The First PSU and UAD Joint Seminar on Pharmaceutical Sciences

"Building Collaboration on Herbal Medicine Innovation"

Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, Thailand

7th November 2013

Scientific Program
The First PSU and UAD Joint Seminar on Pharmaceutical Sciences
"Building Collaboration on Herbal Medicine Innovation"
Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Thailand
7th November 2013

08.30 - 09.00	Registration
09.00 - 09.15	Opening Remark *Dean of Faculty of Pharmaceutical Sciences / Prince of Songkla University*
Chairperson:	Juraithip Wungsintaweekul
09.15 - 09.35	Synergistic interaction of polysaccharide mixtures investigated by rheology, DSC and NMR for sustained drug delivery system *Vimon Tantishaiyakul / Prince of Songkla University*
09.35 - 09.55	The Comparison of Antibacterial Activity of Kefir Cow's Milk and Kefir Goat's Milk against *Escherichia coli* *Dwi Suhartanti / Universitas Ahmad Dahlan*
09.55 - 10.15	The Minimalization of Ulcerogenic Effect of Mefenamic Acid by Solid Dispersion Formation of Mefenamic Acid-Hydroxypropyl Methylcellulose *Iis Wahyuningsih / Universitas Ahmad Dahlan*
10.15 - 10.40	Coffee break
Chairperson:	Kamonthip Wiwattanawongsa
10.40 – 11.00	Fruit Kimchi Indonesian Taste Fajar Satria/*Universitas Ahmad Dahlan*
11.00 - 11.20	Preparation of brazilin rich extract from *Caesalpinia sappan* heartwood and its antioxidant and antibacterial activity *Nilesh Prakash Nirmal / Prince of Songkla University*
11.20 - 11.40	Analytical Method Validation Calcium Pantothenate in Multivitamin Tablet Use Bacteria *Lactobacillus plantarum* with Turbidimetric Method *Muharom Eko Prasetyo / Universitas Ahmad Dahlan*
11.40 – 12.00	Can mucosal innate immunity be enhanced by plant-derived compounds? *Aornrutai Promsong and Surada Satthakarn / Prince of Songkla University*
12.00 - 13.00	Lunch
Chairperson:	Chatchai Wattanapiromsakul
13.00 - 13.20	Effect of Bengkuang (*Phichyrhizus erosus*) Extract Concentrations on *Aedes aegypti*'s Larvae *Nani Aprilia / Universitas Ahmad Dahlan*
13.20 – 13.40	Quantitative HPLC analysis of chamuangone in *Garcinia cowa* leaf extracts *Pharkphoom Panichayupakaranant / Prince of Songkla University*

13.40 – 14.00	Antioxidant Activity as Free Radical Scavenger of Green Algae (*Spirogyra* sp.) Ethanolic Extract Using DPPH method *Nina Salamah / Universitas Ahmad Dahlan*
14.00 - 14.20	Antibacterial Compounds from *Artocarpus heterophylus* Heartwood *Abdi Wira Septama / Prince of Songkla University*
14.20 - 14.40	Antibacterial Activity of Aqueous Extract of *Stelecarpus burahol* leaves *Novi Febrianti / Universitas Ahmad Dahlan*
14.40 - 15.00	Time Optimization of Antibiotic Production of Actinomycetes Isolates (P301 and P302) Based on Their Antibacterial Activity against *Staphyllococcus aureus* and Bioautography Profile Nanik Sulistyani / *Universitas Ahmad Dahlan*
15.00 - 15.20	Coffee break
Chairperson:	Pharkphoom Panichayupakaranant
15.20 - 15.40	Green Extraction of rhinacanthin-C from *Rhinacanthus nasutus* leaves *Karun Shakya / Prince of Songkla University*
15.40 - 16.00	Effect of Turmeric (*Curcuma longa* Linn.) Rhizome Standardized Extract on Brain Malondialdehyde Level in Trimethyltin Induced Dementia of Rat *Sapto Yuliani / Universitas Ahmad Dahlan*
16.00 - 16.20	Antioxidant and estrogenic activities of commercial remedies for woman in Thailand *Athip Sakunphueak / Prince of Songkla University*
16.20 - 16.40	The Effect of Giving Dengue Fever Booklet on the Level of Knowledge and Practice in the *Ae. aegypti*'s Breeding Places Eradication on the Women in Sorosutan Village of Yogyakarta *Tri Wahyuni Sukesi / Universitas Ahmad Dahlan*
16.40 - 16.50	Anticancer Compounds in Ant Plants (*Myrmecodia pendans*) *Aminatun Wakhidah / Universitas Ahmad Dahlan*
16.50 - 17.00	Anticancer Compounds in Some Species of Sponges *Rizky Restu Romadhona/ Universitas Ahmad Dahlan*

