# PROCEEDING



Faculty of Pharmacy UGM Yogyakarta Indonesia October 2009







## PROCEEDING

# The International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

# **Editors** :

Pudjono Hilda Ismail Ronny Martien Triana Hertiani Ritmaleni

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ii

### CONTENTS

Preface from the Editor	i
Organizing Committee	ii
Welcome Message Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences	
From the committee	111
Remark of the Dean Facultyi	v
Senior Vice Rector For Education	V
CONTENT	vi
Pharmacogenetics : in case of cytochrome P450 oxidases (CYPS) related to adverse drug reactions Arum Pratiwi, Harianto Lim and Ronny Martien	1-4
Interaction of turmeric and garlic extract combination against free radical scavenging activity Patonah, Daryono H. Tjahjono, Elin Yulinah S. and I Ketut Adnyana	5 – 6
Influenced of Kojic Acid and B-Cyclodextrin on SPF Value Sunscreen Product Contained Oxybenzone and Octyl Dimetyl Paba (3:7) (In vanishing cream base formulation) Diana, Tristiana Erawati, Widji Soeratri and Noorma Rosita	7 – 14
Isolation and Antimicrobial activity of endophytic fungi Kabatiella caulivora var B isolated from Alyxia reinwardtii BL Noor Erma Sugijanto, Dian Anggraeny and Noor Cholies Zaini	15 – 17
Rapid and Simple Luciferase Reporter Gene Assays for the Discovery of Peroxisome Proliferator-Activated Receptor $\alpha$ and $\gamma$ Agonists and Nuclear Factor- $\kappa$ B Inhibitors from Medicinal Plants. <b>N. Fakhrudin, S. Vogl, P. Picker, E. H. Heiss, J. Saukel, G. Reznicek, B. Kopp, A. G. Atanasov</b> and V. M. Dirsch	18 – 24
Identification of components of essential oil from <i>Cananga odorata</i> which penetrated into the rat skin /(wistar strain) in the practice of <i>Timung</i> (development of <i>Timung</i> as alternative healing) Mangestuti Agil, Esti Hendradi and Budiastuti	25 – 29
In Vivo Antihyperglycemic Test of Albedo Durian ( <i>Durio zibethinus</i> M) Extract on Aloxan- Induced Diabetic White Rat ( <i>Rattus norvegicus</i> ) F. M. Cahyani, I. Susanti, R. Ratna, Y. D. Panggi and Y. Pravitasari	30 - 33
Effect of Pasak Bumi's Root ( <i>Eurycoma longifolia,</i> Jack) on Sperm Output in Rats <b>Farida Hayati and Mustofa</b>	34 – 37

