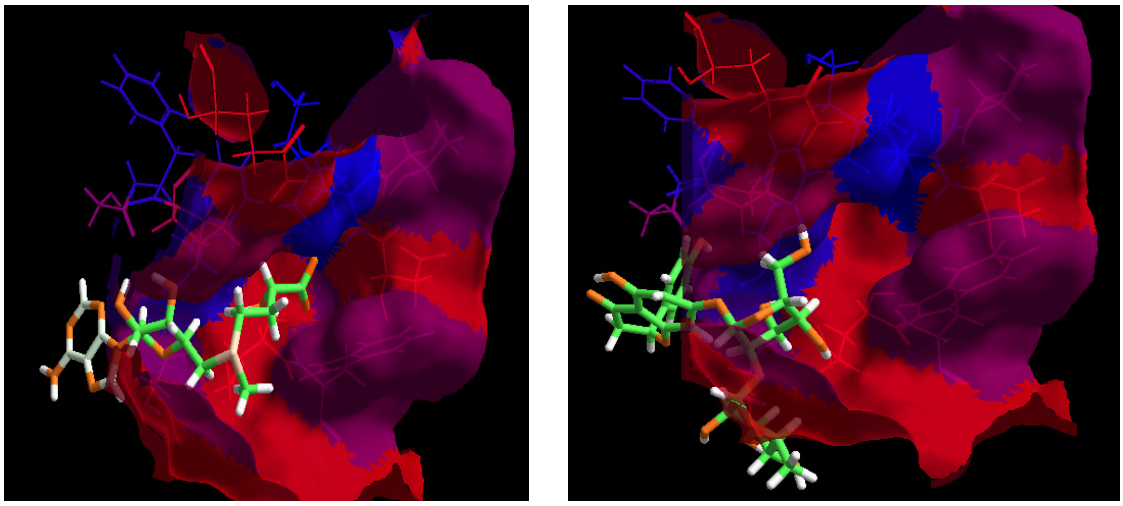
Based on docking results, hydrogen bonding, hydrophobicity and electrostatic properties above can be concluded that naringin has stable binding affinity better than other flavonoids, but if compared with native ligand of 1HMY receptor, naringin stability is not better. This shows that anticancer activity of naringin has the largest contribution of other flavonoid compounds.



(a) (b)

Figure 1. HBond interaction of DNA MTase receptor with SAM (a) dan Naringin (b)

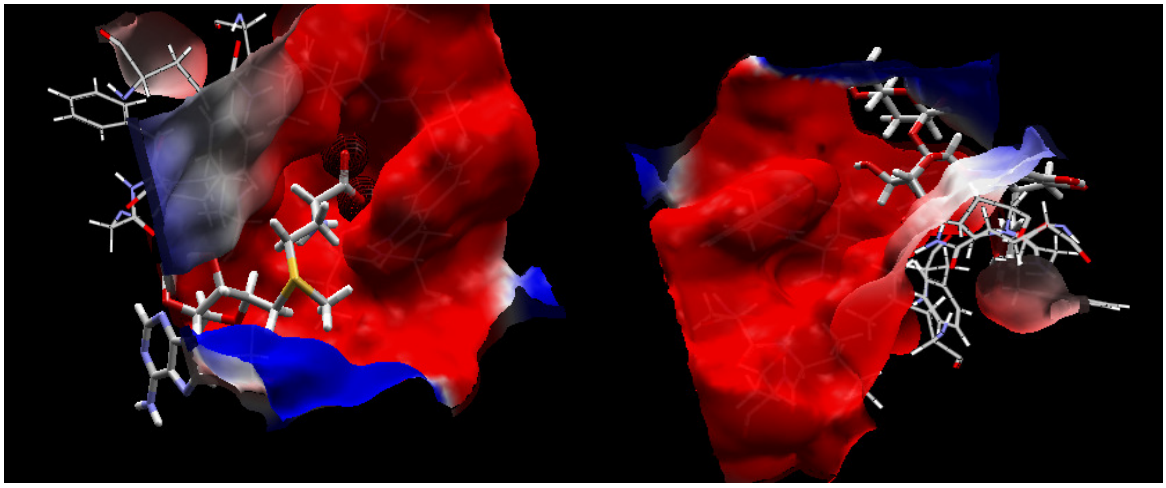
Based on hydrogen bonding interactions native ligand SAM with receptor shows interaction of eight hydrogen bonds with Tyr 285, Gly 23, Gly 78, Leu 21, Ser 305, Glu 40, Trp 41 and Ile 61. While on naringin, this interaction is supported by seven hydrogen bonds with residues : Cys 76, Asp 16, Glu 119, Glu 40, Arg 165, Phe 18 and Gln 30. Hydrogen bonds with residues was high so influence stability bonds between receptor with SAM.