Antioxidant Activity as Free Radical Scavenger of Green Algae (*Spirogyra* sp.) Ethanolic Extract Using DPPH method

Nina Salamah[1], Wahyu Widyaningsih[2], Innayah Izati[3]
[1]Departement of Analytical Chemitry, Pharmacy Faculty, Ahmad Dahlan University, Indonesia
[2]Departement of Pharmacology, Pharmacy Faculty, Ahmad Dahlan University, Indonesia
[3]Pharmacy Faculty, Ahmad Dahlan University, Indonesia
E-mail: syifaniputri@yahoo.com

Introduction:
Antioxidants are compounds that can inhibit the oxidation reaction, to scavenge free radicals and highly reactive molecules. Green algae is the largest division consisting of more than 500 genera and about 15,000 species. *Spirogyra* sp. is an example of species that lives in freshwater. This study aims to determine the antioxidant activity as scavenger free radicals of the ethanol extract of green algae *Spirogyra* sp. by using DPPH (1,1-diphenyl-2-pikrilhidrazil).

Materials and Methods:
Ethanol extract prepared from the powder of green algae *Spirogyra* sp. by maceration method. Trials of antioxidant activity in DPPH visible spectrophotometry. Gallic acid used as a positive control. The free radicals scavenging expressed with ES_{50}. Based on the data, ES_{50} ethanol extract of *Spirogyra sp and gallic acid* were analyzed with SPSS 16.0 correlation.

Results:
The result showed that ethanolic extract of *Spirogyra* sp. have activity as scavenger free radicals. The ES_{50} value of *Spirogyra* sp. was (92,83 ± 0,58) µg/ml and then the positive control, gallic acid was (0,73 ± 0,04) µg/ml.

Conclusion:
Based on statistical analysis, the ES_{50} value of gallic acid and ethanolic extract of *Spirogyra* sp. are significantly difference.

Keywords: *Spirogyra*, green algae, gallic acid, DPPH, antioxidant

Antioxidant ACTIVITY AS FREE RADICAL SCAVENGER OF GREEN ALGAE EXTRACT Ethanolic DPPH *Spirogyra sp*METHOD USING

Nina Salamah, Wahyu Widyaningsih, Hari Susanti, Innayah Izati
Faculty of Pharmacy, University of Ahmad Dahlan Yogyakarta
Email: syifaniputri@yahoo.com

ABSTRACT

Antioxidants are compounds that can inhibit the oxidation reaction, to scavenge free Radicals and highly reactive molecules. Green algae is the largest division Consisting of more than 500 genera and about 15,000 species. Spirogyra sp is an example of s pecies that lives in freshwater. This study aims to Determine the antioxidant activity as scavenger free Radicals of the ethanol extract of green algae Spirogyra sp by using DPPH (1,1-diphenyl-2-pikrilhidrazil).

Ethanol extract prepared from the powder of green algae Spirogyra sp by maceration method. Trials of antioxidant activity in the DPPH visible spectrophotometry. Gallic acid used as a positive control. The free Radicals scavenging Expressed with ES_{50}. *Based on the data, the* ES_{50} *ethanol extract of* Spirogyra sp and gallic acid *were Analyzed with SPSS 16.0 correlation.*

The result Showed that Ethanolic extract of Spirogyra sp have activity as scavenger free Radicals. The ES_{50} *value of Spirogyra sp was (92.83 ± 0.58) mg / ml and then the positive control, gallic acid was (0.73 ± 0.04) mg / ml.*

Based on statistical analysis, the ES_{50} *value of gallic acid* and *Ethanolic extract of Spirogyra sp are Significantly difference.*

Keywords: Spirogyra sp, Green algae, gallic acid, DPPH, antioxidant

Introduction

Free radical is one of the causes of cell or tissue damage which causes the degenerative disease, autoimmune disease and cancer (Sadikin, 2001). Free radicals are formed in the body due to side products of metabolic processes or because of exposure through inhalation and spread throughout the body.