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

vi

The Influence of Arbutin 3% and Sesame Oil (3,5,7 % w/w) on SPF Values of Oxybenzon and Dudimate O (3:7% w/w) in carbomer Gel Base Noorma Rosita, Tristiana Erawati and Rafi Jikrona	38 - 43	
Sulochrin as α -glucosidase inhibitor <i>lead compound</i> Rizna Triana Dewi, Ahmad Darmawan, Sofna D.C Euglemaker, Vari 16 dyoni, Morica Angelina and Minarti	44 – 48	
The Practice of Complementary Indigenous Malay Therapies In Rural Areas: Do Users' Attitudes, Beliefs And Perceptions Significantly Differ From Non-Users? Pei Lin Lua, Rohayu Izanwati Mohd Rawi, Suffian Mohamad Tajudin, Norlida Mamat and Ahmad Zubaidi Abdul Latif	49 – 54	
An Interventional Pilot Study: Effect Of Dark Chocolate Consumption On Anxiety Level Among Female Nursing Students Sok Yee Wong, Pei Lin Lua, Rohayu Izanwati Mohd Rawi, Rokiah Awang, Ahmad Zubaidi and Abdul Latif	55 – 62	
The Anti-proliferation Assay of Bioactive Fraction from <i>Curcuma zedoaria</i> Rhizome Ros Sumarny, Priyosoeryanto B. P., letje W., Latifah K. D. and Chairul	63 - 67	
Studies of Sub-acuteToxicity Assay from <i>Acorus calamus</i> L. in Experimental Animal Models Banjarnahor S.D.S, Sri Hartati and Megawati	68 - 71	
Antioxidant Properties and Phenolics Content of <i>Mikania scandens</i> L.(Wild) Sumi Wijaya, Ting Kang Nee, Khoo Teng Jin and Christophe Wiart	72 - 77	1
The Influence of Olive Oil Addition on Increasing of Arbutin Penetration in the Carbomer- 111/11/(2):00ation on Inhibition of Enzyme Tyrosinase Activity) Widji Soeratri, Tristiana Erawati, Noorma Rosita and Fahriyatul Wahyuni	78 - 81	
The difference of antioxidant activity of various tea ( <i>Camellia sinensis</i> L.) methanol extract Wahyu Widowati, Tati Herlina and Hana Ratnawati	82 - 88	
Chemical Stability of Cisplatin and Ondansetron During Simulation of hemotherapy Administration Yahdiana Harahap, Rizka Andalusia and Armon Fernando	89 – 94	
The Effects of Cassava Starch ( <i>Manihot utilissima</i> , Pohl.) as a Binder on Physicochemical Characteristics of Acetaminophen Tablet Formulation Yandi Syukri, Tri Rahayu Ningsih and M. Hatta Prabawa	95 – 98	
Drug Interaction Study in Hospitalized Hepatic Cirrhosis Patient in Dr. Ramelan Navy Hospital Amelia Lorensia, Aziz Hubeis, Widyati and Hary Bagijo	99 – 102	
The Effect of Cold Storage in Krebs-Henseleit Buffer in the Viability and Metabolic Activities of Precision Cut Intestinal Slices <b>Dewi Setyaningsih, AA Khan and GMM Groothuis</b>	103 - 110	

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009 The Effect Of b-Cyclodextrin And Oxybenzone-Octyl Dimethyl Paba (3:7% W/W) Addition 111-116 On The Penetration Of Kojic Acid In Vanishing Cream (Based on Activity Inhibition of Tyrosinase)

## Diana Winarita, Tristiana Erawati, Noorma Rosita and Widji Soeratri

The profile of knowledge and self-medication in handling cough symptoms by students of 117-120 pharmacy at Airlangga university Elida Zairina, Liza Pristianty and Lestriana Kusumasari

The Characteristics and Release of Diclofenac Sodium of Niosome System in Carbomer 940 121 – 128 Gel Base Preparation (Niosome System of Diclofenac Sodium-Span 60-Cholesterol with Molar Ratio 1:5:5) Esti Hendradi, Tutiek Purwanti, Bety Nurfia Puspitarini and Bianda Ida Kurnia

Red Betel Vine (Piper Crocatum) Essential Oil as Antituberculosis Farida Juliantina Rachmawaty	128 - 133				
Effect of Pasak Bumi's Root ( <i>Eurycoma longifolia</i> , Jack) on Sperm Output in Rats <b>Farida Hayati and Mustofa</b>					
The Influence of Sesame Oil Addition on Arbutin Release from Carbomer-940 Gel Bases Hanifa Rahma, Tristiana Erawati and Noorma Rosita					
Phytochemical Screening and Determination of Antioxidant Activity of Fractions from Ethyl Acetate Extract of Phyllanthus acidus (L.) Skeels Leaf Hindra Rahmawati, Hesty Utami and Moordiani	142 - 145				
Study on Antihyperglicaemic Activitiy of Ethyl Acetate Extract of Sidaguri ( <i>Sida rhombifolia</i> L.) Stem onAlloxan-Induced Diabetic Mice ( <i>Mus musculus</i> L.) Irma Ratna K, Muktiningsih, Suhartono, Natalia Elisabeht and Muhammad Ali Zulfikar	146 - 152				
The Influence of Arbutin and Olive Oil as an Enhancer in Characteristic and SPF Value of Sunscreen (Combination of Oxybenzone and Octyldimethyl Paba in <i>Carbomer</i> 940 Gel Base) Josephine Paramita Ayuningtyas, Tristiana Erawati, Noorma Rosita and Widji Soeratri	153 - 160				
The Effect of Secondary Emollients Triethylhexanoate, Isopropyl myristate, and Propyleneglycol Isostearate on in-vitro skin penetration of tocopheryl acetate cream using Franz-diffusion cell Joshita Djajadisastra, Sutriyo and Fraida Aryani	161 – 165				
Immunomodulatory activity of Plantago major L. on IgM titer of mice Kartini, A. Kirtishanti, Dessy, Fauziah and Isnaini	166 - 169				
Antibacterial activities of <i>Aleurites moluccana</i> (Euphorbiaceae) Othman Abd Samah and Rasyidah Mohamad Razar	170 - 178				
Total synthesis and revised structure of benzophenone glucopyranosides from phaleria	179 - 185				

