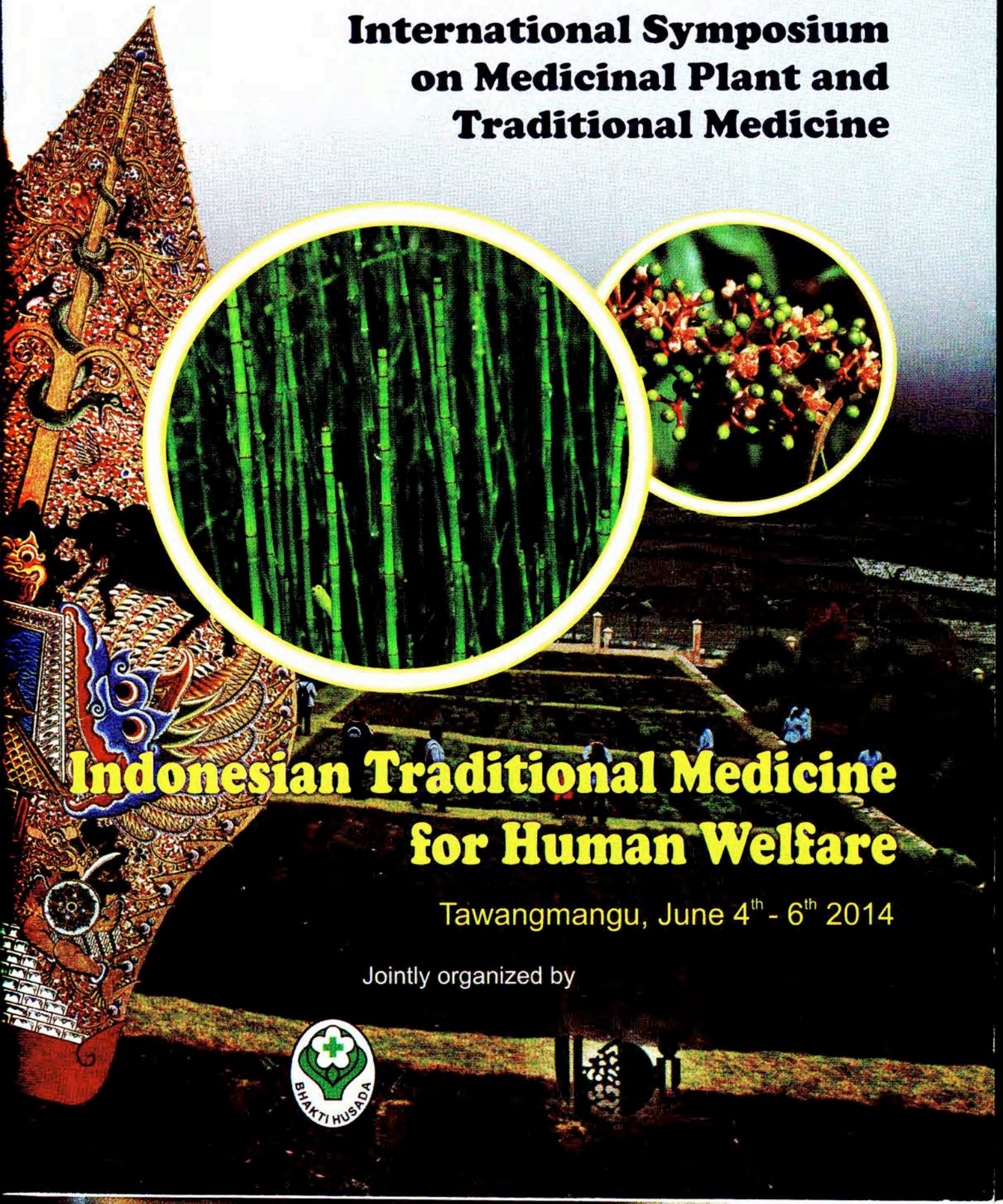
PROGRAM BOOK AND ABSTRACTS

International Symposium on Medicinal Plant and Traditional Medicine



Indonesian Traditional Medicine for Human Welfare

Tawangmangu, June 4th - 6th 2014

Jointly organized by

BHAKTI HUSADA

DAY 2, JUNE 5th 2014
ROOM 2
MEDICINAL PLANT PHYTOCHEMISTRY
Moderator : Dr. Rifatul Wijhati
Assistant : Mery

