PROGRAM BOOK AND ABSTRACTS

International Symposium on Medicinal Plant and Traditional Medicine

Indonesian Traditional Medicine for Human Welfare

Tawangmangu, June 4th - 6th 2014

Jointly organized by

BHAKTI HUSADA

ORAL PRESENTATION SCHEDULE
DAY 2, JUNE 5th 2014

ROOM 1 (SINEMA)
BOTANY AND CULTIVATION TECHNOLOGY
Moderator : Dr. Usman Siswanto
Assistant : Devy

No.	KODE	TITLE	AUTHOR/s	TIME
1.	O-MPB-009	In vitro conservation of several endangered medicinal plants	Sitti Fatimah Syahid and Natalini Nova Kristina	13.10 -13.20
2.	O-MPB-010	Effect of watering frequency on growth and anthocyanins content of light red roselle and deep red roselle	Edi Purwanto, Andri Eko Permadi and Sumani	13.20 -13.30
3.	O-MPB-011	Role of biotechnology in medicinal plants development	Arini Putri Hanifa and Acima	13.30 -13.40
4.	O-MPB-013	Accessions relationship of purwoceng (*Pimpinella pruatjan* Molkenb.) based on morphological caracters	Harto Widodo, Azizatur Rahmah, Rina Sri K	13.40 -13.50
5.	O-MPB-014	Variation in morphological characters, yield components and essential oil contents of java turmeric (*Curcuma xanthorrhiza* Roxb.) accessions from Jawa	Nurliani Bermawie, Natalini Nova Kristina and Susi Purwiyanti	13.50 -14.00
6.	O-MPB-016	The study of sunflower (*Helianthus annuus* L.) and galangal (*Kaempferia galanga* L.) on growth and yield in intercropping system	Dian Susanti* and Rahma Widyastuti	14.00-14.10
		Discussion		14.10-14.40
7.	O-MPB-012	Study on water availability and shade on rate of growth and valeric acid content of valerian [*Valeriana javanica* (Bl.) DC.]	Fauzi, Yuli Widiyastuti, Bambang Pujiasmanto	14.40 –14.50
8	O-MPP-014	Virtual screening of compounds from Indonesian herbal database for potential human epidermal growth factor receptor inhibitors	Yoga Mulia Pratama, Luthfi Saiful Arif, Fitria Dewi Larassuci	14.50 -15.00
9	O-MPB-015	Intraspecific variation of ekinase accessions *(Echinacea purpurea* (L.) Moench) from mass selection year I based on ISSR analysis	Dyah Subositi and Fauzi	15.00 -15.10
10.	O-MBM-019	Cytotoxic activity of mistletoe (*Scurrula atropurpurea* Bl. Dans.) extract against breast cancer cells MCF-7	Slamet Wahyono* and Nita Supriyati	15.10 -15.20
11.	O-MPP-011	Molecular docking of antioxidant compounds: groups of flavonoid and phenolic from eight Indonesian medicinal plants	Desy Nurmalitasari, Agni Hikmawati, Laily Hidayati, Broto Santoso	15.20-15.30
		Discussion		15.20-16.45

POKJANAS

CERTIFICATE

BAKTI HUSADA

This is to certify that

DESY NURMALITASARI

has participated in

The 46th Symposium of National Working Group of Indonesia Medicinal Plant
International Symposium on Medicinal Plant and Traditional Medicine
Theme: Indonesia Traditional Medicine for Human Welfare
4 - 6 June 2014, Tawangmangu, Indonesia, as:

ORAL PRESENTER

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National Working Group of Indonesia Medicinal Plant

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The 46th Meeting of National Working Group on Indonesia Medicinal Plants
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ON MEDICINAL PLANTS AND TRADITIONAL MEDICINE
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Surat Keterangan

Pengalihan Ijin Publikasi Mandiri

Kami, panitia pelaksana kegiatan ***International Symposium on Medicinal Plant and Traditional Medicine*** "Indonesia Traditional Medicine for Human Welfare", memberikan ijin Pengalihan Publikasi Mandiri secara online artikel dari:

Nama Penulis : Desy Nurmalitasari, Agni Hikmawati, Laily Hidayati, Broto Santoso
Asal Institusi : Fakultas Farmasi Universitas Muhammadiyah Surakarta
Judul Artikel : **Molecular Docking of Antioxidant Compounds: Groups of Flavonoid and Phenolic from Eight Indonesian Medicinal Plants**.

untuk dipublikasi secara Mandiri oleh yang bersangkutan. Artikel tersebut telah dipresentasikan secara oral dalam ***International Symposium on Medicinal Plant and Traditional Medicine*** "Indonesia Traditional Medicine for Human Welfare" yang diselenggarakan oleh Medicinal Plant and Traditional Medicine Research and Development Centre (MPTMRDC/B2P2TOOT) - NiHRD, Ministry of Health Republic of Indonesia in colaboration with National Working Group of Indonesian Medicinal Plant (POKJANASTOI)pada tanggal 4-6 Juni 2014 di Auditorium of Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC/B2P2TOOT) Tawangmangu. Demikian surat ini dibuat agar dapat dipergunakan sebagaimananya mestinya.