The human body has a system of antioxidant to ward off free radical reactivity that continuously formed by the body. The damage caused by free radicals is essentially caused by the amount of reactive oxygen compound exceed the amount of antioxidant in the body (Winarsi, 2007). The damage can be prevented if there is supply of exogenous antioxidant. By the acquisition source there are two kinds of antioxidants namely natural antioxidant and synthetic antioxidant (Dalimartha dan Soedibyo, 1999). Synthetic antioxidants such as Butyl Hydroxy Aniline (BHA), Butyl Hydroxy Toluene (BHT) and Propyl Galat have it drawbacks it caused exposure time can increase the occurrence of live damage and carcinogenic.

Weaknesses that contained in synthetic materials should be reduced to finding a natural antioxidant compound with high activity and side effects as low as possible (Pourmorad dkk, 2006). Natural ingredients are the main source of natural antioxidant compounds that contain different antioxidants such as carotenoids, vitamins, phenolic compounds, flavonoids, glutathione, and endogenous metabolite that function as antioxidants singlet and triplet, anti dot to free-radical, and enzyme inhibitors (Zhang dan Hamauzu, 2004).

Melatonin and phenolic compounds have antioxidant activity. The Antioxidant counteract directly the oxygen radical and nitrogen such as superoxide ($\bullet O_2$), hydroxyl (•OH), and nitric oxide (•NO). The chemical content of green algae are melatonin compound (Hardeland, 2003), carotenoid, phenolic compounds, *phycobiliprotein* pigment, polysaccharides, and unsaturated fatty acids (Shalaby, 2011).

So far, the use of algae as a trade commodity or raw industry material is still relatively small when compared to the diversity of algae that exist in Indonesia. Whereas chemical component contained in the algae is very beneficial to the food industry raw material, cosmetic, pharmaceutical and others (Putra, 2006). Therefore green algae is require to optimization the function.

There are various species of green algae that exist in Indonesia. The habitat differences led to the differences in the antioxidant activity of green algae species. *Spirogyra sp* is example of species which is live in freshwater. Therefore, it is necessary to provide activity test the antioxidant ethanol Extract of *Spirogyra sp* with DPPH method. This method is simple, fast, and easy to activity screening of radical capturing of some compounds, in another hand this methods was proved to be accurate, reliable and practical (Prakash *et al.,* 2001).

Research Method

Material and Equipment

Spektrofotometer UV-Vis (Shimadzu UV PharmaSpec 1700). *Spirogyra sp* which was obtained from Ketandan Village, North Klaten, on March *2013,* the melatonin standard (Calbiochem), gallic acid standards (Sigma), tanat acid standard (Merck), quercetin standard (Merck), ethanol pa (Merck), and DPPH (Sigma).

Research Procedure

Main Ingredient Preparation

Green algae *Spirogyra sp* was put in a container that filled with water and allowed to stand for 12 hours of irradiation phase and 5 hours in the darkness phase. Then was dried with a dryer cupboard for 46 hours at a temperature of 40° -50° C. Green algae that has been dried proceed become a powder and then was sieved with a sieve number 40 to obtain a uniform powder size.

Determination of Moisture Content

Water content of Green algae powder was determined by *Moisturizer Halogen Analyzer* tool.

Manufacturing of Ethanol Green Algae *Spirogyra sp* Extract

Total of 250.0 grams of powder was vut into the petroleum ether. In the next step the powder was macerated with ethanol for 24 hours, and filtered with a Buchner funnel and the filtrate obtained was concentrated by *vacuum rotary evaporator.*

Determination of Drying Extract

2.5 grams of extract was brought into the crucible. The crucible which was already contains extract was heated in an oven at 110°C for 2 hours to obtain a constant weight. After completion of the process, then the crucible was inserted into eksikator, was allowed to cool and then weighed.