No.	KODE	TITLE	AUTHOR/s	TIME
1.	O-MPP-009	Analysis of secondary metabolites profile of lempuyang gajah (*Zingiber zerumbet* Smith) ethanol extract using gas chromatography mass spectroscopy with derivatization	Dedi Hanwar, Mutia Sari Dewi, Andi Suhendi, Ika Trisharyanti D.K	13.10 -13.20
2.	O-MPP-013	Optimization of chitosan nanoparticles preparation of rosella (*Hibiscus sabdariffa* L.) calyx extract	Muhammad Ikhwan Rizki, Nurkhasanah, Tedjo Yuwono	13.20 -13.30
3.	O-MPP-012	Purple sweet potato leaves: antioxidant activity by DPPH, CUPRAC, FTC, TBA methods and molecular docking profile using DOCK6	Hidayah Annisa Fitri, Titis Rahayu, Broto Santoso*, Andi Suhendi	13.30 -13.40
4.	O-MPP-016	Stability characterization of β-carotene from ambon banana (*Musa paradisiaca sapientum*) peel: it's potency as vitamin A supplement	Suparmi, Harka Prasetya, Martanto Martosupono and Lasmono Tri S.	13.40 -13.50
5.	O-MPP-018	Antioxidant activity and total phenolic content of bangle (*Zingiber cassumunar* Roxb.) rhizome	Lia Marliani, Winasih R., Anju Sinurat	13.50 -14.00
6.	O-MPP-019	Isolation and characterization secondary metabolites from the root culture of *Morus cathayana* and its toxicity	Ni Luh Putu Yuniantari, Euis Holisotan Hakim	14.00 -14.10
		Discussion		14.10- 14.40
7.	O-MPP-010	Screening of volatile compounds of brotowali (*Tinospora crispa*) and antifungal activity against *Candida albicans*	Warsinah and Harwoko	14.40 -14.50
8.	O-MPP-015	Determination of antioxidant activity using DPPH method to the ethyl acetate fraction of velvet apple (*Diospyros blancoi* A. DC.) leaf methanol extract	Yulio Nur Aji Surya and Yohanes Dwiatmaka	14.50 -15.00
9.	O-MPP-017	Profil radical scavenger and antibacterial activities of stigmasterol and stigmasta-4,22-dien-3-on from stems of *Polygonum pulchrum*	Sahidin, Nohong, Marianti A. Manggau	15.10-15.20
10.	O-MBM-013	In vitro anti fungal activity of essential oil and extract of sirih manado leaves (*Piper betle* L.) againts *Candida albicans*	Nita Supriyati, Fijri Cahyani	15.20–15.30
11.	O-MBM-018	Phytochemical and cytotoxic evaluation of krangean fruits [*Litsea cubeba* (Lour.) Pers.] extracts against cancer cell line	Yuli Widiyastuti, Sari Haryanti, and Elok Widayanti	15.30-15.40
		Discussion		15.40– 16.00

POKJANAS

CERTIFICATE

BAKTI HUSADA

This is to certify that

HIDAYAH ANISA FITRI

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

Secretary General,
National Working Group of Indonesia Medicinal Plant

Indah Yuning Prapti, MPH

Director General,
National Institute of Health Research and Development,
Ministry of Health Republic of Indonesia

Prof. dr. Tjandra Yoga Aditama, Sp.P(K)., DTM&H., MARS., DTCE.

The 46th Meeting of National Working Group on Indonesia Medicinal Plants
INTERNATIONAL SYMPOSIUM
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 : Hidayah Annisa Fitri, Titis Rahayu, Broto Santoso, Andi Suhendi
Asal Institusi : Fakultas Farmasi Universitas Muhammadiyah Surakarta
Judul Artikel : **Purple Sweet Potato Leaves: Antioxidant Activity by DPPH, CUPRAC,FTC, TBA methods and Molecular Docking Profile using Dock6**

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

Purple Sweet Potato Leaves: Antioxidant Activity by DPPH, CUPRAC, FTC, TBA methods and Molecular Docking Profile using Dock6

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Abstract

Purple sweet potato leaves is one of crop plants that has natural antioxidants potency because it contains phenolic and flavonoid compounds. The aim of this experiment was to determine the effect of leaf drying method using direct sunlight 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. All chemical compounds contained in plant was obtained from Universal Natural Product Database (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 of docked molecules demonstrated that they had strong binding affinity with protein 1TDI, 1YVL, 4NOS and 18GS compared to their native ligands. This proves that the content of phenolic and flavonoid compounds from purple sweet potato does have antioxidant activity through multiple lines of mechanisms of action that need to be confirmed in further laboratory testing.