Tawangmangu, 27 Februari 2015

Panitia Pelaksana

Nagiot Cansalony Tambunan
Ketua Panitia

O-MPP-011

MOLECULAR DOCKING OF ANTIOXIDANT COMPOUNDS: GROUPS OF FLAVONOID AND PHENOLIC FROM EIGHT INDONESIAN MEDICINAL PLANTS

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Indonesia is a well-known country with a variety of resources vegetables and fruits are abundant. Most of them are used as a prospectus cure for cancer within antioxidant mechanisms. Some parts of these plants are eucalyptus leaves, bay leaves, guava leaves, mangosteen skin, Kepel leaves, peanut shell, yellow leaves and seeds of rambutan known to have antioxidant activity. This recent study aimed to gain information about the chemical interaction studies of the ligand-protein by molecular docking of flavonoid and phenolic compounds with a protein target that is responsible for antioxidant activity. The eight extracts obtained by maceration using 96% ethanol were tested their antioxidant activity with DPPH method to obtain their IC_{50} values. Flavonoids and phenolic compounds of each plant were obtained from the database of Universal Natural Product Database (http://pkuxxj.pku.edu.cn/UNPD) and other literature. The 99-selected compounds were performed molecular docking against the 6-protein target using Dock6. The results were obtained in the form of colour pasta extracts with IC_{50} values (and yield) for eucalyptus leaves, bay leaves, guava leaves, mangosteen skin, Kepel leaves, peanut shell, yellow leaves and seeds of rambutan was 29.4 (33.36); 43.9 (25.71); 82.6 (30.0); 170 (38.44); 190 (22.71); 240 (6.09); 790 (26.39) and 970 (8.55) µg/mL (% w/w) respectively. Eucalyptus leaf extract has the greatest antioxidant activity by DPPH method. This result correlates with its binding activity of molecular docking. A compound, namely (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid (eucalyptus) is highly active against all protein targets followed by murrapanine (yellow) compared to the native ligand of the protein target. All other molecules of the six remaining extract did not contain molecule that has a dominant binding affinity of ligand-protein. This could be happened because the antioxidant activity of each extract was admitted as resultant of several molecules or there are other molecules that are not incorporated in the flavonoid and phenolic groups that have antioxidant activity. However, further research is needed to proof that antioxidant activity is the resultant of several compounds.

Keywords: Indonesian Medicinal Plants, Antioxidant, DPPH, Molecular Docking, Dock6

O-MPP-012

PURPLE SWEET POTATO LEAVES: ANTIOXIDANT ACTIVITY BY DPPH, CUPRAC,FTC, TBA METHODS AND MOLECULAR DOCKING PROFILE USING DOCK6

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Purple sweet potato leaves is one of crop plants that has natural antioxidants potency because it contains of phenolic and flavonoid compounds. The aim of this experiment was to determine the effect of leaf drying method at room temperature,by oven, freeze drying, and liquid nitrogen to the antioxidant activity of the ethanol extract and its molecular docking profile. The antioxidant activity of the four types of extracts was measured using DPPH, CUPRAC, FTC, and TBA method. All molecules contained in plant was obtained from UNPD and performed molecular docking using Dock 6. The experimental results showed that the differences in leaf drying methods affected the total phenolic and flavonoid content of the ethanol extract. The measurement of antioxidant activity of extracts which measured by four different methods also showed varying results profile. It can be concluded that the differences in the method of drying, the type of oxidant generated in the antioxidant measurement method and the treatment for each antioxidant measurement methods of had significant impact on its antioxidant activity. The top ten molecules, gained from molecular docking, exposed that they have strong binding affinity with protein 1TDI, 1YVL, 4NOS and 18GS compared

Molecular Docking of Antioxidant Compounds: Groups of Flavonoid and Phenolic from Eight Indonesian Medicinal Plants

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Abstract

Indonesia is a well-known country with a variety of resources vegetables and fruits. Most of them are used as a prospectus cure for cancer within antioxidant mechanisms. Some parts of these plants are eucalyptus leaves, bay leaves, guava leaves, mangosteen skin, Kepel leaves, peanut shell, yellow leaves and seeds of rambutan known to have antioxidant activity. This recent study aimed to gain information about the chemical interaction studies of the ligand-protein by molecular docking of flavonoid and phenolic compounds with a protein target that is responsible for antioxidant activity. The eight extracts obtained by maceration using 96% ethanol were tested their antioxidant activity with DPPH method to obtain their IC_{50} values. Flavonoids and phenolic compounds of each plants were obtained from the database of Universal Natural Product (http://pkuxxj.pku.edu.cn/UNPD) and other literature. The 99-selected compounds were performed molecular docking against the 6-protein target using Dock6. The results were obtained in the form of colour pasta extracts with IC_{50} values (and yield) for eucalyptus leaves, bay leaves, guava leaves, mangosteen skin, kepel leaves, peanut shell, yellow leaves and seeds of rambutan was 29.4 (33.36); 43.9 (25.71); 82.6 (30.0); 170 (38.44); 190 (22.71); 240 (6.09); 790 (26.39) and 970 (8.55) µg/mL (% w/w) respectively. Eucalyptus leaf extract has the greatest antioxidant activity by DPPH method. This result correlates with its binding activity of molecular docking. A compound, namely (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid (eucalyptus) is highly active against all protein targets followed by murrapanine (yellow) compared to the native ligand of the protein target. All other molecules of the six remaining extract did not contain molecule that has a dominant binding affinity of ligand-protein. This could be happened because the antioxidant activity of each extract was admitted as resultant of several molecules or there are other molecules that are not incorporated in the flavonoid and phenolic groups that have antioxidant activity. However, further research is needed to prove that antioxidant activity is the resultant of several compounds.

Keywords: Indonesian Medicinal Plants, Antioxidant, DPPH, Molecular Docking, Dock6

INTRODUCTION

Antioxidants are compounds that mainly have low molecular mass and they can reduce the damaging effects of radical compounds in general (Langseth, 1995). These compounds stabilize radicals by completing the lack of electrons with the result that stop their chain reaction (Windono et al., 2001). Small number of antioxidants could inhibit or delay the oxidation process.

Based on its mechanism of action, antioxidants can be classified into three groups. Primary antioxidants donate a hydrogen atom rapidly to a lipid radical, for example flavonoids, tocopherol, thiol compound that can break the chain propagation reaction by donating electrons to the peroxide radicals in fatty acids. Secondary

antioxidant, which able to eliminate the process of initiation of oxygen and nitrogen radicals or to react with molecules or enzymes that initiate radical reactions such as, oxidase and reductase causing non reactive radical species. The examples of this category are sulphite antioxidants, vitamin C, beta-carotene, uric acid, bilirubin, and albumin. Tertiary antioxidant is a type of antioxidants that could repair the damaged cells and tissues caused by radicals. Sulphoxide methionin reductase could be as an example (Aviram and Vaya, 2001).