(a) (b)

Figure 2. Hydrophobicity visualization of DNA MTase complexed with ligand (a) *SAM* (b) Naringin

Figure 2 shows hydrophobicity surface of protein (residue), where blue color in surface shows hydrophobic residue and red color indicates hydrophilic residue. Ligand binding should have similar properties with residue. SAM ligand binding shows hydrophilic groups that bound hydrophilic surface, and there are some hydrophobic groups that bound in hydrophilic surface. In contrast to with naringin, which only a small groups corresponding to hydrophobicity. This suitability give stability interaction between receptor with ligand.



(a) **(b)**

Figure 3. Electrostatic visualization of DNA Mtae complexed with ligands (a) *SAM* (b) Naringin

Figure 3 shows electrostatic surface that was associated with partial charge of protein (residue), where blue color indicates positive partial charge residue so that presence of negative partial charge of ligand will lead to partial stability of ligand and receptor binding and *vice versa*. If they analyzed both of them where have electrostatic interactions not good, because in both of groups have negative partial charge residue on negative partial surface so that the interaction did not contribute to stability of ligand and receptor.

Table 3. Molecular physicochemical properties of SAM and flavonoid compounds

Compound	Log P	ClogP	MR	CMR
Native Ligand	-	-5,50603	-	10,0026
Kaempferol	0,74	2,09989	74,7	7,2866
Galangin	1,13	2,76403	72,88	7,1335
Quercetin	0,35	1,50375	76,51	7,4397
Robinetin	0,35	0,57751	76,51	7,4397
Fisetin	0,74	1,24365	74,7	7,2866
Kaempferide	0,74	2,09989	74,7	7,2866
3-hydroxyflavone	1,91	3,043	69,26	6,8273
Morin	0,35	1,13375	76,51	7,4397
Naringin	-1,1	-0,0905768	137,52	13,6506
Coumestrol	2,05	3,13683	69,3	7,0591
Daidzein	2,13	2,0753	69,93	6,9804

4. CONCLUSION

The main aim of molecular docking studies on DNA Methyltransferase (DNA MTase) receptor flavonoid, but has higher mean docked energy when compared with native ligand. The result shows that naringin has good inhibiting affinity compared to other and flavonoid compounds selects active inhibitor for cancer. Docking result illustrated *naringin* has less mean docked energy when compared to other test flavonoid compounds. With application of bioinformatics tools we conclude that naringin compound has the best of binding affinity than other flavonoid compounds

which has higher activity of antioxidant. Thus compound can be designed by approved drug design methods to produce naringin derivates that has higher activity than other flavonoids.

5. REFERENCES

1. Middleton, E.J., Kandaswami, C., Theoharides, T.C.. 2000. The effects of plant flavonoids on mammalian
4. gastrointestinal injury in rats, and the protective ability of a novel grape seed proanthocyanidin extract. *Nutrition Research* 19(8) : 1189-1199
5. Burda, S., Oleszek, W. 2001. Antioxidant and antiradical activitiesof flavonoids. *J. Agric. Food Chem*, 49, 2774-2779.

cells : implication for inflammation, heart disease, and cancer. *Pharmacol Rev* , 52, 673-651

2. Middleton, E.J.1998. Effects of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.* , 439, 175-182
3. Manashi, B., Milnes, M., William, C., Balmoori, J., Ye, Xumei, Stohs, S., Bagchi, D.. 1999. Acute and chronic stress-induced oxidative
6. Momparlier, R. L., Bovenzi, V. 2000. DNA methylation and cancer. *J Cell Physiol,* 183(2) : 145-154
7. Thomsen R., Christensen M. H. 2006. MolDock: A New Technique for High-Accuracy Molecular Docking. *J. Med. Chem.,* 49(11), pp 3315 – 3321
8. Purnomo, Hari. 2012. *Kimia Komputasi*. Yogyakarta : Pustaka Pelajar