Keywords: Purple Sweet Potato, DPPH, CUPRAC, FTC, TBA, Dock6, Molecular Docking

INTRODUCTION

Purple sweet potato leaves (*Ipomoea batatas* L.) has been widely used as food source in Indonesia. Based on research by Islam (2006), purple sweet potato leaves had higher polyphenol compounds than other vegetables such as spinach, broccoli, cabbage and lettuce. Islam *et al*. (2002) have been successfully identified polyphenol compounds in the purple sweet potato leaves. The polyphenol compounds are anthocyanin and phenolic acid. Anthocyanins are member of flavonoids group, while the phenolic acid is member of phenolic group (Dai and Mumper, 2010). There are 15 types of its anthocyanins compounds, they are are cyanidine and acylated peonidine glycoside. The phenolic acid consists of kaffeic acid and 5 types of caffeoylquinate derivatives, namely 3-mono-O-caffeoylquinic acid (chlorogenic acid, CHA), 3,4-di-O-caffeoylquinic acid (3,4-diCQA), 3,5-di-O-caffeoylquinic acid (3,5-diCQA), 4,5-di-O-caffeoylquinic acid (4,5-diCQA), and 3,4,5-tri-O-caffeoylquinic acid (3,4,5-triCQA) (Islam, 2006). The relative levels of the phenolic acid content starting from the most

numerous are 3,5-diCQA, 4,5-diCQA, chlorogenic acid, 3,4-diCQA, 3,4,5-triCQA, caffeic acid (Islam *et al*., 2002).

Several studies have been investigated methanol, ethanol and water extract of *I. batatas* leaves that grow in some countries such as Malaysia, America, and Croatia. They showed that a total polyphenol compound in it has potent antioxidant activity (Hue *et al*., 2012; Islam *et al*., 2009; Koncic *et al*, 2012). In clinical studies, consumption of *I. batatas* L leaves has proven immune system and antibodies enhanced (Chen *et al*., 2005), decreased lipid peroxidation and DNA damage (Chen *et al*., 2008), reduced the risk of damage caused by oxidative reactions in the body and prevented the secretion of inflammatory pre-cytokines (Chang *et al*., 2010).

There are several factors can affect the antioxidant potential of purple sweet potato leaves. First is a drying method of leaves before they were extracted and tested. In addition to the leaves drying by direct sunlight, there are various methods of drying by using a tool or material that can improve the efficiency and effectiveness of the drying process. Oven, freeze-drying and liquid nitrogen methods are other drying technique, which have advantages over the method of drying by direct sunlight.

The aims of this study was to determine the antioxidant activity of the ethanol extract of purple sweet potato leaves which dried by four different drying methods and its molecular docking profile. They are direct sunlight at room temperature; using an oven at 40^{o}C; freeze drying and liquid nitrogen. The amount of antioxidant activity of four type extracts was analysed by spectrophotometer with reagent of DPPH, CUPRAC, FTC and TBA. CUPRAC and DPPH method used to determine the antioxidant activity of samples against reactive oxidant of DPPH and form CUPRAC complex compound, while the FTC and TBA method used to determine antioxidant activity of the extracts against lipid peroxidation reaction. Molecular docking has been accomplished using Dock 6.

MATERIAL AND METHODS

Molecular docking was carried out using MacBook Pro 13-inch mid 2012 with Mac OS X Mavericks 10.9 (64bits) as operating system. Computational system has been built using MacPorts 2.2.1, XCode 5.1, Chimera 1.8, Dock6.6, OpenBabel 2.3.2, PyMOL 1.7.1.1 and LigPlot+ 1.4.5 for education. Purple sweet potatos leaves (*I. batatas* L.) have been collected from Tawangmangu region, Karanganyar, Jawa Tengah. Materials for antioxidant assay were gallic acid, quercertin, DPPH (2,2-diphenyl-1-picrylhydrazyl), absolute ethanol, ethanol for extraction, oleic acid, iron (II)

chloride, ammonium thiocyanate, Folin–Ciocalteu reagent, phosphate buffer of pH 7, thiobarbituric acid (TBA), CUPRAC reagent, aquadest, freeze dryer, rotary evaporator, spectrometer, micropipette, glassware and analytical balance. Chemical materials were pro analysis grade.

Extraction process was done using maceration method. Total phenolic and flavonoids compounds in ethanol extract were determined according to Singleton *et al*. (1999) and Quettier *et al*. (2000) that has been modified. Phenolic content of the extract is expressed as gram of Gallic acid equivalent (GAE) per gram of extract (g GAE/g extract) while the levels of flavonoids is expressed as equivalent gram of quercetin (QE) per gram of extract (g QE/g extract). Antioxidant activity assay was done using DPPH, FTC and TBA as described by Stanković (2011) and Rezaeizadeh *et al*. (2011) that has been adapted. The antioxidant activity with DPPH expressed as IC_{50}, i.e. the concentration of the extract that can reduce 50% of radical. Potent antioxidant is indicated by small IC_{50} value. Method of FTC and TBA expressed antioxidant activity as percent of inhibition of sample against lipid peroxidation. CUPRAC method has been performed as described by Apak *et al*. (2007) *cit*. Widyastuti (2010) that has been adjusted. The amount of the antioxidant activity of CUPRAC was stated in µmol/g dry Trolox. The greater antioxidant activity of the sample showed with smaller value of result that was required to inhibit oxidation.