Determination of Extract Ash Levels

2.5 grams of extract was brought into the crucible. Porcelain crucible was inserted in a furnace, and then heated at 600° C for 8 hours to ashes.

The Qualitative Test for Antioxidant Compounds

Polyphenols test

Number of samples were dissolved in 96% ethanol, were added $FeCl_3$ 1% as much as 3 drops when color green, blue or black arise it shows the presence of polyphenols.

Alkaloid test

Number of samples were dissolved in 96% ethanol, were added 3 drops of Dragendorff reagent. If using Dragendorff reagent it will form precipitate with brownish orange color, it means the extract has alkaloids contain.

Flavonoids test

0.5 ml of sample solution was dropped on filter paper and allowed to dry. The dried droplet was steamed by ammonia. The color was change from pale yellow to intense yellow it means they have flavonoids.

Tannins test

Numbers of samples were dissolved in 96% ethanol, the solution was added a solution of 2% NaCl (1 ml), if there is sediment then filtered through filter paper. The filtrate was added 1% (5 ml) gelatin solution when there is sediment it means it has the tannin or tannin substance.

Capturing Activities of Free Radical Test by Quantitative and DPPH Method

Determination of *Operating Time*

Respectively of 1.0 ml of sample solution of ethanol *Spirogyra sp* extract and control positive Gallic acids were added with 1.0 ml DPPH 0.15 mM. Absorbance was observed for 0-120 min at a wavelength of 517 nm.

Determination of the wavelength of the maximum absorbance

Respectively of 1.0 ml of sample solution of ethanol *Spirogyra sp* extract and positive control of Gallic acid 1 mg / ml were added 1.0 ml DPPH 0.15 mM. It was kept in the dark until *the Operating Time,* and then absorbance was observed at a wavelength of 450-650 nm. Determination of the wavelength of maximum absorbance was also performed for negative control.

Measurement of absorbance of the solution

1.0 ml of each samples solution and the positive control solution of various concentrations of Gallic acid were added with 1.0 ml of DPPH solution, the solution was then stored in a dark place until *the Operating Time.* The measurement absorbance was read

at *Operating Time* and wavelength at maximum absorbance which has been obtained, as a blank was used the absolute ethanol p.a.

Data Analysis

The absorbance data was calculated for the percentage of capture of free radical. Percentage data of free radical captured which had obtained and concentrations of compound test, then was followed by made a linear regression equation to determine the price of ES_{50} *(effective scavenging)* which is the concentration of test compound that is capable for capturing free radicals by 50%. From Price of ES_{50} was analyzed statistically with normality test and homogeneity of data with 95% confidence level.

Results and Discussion

The powder of green algae which had experience on the drying process is necessary to define the water content in order to determine the water content in the powder. The quality requirement of powder water content should not be more than 10% (DepKes RI, 1995). The moisture content *of Spirogyra sp* was of 7.09%. Water contents of each powder had qualified for the moisture content which was no more than 10%.

Determination of extract drying shrinkage has aim to allow the maximum limits (range) of the amount of the compound which was lost in the drying process. The drying of extract shrinkage should not be more than 10% (DepKes RI, 2008). According the research result, the drying shrinkage of *Spirogyra sp* extract was 9.59%. All drying shrinkage of each extracts had qualified for the quality of the drying shrinkage of extract which was no more than 10%.

Determination of ash content extracts was aim to provide an overview of the internal and external mineral content that derived from the initial process up to the extract constructed. The value or ranges that allowed which is associated with purity and contamination (Emilan et al, 2011). Ash content *of Spirogyra sp* extract was 6.52%.