The compounds that have antioxidant activity in plants are phenolic and flavonoid groups. They work by donating redox capability which play an important role in captivating and deactivating radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Other compounds that can be found in plants belonging to the group of antioxidants, among other polyphenols group, bio-flavanoid, vitamin C, vitamin E, *β*-carotene, catechin and resveratrol (Hernani and Raharjo, 2006).

Indonesia is a well-known country with a variety vegetables and fruits. They could be used as a source of natural medicine. Some plants such as eucalyptus leaves (*Melaleuca leucadendron* L), bay leaves (*Syzygium polyanthum*), guava leaves (*Psidium guajava* Linn), mangosteen skin (*Garcinia mangostana*), kepel leaves (*Stelechocarpus burahol*), peanut shell (*Arachis hypogaea*), yellow leaves (*Murraya paniculata*) and seeds of rambutan (*Nephelium lappaceum*) are known to have antioxidant activity. Guava has phenolic compounds such as mirisetin, epigenin, ellagic acid and anthocyanins (Miean and Mohamed, 2001). Saponins, flavonoids, tannins and essential oils have been found in eucalyptus (Thomas, 1992). Ethanol extract of its leaves has antioxidant activity (Indrayana, 2008). Belami *et al*., (1997) indicated that bay leaves contain mainly flavonoids, quercetrin, which are known to have very strong activity as an antioxidant and anti-cancer (Ding *et al*., 2010). Some studies showed that flavonoids and phenolic in plants and its extract have antioxidant activity but there is no correlation between the concentration of flavonoids and phenolic and their antioxidant activity (Zheng and Wang, 2001).

Gupta *et al*. (2013) stated that there were six-protein targets, which have been used to predict antioxidant activity of molecules. These proteins are enzyme that responsible for antioxidant mechanism in human body. They were ordered in transferase (18GS, 1TDI, 3LJR, 1JNK), oxidoreductase (4NOS) and signalling protein (1YVL) class. Molecular docking could be able to bind and emphasise the evidence of chemical interaction that will occur between the results of laboratory and computational

chemistry. Molecular docking uses the three-dimensional structure of the receptor to screen and acquire conformation of molecules as ligands that have the highest binding affinity to protein. The method could gain the information about relations between the conformations of ligands with the active site of protein (McGovern and Shoichet, 2003).

This research has the following objectives: measuring the ability of antioxidant activity by molecular docking, knowing the types of medicinal plants that contain the best antioxidants and their molecular docking profile. This recent study has been performed to gain information about the chemical interaction studies of the ligand-protein by molecular docking. The flavonoid and phenolic compounds in eight medicinal plants have been docked to proteins target that are responsible for antioxidant activity.

MATERIAL AND METHODS

Materials for molecular docking were MacBook Pro with Intel-i7 processor, 8GB RAM, Intel HD Graphics 4000 and Mac OS X Mavericks 10.9 (64bits). Computational system has been built using MacPorts 2.2.1, XCode 5.1, Chimera 1.8, MarvinSuite 6.2, Dock6.6, iBabel 3.3 or OpenBabel 2.3.2, PyMOL opensource version 1.7.1.1 and LigPlot+ 1.4.5 for education. Materials for antioxidant assay were DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, aquadest, spectrometer, glassware and analytical balance. Chemical materials were pro analysis grade.

Molecular docking has been performed using Dock6 to calculate docking score of ligands. They have been gained from UNPD database (Universal Natural Product Database at http://pkuxxj.pku.edu.cn/UNPD/) and some articles (Kato *et al*., 2013; Sunarni *et al*., 2007). Chimera has been used to prepare protein, ligands and other components as explained at http://www.bioinformatics.iastate.edu/. Molecules of bay leaves and kepel leaves have been generated utilizing MarvinSuite. Two-dimensional structures of them were drawn with MarvinSketch. Their 3D-structure was produced by MarvinSpace. Docking results was obtained as Grid Score (kcal/mol) using Dock6.6 with four types calculation based on the center of binding-site gridbox and flexibility of ligand during process. They are flexible ligands with default Dock6 calculation or ligand centre for gridbox and rigid ligand using default Dock6 calculation or ligand centre for gridbox. Protein residue and ligand contact were generated using LigPlot+. PyMOL was applied to capture the conformation of docked ligands inside protein.

Ethanol extract of plants were obtained using maceration method and evaporation to acquire concentrated extract. Antioxidant assay was carried out by

preparing of the requirement reagent in ethanol (0.4 mM DPPH, concentration series of each plant extract). After 45 minutes incubation, fixed solutions were measured for their absorption intensity at 517 nm. Radical scavenging activity against DPPH was expressed as IC_{50} value.

RESULTS AND DISCUSSION

Ligands gained from database must be appropriate to phenolic or flavonoid groups. The result revealed that there were 99 molecules in total for the molecular docking studies. The number for molecules from eucalyptus leaves, bay leaves, guava leaves, mangosteen skin, kepel leaves, peanut shell, yellow leaves and seeds of rambutan are 7, 3, 11, 43, 1, 14, 18 and 6, respectively.