Molecular docking has been performed using Dock6 and the result state as Grid Score (kcal/mol). Ligands have been obtained from UNPD database (Universal Natural Product Database at http://pkuxxj.pku.edu.cn/UNPD/). Chimera has been used to prepare protein, ligands and other components that needed as described by Kerrigan at http://www.bioinformatics.iastate.edu/. Docking calculations are divided into four types based gridbox center of protein binding-site pocket and the flexibility of the ligand during the process. The conformation of docked ligands and its interaction with protein residue were generated using PyMOL and LigPlot+.

RESULTS AND DISCUSSION

The plant material, which used in this experiment, had previously been authenticated, so the leaves, which used in this experiment, were indeed the leaves from purple sweet potato plants (*Ipomoea batatas* L.). The leaves were taken from potato plantations in Karanglo, Karanganyar, Jawa Tengah. Differences drying method produces differences leaves characteristic, macerate colour, physical properties of the ethanol extract, drying time, and the manufacture time for each extract. The amount of

extracts produced from different type of dried leaves was different. The difference can be seen in Table 1. This could be happened because the ability of drying method to remove water from leaves is different and time to dry the leaves are different as well. Indirectly, the required time for extractions increase and its efficiency also reduce.

Table 1. The influence of drying method on leaves characteristics, extracts and extraction time.

Drying Method	Drying time (days)	Dry leaves characteristics	Extraction time (days)	Yield (%)	Extract characteristics
Oven 40^{o}C (EA)	4	Green, easy to be crushed	17	23.097	Thick and sticky like taffy, more sticky than extract from leaves dried under direct sunlight, brown
Direct Sunlight (EB)	27	Brown to black, rather tough even though it is dry	26	10.87	Thick and sticky like taffy, brown
Freeze Drying (EC)	2	Green, easy to be crushed	9	25.565	Thick and sticky like taffy, green
Liquid Nitrogen (ED)	<1	Green, easy to be crushed	9	6.902	Thick and sticky like taffy, green to brown

The principal of drying leaves without using heat energy such as freeze-drying was removes water content by means of sublimation in cold temperatures (Hariyadi, 2013; Liu and Rouse, 2005). This is the reason why the drying process becomes faster and can produce a dry leaves without loss its original content than fresh leaves. Heat drying methods such as oven and direct sunlight at room temperature use evaporation principle to remove the water. Heat could make crust on the surface of the leaf that causes to take longer time for water in the leaves could diffuse completely into the environment. This is why dry leaves undergo case hardening (Hariyadi, 2013). Water removal using direct sunlight at room temperature depends on the climate condition. This method has always been chosen even though drying process takes a long time. Water may be still trapped inside leaves. In addition, case hardening and enzymatic process occurred during the drying process that has been proven with brown to black of dry leaves and tough surface.

Dry leaves characteristics apparently also impact appearance of extracts. Based on Table 1, it can be concluded that the “cool” drying method of the leaves can preserve the characteristic of fresh leaves. Heat drying method gave different colour of extract. It can be explained that yield of extract obtained from freeze-drying leaves is the highest one. Dry leaves resulted from liquid nitrogen method has contradictive

yield. Liquid nitrogen only freezes the content of leaves including the water without removing them.

Ethanolic extract from freeze-dried leaves has the highest total flavonoid content, but it has the lowest concentration on phenolic content. On the contrary, ethanolic extract from oven-dried leaves has the highest phenolic content and the lowest one on flavonoid content (Table 2). This result proved that heat could reduce the flavonoid content and increase the phenolic compounds. Cooling system of drying method of leaves provided higher flavonoid content but phenolic content of freeze-dried leaves has 40% lower than liquid nitrogen of drying method.

Non-heated drying prevented the leaves content such as polyphenol compounds and chlorophyll from damage and the solvent could extract the compounds optimally. This is one of reason why yield of extract from liquid nitrogen of drying was the lowest than other because it has the highest total mass of compounds including water itself. Otherwise, total of extracted compounds was the same in quantities. Another premise state that chlorophyll could be there and also extracted.

Table 2. Flavonoid and phenolic content of leaves of purple sweet potato and its antioxidant activity using DPPH, CUPRAC, FTC and TBA.

Drying Method	Flavonoid content (QE g/g extract)	Phenolic content (GAE g/g extract)	DPPH assay (IC_{50}, ppm)	CUPRAC assay in 100 ppm (µmol/g dry Trolox)	FTC assay in 400 ppm (% inhibition)	TBA assay in 400 ppm (% inhibition)
Oven 40°C (EA)	0.467	3.023	93.945	34.326	27.24	78.78
Direct Sunlight (EB)	0.804	1.779	64.525	15.772	8.68	76.03
Freeze Drying (EC)	0.993	0.593	223.988	37.734	35.19	64.34
Liquid Nitrogen (ED)	0.933	1.055	86.418	9.183	42.87	69.82
Vitamin C	-	-	3.434	0.330 (80 ppm)	0	0
Vitamin E	-	-	14.913	0.230	72.17	70,57

Note: Best antioxidant can be showed with the lowest value of DPPH and CUPRAC and the highest value of FTC and TBA.