Table 1. Qualitative results Test of the antioxidant compounds

Qualitative test	Sample	Reagent	Theory	Result	Conclusion
Polyphenols	Error acid	FeCl3	complex green, purple, blue or solid black	blue-black complex	+
	Spirogyra sp			blackish green complex	+
Alkaloids	Melatonin	Dragendorff	brownish orange precipitate	brownish orange precipitate	+
	Spirogyra sp			brownish orange precipitate	+
Flavonoids	Quercetin	Ammonia vapor	intensive yellow	intensive yellow	+
	Spirogyra sp			no color change	-
Tannins	Acid Tannates	2% NaCl 1% gelatin	E ndapan	white precipitate	+
	Spirogyra sp			green precipitate	+

For the testing of capturing free radical activity of green algae *Spirogyra sp* extracts was performed by using DPPH method. The consideration of the method was because the preliminary test with the capturing activity of free radical was used DPPH reagent; the sample was able to capture the DPPH free radical which was marked with a reduction of DPPH purple color. The DPPH method is one of the most common methods that used to evaluate the antioxidant activity, particularly for phenol compound or polyphenol. (Yamaguchi et al, 1998). The reduction on the intensity of the color was due to reacting of radical molecules 1,1-diphenyl-2-pikrilhidrazil with one of hydrogen atom that was released by the sample so that it was constructed the 1,1-diphenyl-2- pikrilhidrazin compound with yellow stable. The phenolic compound which was contained in the sample was loss of H atom it will be forming as a new free radical which is stable and not reactive because of the resonance effect of the aromatic core.

The early stage of testing compounds by using visible spectrophotometry was to determine the operational time *(operating time)*. At the time of operation was showing that the optimal DPPH had reacted with the test compound. Base on the study, the Gallic acid showed a steady uptake in the minute 83 up to minute 97, and a solution of ethanol *Spirogyra sp* extract in the minute 52 up to minute 75.

The next stage was the determination of the maximum absorbance wavelength. The wavelength of maximum absorbance that used was the wavelength of maximum absorbance of DPPH negative control, because in principle the absorbance which was measured was the absorbance of DPPH solution which did not react with antioxidant compounds.

Theoretically the wavelength of absorbance for DPPH solution is 515-517 nm. Base on the study, it was obtained the wavelength of maximum absorbance DPPH of positive control for Gallic acid with 1 mg / ml concentration, the ethanol *Spirogyra sp* extract with a 50 ug / ml concentration, respectively were 515.4 nm; 515.6 nm.

In testing of the free radical activity of negative control was always read the absorbance before reacting DPPH by sample and the positive control. This was because to avoid shifting the DPPH absorbance values due to the effect of storage and other influences. In addition, the negative control value will affect to the calculation of percent capture of free radicals. DPPH concentration used was 0.15 mM with absorbance values for antioxidant activity test positive of control Gallic acid, ethanol *Spirogyra sp* extract respectively 0.812; 0.796. Test solution with the concentration of each was added a solution of 0.15 mM DPPH allowed to stand in a dark place during operating time, and then was tested the activity of free radicals capture on the wavelength of maximum absorbance of the negative control. The comparison of the amount of DPPH solution and test solution was 1:1 (v / v).

To compare the capture activity of free radical DPPH between positive control Gallic acid and ethanol *Spirogyra sp* extract was used a potential parameter of capture of free radical activity. The activity parameter of Capture of free radical that used was ES_{50}.

Table I I. Percent capture of free radical and ES_{50} Gallic acid value

No.	Capture Activities of Free Radical DPPH (%)						R count	Linear Regression Equation	ES_{50} (ug / ml)
	0.25 ug / ml	0.50 ug / ml	0.75 ug / ml	1.00 ug / ml	1.25 ug / ml	1.50 ug / ml			
1	35.22	39.90	53.32	56.28	66.38	75.37	0.9911 *	x + y = 32.3600 26.0967	0.74
2	33.87	45.57	51.72	57.88	65.52	75.12	0.9948 *	x + y = 31.1154 27.7207	0.72
3	35.71	44.70	51.48	57.76	66.38	74.26	0.9988 *	x + y = 30.1794 28.6413	0.71
4	33.25	37.93	50.25	58.00	65.15	73.40	0.9956 *	x + y = 33.1611 23.9807	0.78
5	33.87	45.81	51.48	57.27	65.15	75.25	0.9952 *	x + y = 30.9383 27.7340	0.72
Mean ± LE									0.73 ± 0.04
CV									4.11%

* R count> R table

Table III. Percent capture of free radical and *ES* $_{50}$ ethanol *Spirogyra sp* extract value