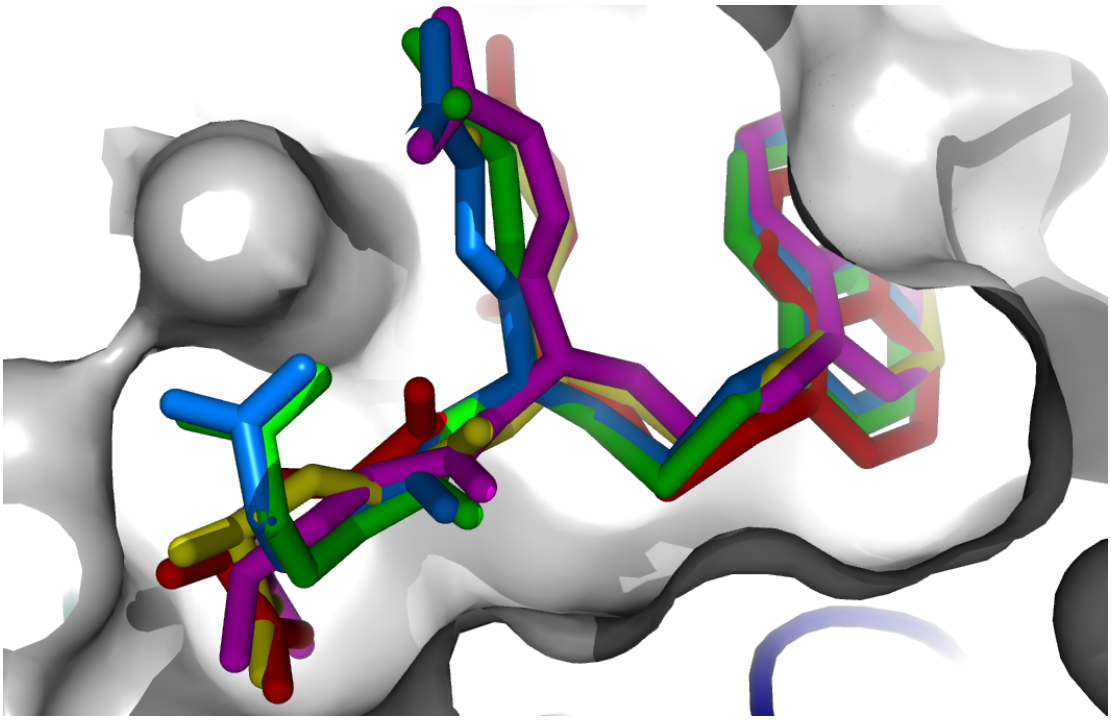

Figure 1. Docked position of native ligands inside 3LJR protein resulted from molecular docking compared to its crystallography data. Red colour represents crystallography ligand, green for flexible ligand that obtained based on default Dock6 calculation for gridbox (RMSD = 0.646), blue for flexible ligand that obtained based on ligand centre for gridbox (RMSD = 0.602), yellow for rigid ligand using ligand centre for gridbox (RMSD = 0.925) and magenta for rigid ligand using default Dock6 calculation for gridbox (RMSD = 0.497).

Scientific evidence regarding chemical compounds in the bay leaves and kepel leaves has not been found to date. This causes the number of molecules of both plants is less representative to be profile of molecular docking. Fortunately, they are included the group of phenolic and flavonoid. They are hydroxychavicol, 4-allyl-1-hydroxy-2-(20allyl-40-hydroxy-50-methoxyphenoxy)benzene and 4-allyl-2-hydro-xy-1-(20-allyl-40-

hydroxy-50-methoxyphenoxy)benzene for bay leaves and 3,7,3',4'-tetrahydroxy-5-methylflavon for kepel leaves (Kato *et al*., 2013; Sunarni *et al*., 2007).

Validation of molecular docking should be done to the native ligand from each protein. The results of molecular docking validation of six proteins with their native ligands indicated that the 3D conformation between native ligand of crystallography with native ligand of docking results was found to be similar position from one to another. Figure 1 is an example of the 3D conformation of docked ligand of the protein 3LJR, a transferase enzyme, namely Glutathione S-transferase theta-2.

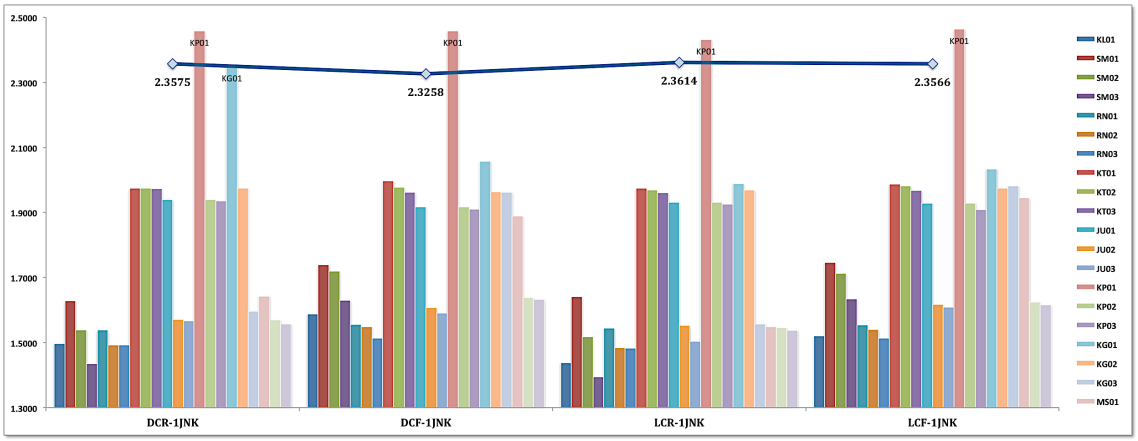

Figure 2. Result of the best three of docked ligands for protein 1JNK represent as –Log (Grid Score) value and compare to dock result of native ligand (blue solid line) for each type of gridbox centre. Result of rigid ligands (DCR) and flexible ligands (DCF) that obtained based on default Dock6 calculation for gridbox and result of rigid ligands (LCR) and flexible ligands (LCF) using ligand centre for gridbox.

Table 1. Result of extraction and radical scavenging activity against DPPH

Name of Plant	Extract Yield (% w/w)	IC_{50} (µg/mL)
Eucalyptus leaves (*Melaleuca leucadendron*, L)	33.36	29.4
Bay leaves (*Syzygium polyanthum*)	25.71	43.9
Guava leaves (*Psidium guajava*, Linn)	30.0	82.6
Mangosteen skin (*Garcinia mangostana*)	38.44	170
Kepel leaves (*Stelechocarpus burahol*)	22.71	190
Peanut shell (*Arachis hypogaea*)	6.09	240
Yellow leaves (*Murraya paniculata*)	26.39	790
Seeds of rambutan (*Nephelium lappaceum*)	8.55	970

Compound KP01 or (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid has the best result of molecular docking even compared to native ligand of 1JNK for all type of gridbox centre (Figure 2). They have similar 3D-conformation when docked with protein (Figure 3). This happened because the chemical interaction between ligand and protein residue is same one to another.