Previously state that purple sweet potato leaves had antioxidant activity mainly because of flavonoid and phenolic contents. They serve as a hydrogen donor group to radical compounds. Hence the antioxidant activity of the extract depends on flavonoid and phenolic compounds. Antioxidant activity of the extracts was determined using four different methods, i.e. DPPH, CUPRAC, FTC and TBA. In this research, vitamin C and vitamin E were used as positive control.

DPPH is a method to measure the antioxidant activity of a compound directly based on the capability of compound to scavenge radical species (Porkoni, 2001 *cit*. Cholisoh and Utami, 2008). The ethanolic extract from freeze-dried leaves had the lowest antioxidant activity with IC_{50} value of 223.988 ppm. This value increased sequentially are ethanolic extract from oven dried-leaves (93.9455 ppm), ethanolic extract from frozen leaves by liquid nitrogen (86.418 ppm) and ethanolic extract resulted from dried leaves with drying method using direct sunlight at room temperature (64.525 ppm), while vitamin C and vitamin E have smaller IC_{50} value of 3.4346 ppm and 14.913 ppm, respectively (Figure 1).

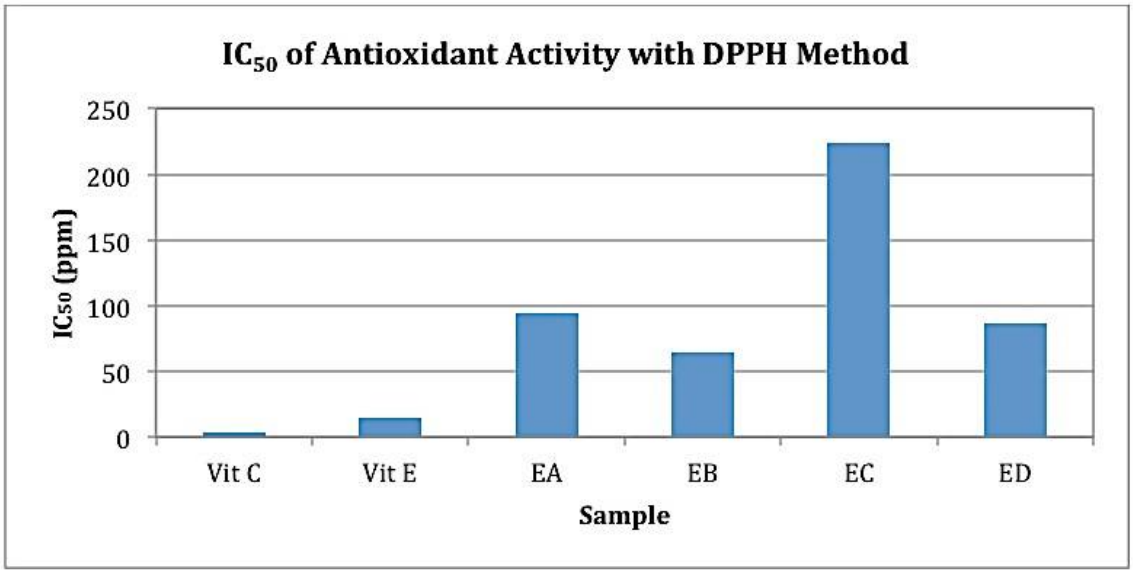

Figure 1. Result of antioxidant assay with DPPH method. Vit C = Vitamin C, Vit E = Vitamin E, EA = ethanolic extract from direct sunlight of dried leaves at room temperature, EB = ethanolic extract from dried leaves by oven at 40^{o}C, EC = ethanolic extract from dried leaves by freeze drying and ED = extract from dried leaves by liquid nitrogen.

The principle of CUPRAC assay is similar to the DPPH method, i.e. stabilization of reactive oxidants CUPRAC-$CuSO_4$ complex with hydrogen groups of flavonoids and phenolic compounds. The results showed that the order of concentration profiles µmol/g dry trolox required by the control and extract at a concentration of 100 ppm (80 ppm vitamin C) to inhibit oxidation almost had the same profile as the DPPH method. Vitamin E, vitamin C, ethanolic extract from frozen leaves by liquid nitrogen, ethanolic extract from direct sunlight at room temperature of dried leaves, ethanolic extract from oven dried leaves and ethanolic extract from freeze-dried leaves have CUPRAC value of 0.230, 0.330, 9.183, 15.772, 34.326 and 37.734 µmol/g dry trolox, respectively. It can be concluded that ethanolic extract from frozen leaves by liquid nitrogen has the highest antioxidant activity. The antioxidant activity of vitamin C decreased probably

due to the presence of buffer that reacts with vitamin C so the amount of vitamin C involved in CUPRAC reaction reduced.