No.	Capture Activities of Free Radical DPPH (%)					R	Linear Regression Equation	*ES* $_{50}$ (ug / ml)
	25 ug / ml	50 ug / ml	75 ug / ml	100 ug / ml	125 ug / ml			
1	20.23	32.04	41.08	52.76	65.45	0.9985 *	y = 0.4446 x + 8.964	92,30
2	19.35	31.53	41.96	52.14	64.95	0.9991 *	y = 0.4472 x + 8.443	92.93
3	20.23	32.66	41.21	52.39	65.20	0.9981 *	y = 0.4386 x + 9.437	92.48
4	19.47	32.04	41.33	52.01	64.45	0.9988 *	y = 0.4397 x + 8.881	93.52
5	19.97	31.66	42.08	52.01	64.82	0.9991 *	y = 0.4402 x + 9.093	92.93
Mean ± LE								92.83 ± 0.58
CV								0.51%

* R count> R table

Table above can be seen that the greater the concentration, causes the greater the percent of free radical DPPH captured. Percent of the data capture of the radical DPPH at different concentrations can be calculated the value of *the ES* $_{50}$. *ES* $_{50}$ average positive control of Gallic acid and ethanol *Spirogyra sp* extract were respectively 0.73 ug / ml and 92.83 ug / ml.

ES $_{50}$ value inversely proportional to the ability of compound to capture free radical DPPH, the smaller *ES* $_{50}$, the greater the ability of a compound to capture free radical by 50%.

Data of *ES* $_{50}$ positive control Gallic acid, ethanol *Spirogyra sp* extract then were analyzed statistically with 95% confidence level. statistical test showed a significant difference between an capturing of free radical between positive control of Gallic acid and ethanol *Spirogyra sp* extract therefore they have a difference of antioxidant activity.

Conclusion

Capture free radical activity of ethanol Spirogyra sp extract was weaker than Gallic acid indicated by ES50 price of ethanol Spirogyra sp extract (152.19 ± 6.11) ug / ml was greater than Gallic acid (3.66 ± 0.43) ug / ml. There was a significant ES50 price difference between the Spirogyra sp, and Gallic acid

Bibliography

DepKes RI[b], 1995, *Materia Medika Indonesia,* Jilid VI, Departemen Kesehatan RI, Jakarta.

DepKes RI, 2008, *Farmakope Herbal Indonesia*, Jilid I, Departemen Kesehatan RI, Jakarta.

Emilan, T., Kurnia, A., Utami, B., Diyani, L.N., Maulana, A., 2011, Konsep Herbal Indonesia: Pemastian Mutu Produk Herbal, *Laporan*, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Indonesia, Jakarta

Hardeland, R., and Poeggeler, B., 2003, Non-vertebrate melatonin, *Journal of Pineal Research*, 34: 233-241

Prakash, A., 2001, Antioxidan Activity, *Medallion Laboratories Analytical Progress*, 19(2): 1-6

Pourmorad, F., Hosseinimehr, S, J., Shahabimajd, N, 2006, Antioxidant Activity, Phenol and Flavonoid Contents of Some Selected Iranian Medical Plant, *African Journal of Biotechnology*, 5 (11): 1142-1145

Putra, S.E., 2006, *Alga Laut sebagai Biotarget Industri*, Chem-is-try diakses tanggal 5 Maret 2013

Sadikin, M., 2001, *Pelacakan Dampak Radikal Bebas terhadap Makromolekul*, Jakarta, Fakultas Kedokteran Universitas Indonesia

Shalaby, E.A., 2011, Algae as Promising Organisms for Environment and Health, *Landes Bioscience*, 6(9): 1338-1350

Winarsi, Heri., 2007, *Antioksidan Alami & Radikal Bebas*, 11-23; 189 Kanisius, Yogyakarta

Yamaguchi, T., Takamura, H., Matoba, T., dan Terao, J., 1998, HPLC Method for Evaluation of Free Radical-scavenging Activity of Food by Using 1,1-diphenyl-2-picrylhidrazyl, *Bioscience, Biotechnology, and Biochemistry*, 62: 1201-1202

Zhang, D, Hamauzu, Y., 2004, Phenolic Compounds and Their Antioxidant Properties an Different Tissue of Carrots (Daucus carota L), *Food, Agriculture & Environment*, 2(1): 95-100