They have two hydrogen bonds with Lys49 and Mg348-Asn150. Furthermore, the same results have been revealed for 1TDI, 1YVL, 3LJR and 18GS protein (Figure 8). Murrapanine or KG01 (yellow leaves) as the second of rigid ligand compared to native for dock screening using default Dock6 calculation for gridbox.

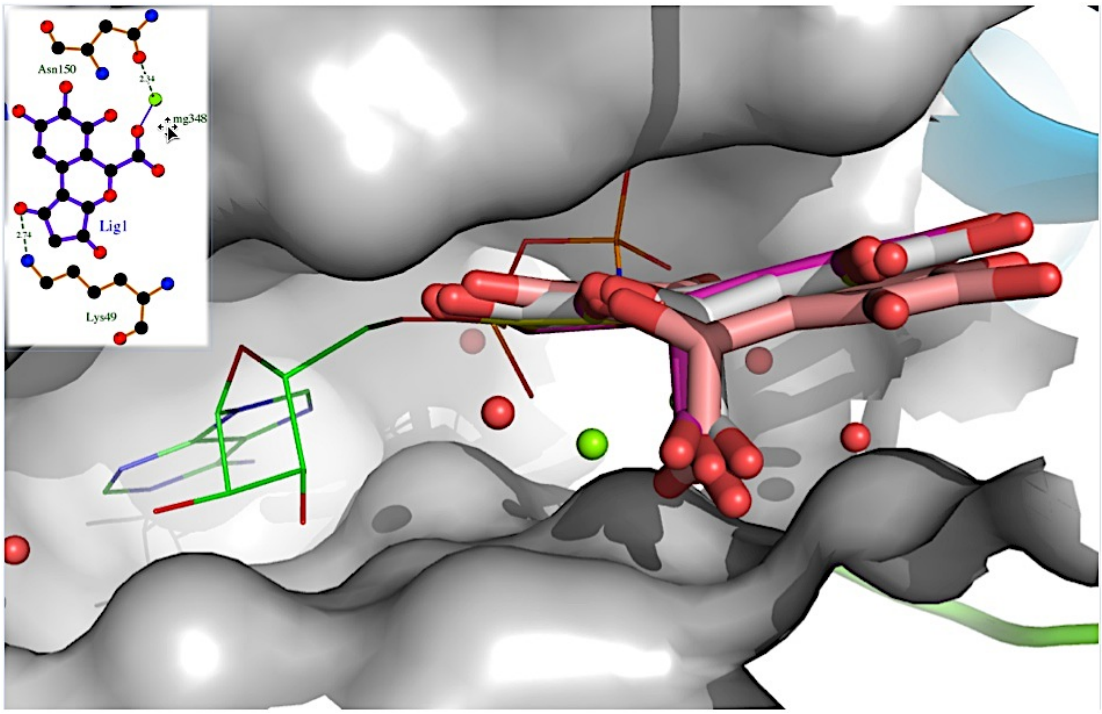

Figure 3. The best of docked ligand conformation with the lowest of Grid Score, namely (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid (drawn in sticks) from eucalyptus compared to native ligand (drawn in lines). One of them has hydrogen bond with Lys49 and Mg348-Asn150 (white box).

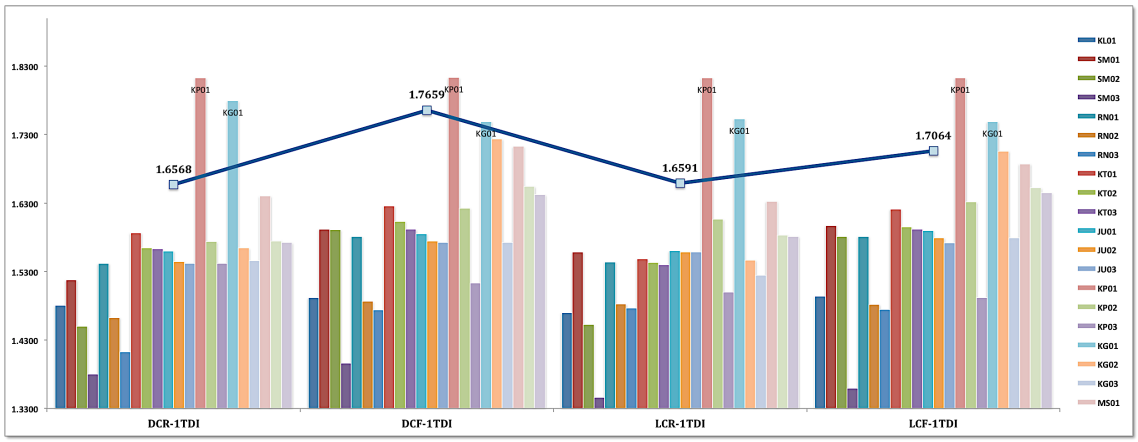

Figure 4. The best three of docked ligands for protein 1TDI represent as –Log (Grid Score) value and compare to dock result of native ligand (blue solid line) for each type of gridbox centre. Result of rigid ligands (DCR) and flexible ligands (DCF) that obtained based on default Dock6 calculation for gridbox and result of rigid ligands (LCR) and flexible ligands (LCF) using ligand centre for gridbox.