FTC and TBA are methods for measuring the antioxidant activity based on inhibition of lipid peroxidation. Absorbance profile is used to determine when the maximum absorbance vs. days reached. Oleic acid is used as fatty acid in this experiment and the positive control used are vitamin C and E, while fatty acid emulsion without using antioxidant compounds is used as negative control. Results showed that the highest absorbance was on the fifth day and dropped on the sixth day (Figure 2).

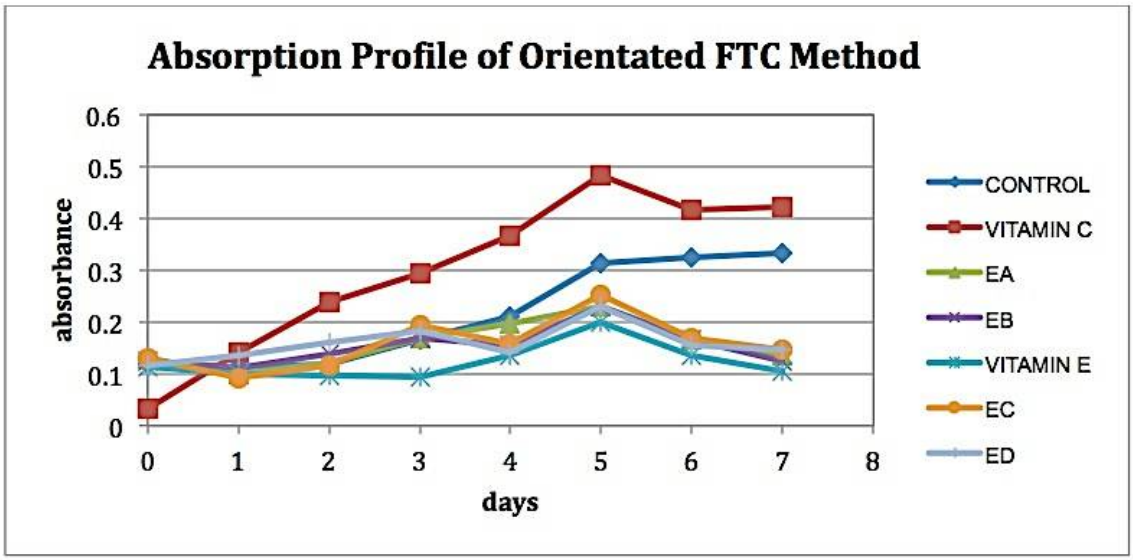

Figure 2. Absorption Profile of Orientated FTC. EA = extract from direct sunlight dried leaves at room temperature, EB = extract from dried leaves by oven at 40^{o}C, EC = extract from dried leaves by freeze-drying and ED = extract from dried leaves by liquid nitrogen.

FTC method used to calculate extracts and standard inhibition against peroxide compound of fat that forms on the day, which showed the maximum absorbance of the sample. TBA method used to calculate extracts and standard compounds against second derivatives of fat peroxide, malonaldehyda compounds, which are formed on the day after the maximum absorbance of the sample. The inhibition percentages of EA, EB, EC, and ED at concentration of 400 ppm against peroxide fat in order are 27.24; 8.68; 35.19 and 42.87 %. Inhibition values (in percentage) of EA, EB, EC, and ED at same concentration against malonaldehyde are 78.78; 76.03; 64.34; and 69.82 %. The antioxidant activity was detected using TBA method is higher than the FTC caused peroxides which formed in the early stages of lipid peroxidation had smaller quantities than its derivative compounds which formed in the second stage

(malonaldehyde) and probably, this compounds were more stable at the same time (Aqil *et al*., 2006).

The standard compounds which gave antioxidant value with the FTC and TBA was only vitamin E, and the results are 72.17 % and 70.57 %, respectively. Vitamin C gives 0 % inhibition against lipid peroxidation. Vitamin C was damaged during the incubation process at a temperature of 40^{o}C. Vitamin C is a thermo-labile compounds and its degradation increase proportionally to the temperature rise (Hartigan-Go, 1996) The continuous heating during method incubation caused oxidation of vitamin C. Kinetic reaction of vitamin C oxidation at a temperature of 40°C followed first order reaction with a reaction rate constant of 4.55 x 10^{-4}/min (Rahmawati and Bundjali, 2012). Vitamin C supposed to be an antioxidant and reduces formed peroxide but in the fact it becomes a reactive oxidant due to oxidation. This can be seen from the higher colour intensity of the emulsion solution of vitamin C than the control and extract when reacted with the FTC and TBA reagent.