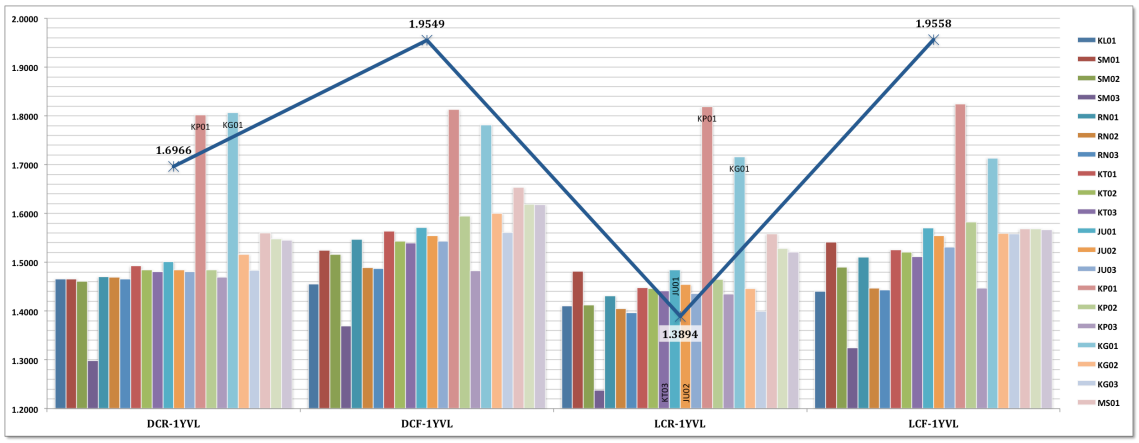

Figure 5. The lowest three of docked ligands for 1YVL as –Log (Grid Score) compare to dock result of native ligand (blue solid line) for each type of gridbox centre. DCR= dock centre rigid, DCF= dock centre flexible, LCR= ligand centre rigid and LCF= ligand centre flexible.

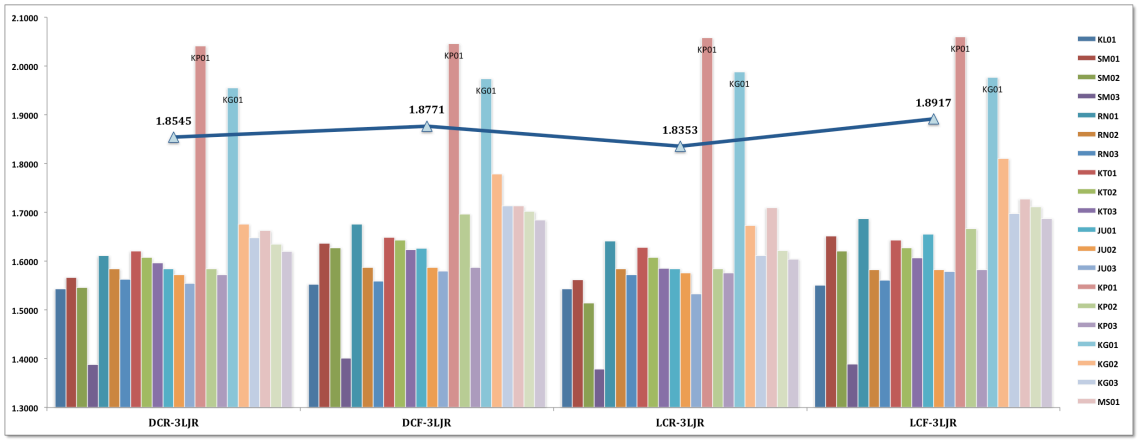

Figure 6. The lowest three of docked ligands for 3LJR as –Log (Grid Score) compare to dock result of native ligand (blue solid line) for each type of gridbox centre. DCR= dock centre rigid, DCF= dock centre flexible, LCR= ligand centre rigid and LCF= ligand centre flexible.

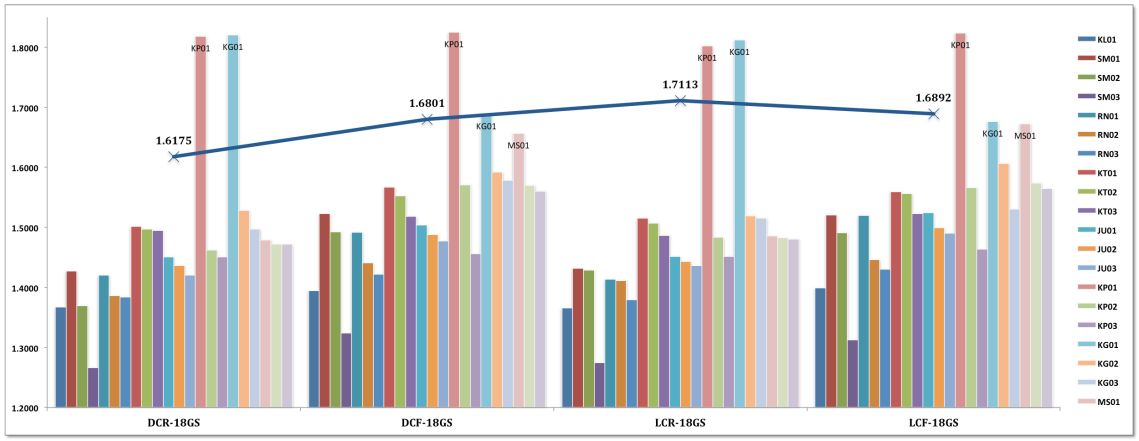

Figure 7. The result of best three of docked ligands in 18GS (–Log [Grid Score]) compare to dock result of native ligand (blue solid line) for each type of gridbox centre. DCR= dock centre rigid, DCF= dock centre flexible, LCR= ligand centre rigid and LCF= ligand centre flexible.