The previous evidence and this result proved that phenolic compounds in the leaves have responsibility for its antioxidant activity. However, based on the results of this studies, it can not be known yet what compound (flavonoids or phenolic) which responsible for the antioxidant activity in extracts. The possibility is flavonoids and phenolic compounds have antioxidant synergistic mutual support effect in donating a hydrogen atom to stabilize the radical compounds. The correlation between total flavonoids and phenolic content in extract with its antioxidant activity can only be proven by DPPH assay, whereas CUPRAC, FTC and TBA assay are not known yet.

The various amount of antioxidant activity of four kinds extracts, affected by leaves drying methods. The conditions of research and other reactive oxidant species that used also affecting the results. DPPH antioxidant assay is a method, which has easiest way to keep the stabile condition during the research because it only needs to be kept out from the light. The antioxidant process on CUPRAC, FTC and TBA methods need special conditions such as pH and temperature to maintain oxidation process. However, is quite difficult to provide a stable condition due the requirement in laboratory and many factors that cannot be controlled by researchers even. That is the reason why the antioxidant assays of extracts have various results.

There were 36 compounds that have been collected from UNPD database (Universal Natural Product Database). They must be including of phenolic or flavonoid groups. Molecular docking should be validated to the native ligand from each protein.

The validation results of six proteins with their native ligands showed that the 3D conformation between crystallography data with native ligand of docking results was found to be similar position from one to another cause their RMSD value satisfied the requirement.

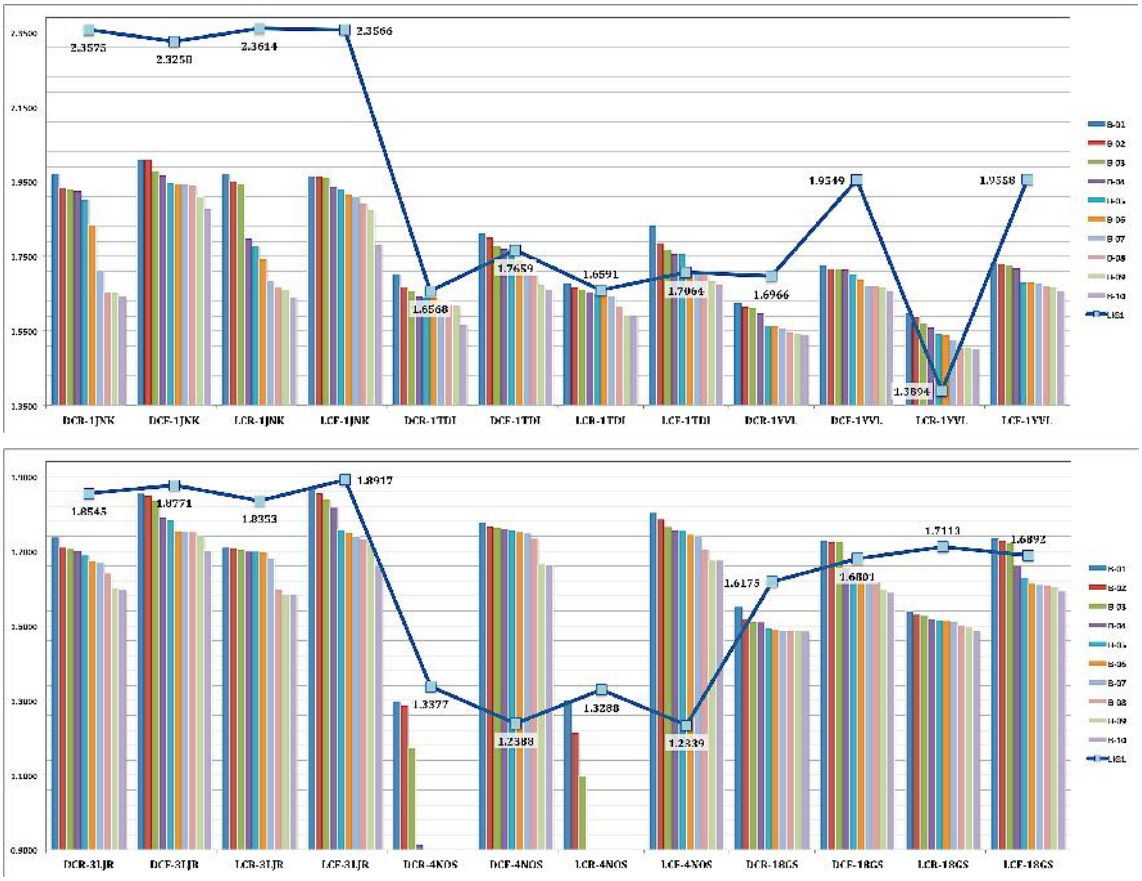

Figure 3. Result of the top ten of docked ligands for six protein represent as –Log (Grid Score) value and compare to dock result of native ligand (blue solid line) for each type of gridbox centre. Result of rigid ligands (DCR) and flexible ligands (DCF) that obtained based on default Dock6 calculation for gridbox and result of rigid ligands (LCR) and flexible ligands (LCF) using ligand centre for gridbox.

The top ten of docked molecules demonstrated that they had strong binding affinity with protein 1TDI, 1YVL, 4NOS and 18GS compared to their native ligands (Figure 3). Various compounds have been gathered from best ten ligands of molecular docking from each protein. There were three compounds, namely 3,5-diCQA, cynarine and 3,4-diCQA that lied on the top of binding affinity more often with protein target. Cynarine has hydrogen bond and hydrophobic interaction with Arg42 and Phe217 of protein 1TDI, respectively (Figure 4). As an example, beta-D-fructofuranosyl-(2->1)-alpha-D-[2-O-L-hystidyl]glucopyranoside has the same interaction with protein, i.e. hydrophobic interaction (1YVL:Trp588, 1YVL:Asp596 and 1YVL:His598) and hydrogen

bonding with protein residue of 1YVL:His598, 4NOS:HOH424 and 4NOS:HEM422. Protein of 18GS has only hydrogen bond interaction with 3,4-diCQA, such as Arg12, Cys100, HOH212 and HOH216.

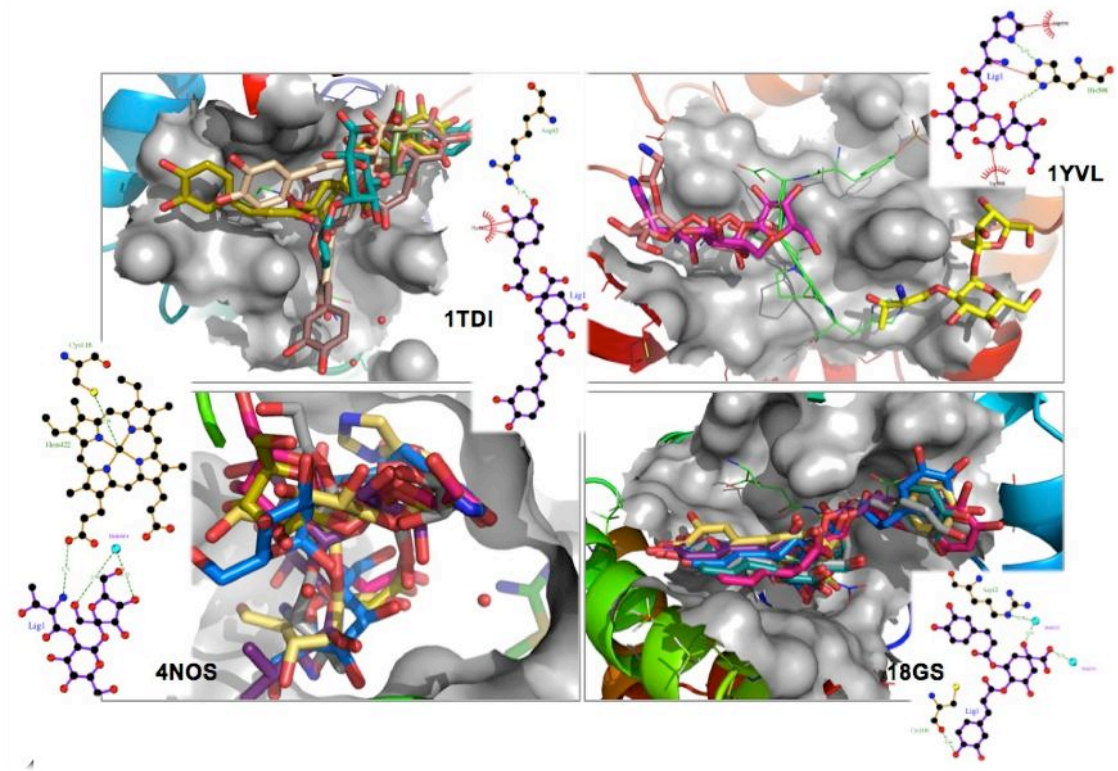

Figure 4. Docked ligands with the best three of Grid Score, ligands were various (drawn in sticks) from eucalyptus compared to native ligand (drawn in lines). White box represent chemical interaction ligand-protein residue.

CONCLUSION

Based on this study, purple sweet potato leaves has potential as a natural antioxidant that need further verification research. Molecular docking also proves that the phenolic and flavonoid content from purple sweet potato does have antioxidant activity through multiple lines of mechanisms of action that needed to be confirmed in further laboratory testing.

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