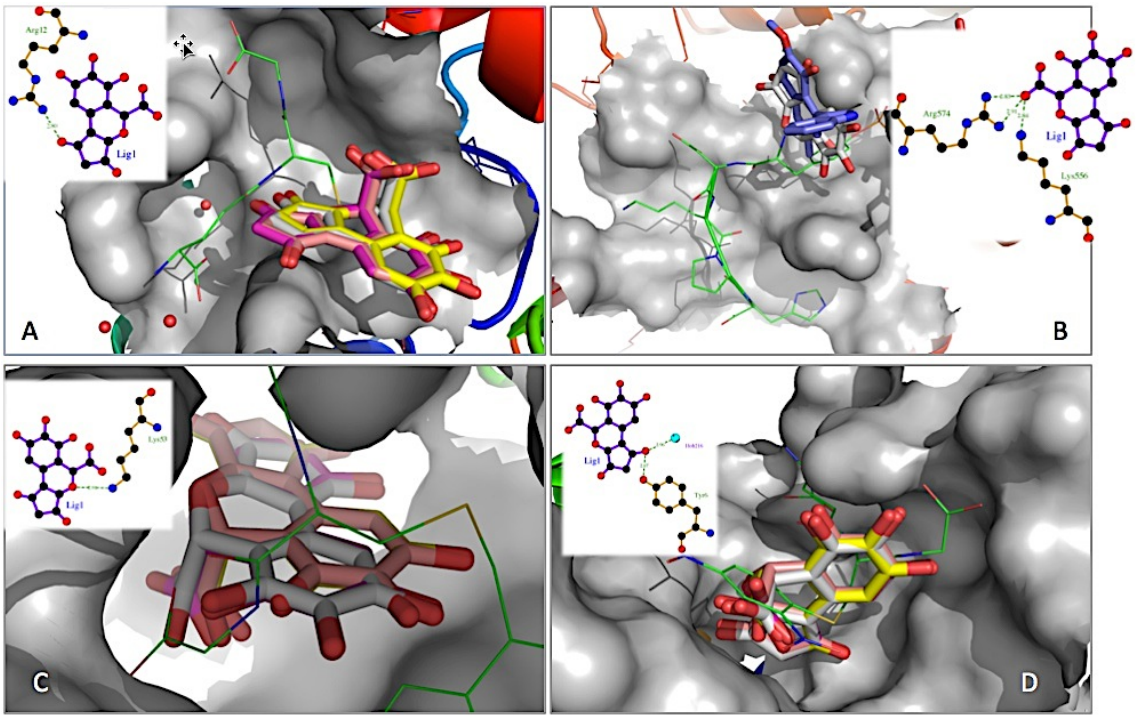

Figure 8. Docked ligands with the lowest of Grid Score, namely (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid (drawn in sticks) from eucalyptus compared to native ligand (drawn in lines). White box represent chemical interaction ligand-protein, such as hydrogen bond with Arg12 (A: 1TDI), Lys556 and Arg 574 (B: 1YVL), Lys53 (C: 3LJR) and Tyr6-HOH216 (D: 18GS).

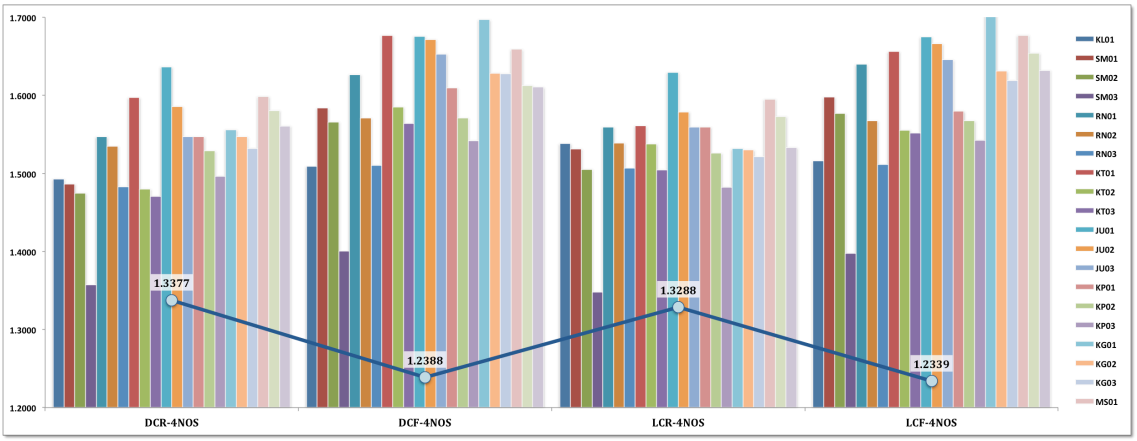

Figure 9. The result of best three of docked ligands in 4NOS (–Log [Grid Score]) compare to dock result of native ligand (blue solid line) for each type of gridbox centre. DCR= dock centre rigid, DCF= dock centre flexible, LCR= ligand centre rigid and LCF= ligand centre flexible.

KP01 also had the lowest Grid Score of molecular docking with 1TDI, 1YVL, 3LJR and 18GS protein and the second place is KG01 (Figure 4-7). These only illustrate as a single molecule profile of molecular docking. Different result has been found in assay of the antioxidant using DPPH (Table 1). As an example, yellow leaves's IC_{50} was the runner-up from the bottom that matched to its average of dock

result. This is the reason why molecular docking result of molecules in other plants did not exceed the result of native ligand. They worked as a group of compounds instead of a single molecule. Mangosteen skin has the biggest yield of extract and the highest number of molecules than other plants. This is the reason why the mangosteen has the large variety of biological activity. Some of them are antioxidant and anti-cancer.

Figure 9 explains that tested ligands had potent binding activity with protein compared to original ligand. Binding site pocket of 4NOS has a small volume space because its ligand only has fifteen atoms. Other non standard molecules beside ligand have been removed from protein when preparation has been completed. Gridbox size of 4NOS determined using default Dock calculation and ligand centre point resulted in a big molecular volume of binding site ligand-protein interaction site. Molecular docking of ligand fulfilled other binding site pocket instead where native ligand three-dimensional position was, due to ligands could replaced the position of these non standard molecules.

Peanut shell and seeds of rambutan extract had yield less than 10% and the others had more than 20% yield of extract (Table 1). Phenolic and flavonoid content in both extracts may be less than that of other plant extracts. This is a cause that their antioxidant activity was low. Mangosteen skin extract had the contradictive result. It had high yield of extract but it did not contribute to its IC_{50} value. It can be occurred when extracted molecules were not including in groups of phenolic or flavonoid and did not have the antioxidant pharmacopore. IC_{50} value of bay leaves and guava leaves did not have correlation with their molecular docking result because their antioxidant molecules were not phenolic or flavonoid.

CONCLUSION

A single compound, namely (5R)-6,7,8-trihydroxy-1,3-dioxo-1H,2H,3H,5H-cyclopenta[c]isochromene-5-carboxylic acid (eucalyptus) is highly active against all protein targets followed by murrapanine (yellow leaves) compared to the native ligand of the protein target. All other molecules of the six remaining extract did not contain molecule that has a dominant binding affinity of ligand-protein. This could be happened because the antioxidant activity of each extract was admitted as resultant of several molecules or there are other molecules that are not incorporated in the flavonoid and phenolic groups that have antioxidant activity. However, further research is needed to verify that antioxidant activity is the resultant of several compounds.

ACKNOWLEDGMENT

Thanks to Directorate General of Higher Education of Ministry of Education and Culture has funded this research through field research of student creativity program 2013.

REFERENCES

Aviram M. and Vaya J. 2001. Markers for Low-density Lipoprotein Oxidant, *Methods in Enzymol.*, 335: 244-56.

Belami D., Gana A. and Kusaardiyani S. 1997. *Flavonoid Utama dan Asam Fenolat Daun Salam. Eugenia Polyantha Wight, Myrtaceae*. Skripsi. Fakultas Farmasi. Institut Teknologi Bandung.

Berman HM., Westbrook J., Feng Z., Gilliland G., Bhat TN., Weissig H., Ilya N. Shindyalov IN. and Bourne PE. 2000. The Protein Data Bank. *Nucl. Acids Res*., 28 (1): 235-242.

Ding M., Zhao J., Bowman L., Lu Y. and Shi X. 2010. Inhibition of AP-1 and MAPK signaling and activation of Nrf2/ARE pathway by quercitrin. *Int. J. Oncol*., 36(1): 59-67.

DOCK6.6 (2013). University of California at San Francisco; San Francisco, CA.

Gupta SK., Pandey R., Karmakar M., Smita S., Pant A., Shukla V., Yadav AK., Negi H. and Gupta MM. (2013). Computational Prediction of Molecular Targets responsible for Antioxidant Activity of D-pinitol in Caenorhabditis elegans. *IJCA Proceedings on National Seminar on Application of Artificial Intelligence in Life Sciences 2013*, NSAAILS, 1: 19-23.

Hernani and Raharjo M. 2006. *Tanaman Berkhasiat Antioksidan*. Penebar Swadaya. Jakarta.

Indrayana R. 2008. *Efek Antioksidan Ekstrak Etanol Daun Salam (Syzygium polyanthum) Pada Serum Darah Tikus Jantan Galur Wistar Yang Diinduksi Karbon Tetraklorida*. Skripsi. Universitas Muhammadiyah Surakarta.

Kato E., Nakagomi R., Gunawan-Puteri MDPT. and Kawabata J. 2013. Identification of hydroxychavicol and its dimers, the lipase inhibitors contained in the Indonesian spice, Eugenia polyantha. *Food Chemistry*, 136(3-4): 1239–1242.

Langseth L. 1995. *Oxidants, Antioxidants, and Disease Prevention*. Internasional life Sciences Institutes (ILSI) Europ. Belgium.

Laskowski RA. and Swindells MB. (2011). LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model*., 51, 2778-2786.

Marvin was used for drawing, displaying and characterizing chemical structures, substructures and reactions, Marvin 6.2.0, January 30th, 2014, ChemAxon (http://www.chemaxon.com).

McGovern SL. and Shoichet BK. 2003. Information Decay in Molecular Docking Screens against Holo, Apo, and Modeled Conformations of Enzymes. *J. Med. Chem.*, 46(14): 2895-907.

Miean KH. and Mohamed S. 2001. Flavonoid (Myricetin, Quarcetin, Kaemfenol, Luteolin, and Apegenin) Content of Edible Tropical Plant. *Journal of Agricultural and Food Chemistry*. 49: 3106-311.

O'Boyle N., Banck M., James C., Morley C., Vandermeersch T. and Hutchison G. 2011. Open Babel: An Open Chemical Toolbox. *Journal of Cheminformatics*, 3(1): 33.

Osawa T. 1994. Novel Natural Antioxidants for Utilization in Food and Biological systems. In: Uritani I., Garcia VV. and Mendoza EM. (Eds.). *Postharvest Biochemistry of Plant Food-Materials in Tropics* (pp. 241-251).

Pettersen EF., Goddard TD., Huang CC., Couch GS., Greenblatt DM., Meng EC. and Ferrin TE. 2004. UCSF Chimera--a Visualization System for Exploratory Research and Analysis. *J. Comput. Chem.*, 25(13): 1605-12.

PyMOL (2014). Molecular Graphics System, Version 1.7.1.1 Schrödinger, LLC.

Sunarni T., Pramono S. and Susidarti RA. 2007. Flavonoid antioksidan penangkap radikal dari daun kepel (*Stelechocarpus burahol* (Bl.) Hook f. & Th.). *Majalah Farmasi Indonesia*, 18(3), 111 – 116.

Thomas ANS. 1992. *Tanaman Obat Tradisional Indonesia*. Kanisus.

Windono T., Soediman S., Yudawati U., Ermawati E., Srielita and Erowati TI. 2001. Uji Peredaman radikal Bebas terhadap 1,1 Diphenyl-2-Picrylhydrazyl (DPPH) dari Ekstrak Kulit Buah dan Biji Anggur (Vitis Vinifera L.). Probolinggo Biru dan Bali. *Artocarpus*. Media Pharmaceutical Indonesian. Fakultas Farmasi Universitas Surabaya.

Zheng W., and Wang SY. 2001. Antioxidant Activity and Phenolic Compounds in Selected Herbs. *J. Agric. Food Chem.*, 49: 5165-5170.