macrocarpa

Phebe Hendra, Yukiharu Fukushi and Yasuyuki Hashidoko

viii

Influence of Tween 80 Concentration in Carbomer/ Tween 80 Aggregate on Kojic Acid 186 – 191 Penetration (Observed on Inhibiting Tyrosinase Activity in Vanishing Cream) Siti Evi Jayanti, Tristiana Erawati and Noorma Rosita

Validation for Result Degradation of Nifedipine Residue with Thin Layer Chromatography-192 – 195 Densitometry and Thin Layer Chromatography-Spectrophotometry Sitti faika and Sudibyo Martono

Synthesis and Biological Activity Test of Antibiotic UK-3 Analogues, 2-Hydroxynicotinyl-196 – 198 Butyl-Serine-Ester and Its Derivatives Ade Arsianti, Kiyomi Kakiuchi, Tsumoru Morimoto, M.Hanafi and Endang Saefudin

Vitamin e content in the dragon fruit Established by high performance thin layer 199–204 chromatography-densitometry Any Guntarti and Warsi

Drug interaction study in hospitalized hepatic cirrhosis patient in Dr. Ramelan navy hospital 205 – 208 Amelia Lorensia, Aziz Hubeis, Widyati and Hary Bagijo

PGV-1 inhibits G2M phase progression in WIDr colon cancer cell 209 – 212 Endah Puji Septisetyani, Edy Meiyanto, Masashi Kawaichi and Muthi' Ikawati

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences 213 – 215 The influence of oleic acid pre-treatment on transport of epigallocathecin gallat in green tea (*Camellia sinensis*, L) extract Across mice skin in vitro **Nining Sugihartini, Achmad Fudholi, Suwidjiyo Pramono and Sismindari** 

Development and Production of Anti Tuberculosis Fixed Dose Combinations (FDCs) 216 – 218 Barokah Sri Utami, Syamsul Huda, Nurliya Irfiani and Badrus S.

The Characteristics and Release of Diclofenac Sodium of Niosome System in Carbomer 940 219 – 224 Gel Base Preparation (Niosome System of Diclofenac Sodium-Span 60-Cholesterol with Molar Ratio 1:5 :5)

Esti Hendradi, Tutiek Purwanti, Bety Nurfia Puspitarini and Bianda Ida Kurnia

The Characteristics and Release of Diclofenac Sodium of Niosome System in Carbomer 940 225 – 231 Gel Base Preparation (Niosome System of Diclofenac Sodium-Span 20-Cholesterol with Molar Ratio 1:5 :5)

Esti Hendradi, Tutiek Purwanti, Anditasari and Srimaryati

An Interventional Pilot Study: Effect Of Dark Chocolate Consumption On Anxiety Level 232 – 239 Among Female Nursing Students Sok Yee Wong, Pei Lin Lua, Rohayu Izanwati Mohd Rawi, Rokiah Awang and Ahmad Zubaidi Abdul Latif

Antiemetics utilization in cancer patients with high emetogenic cytotoxic drugs in two 240 – 243 govermental hospital in indonesia **Dyah Aryani Perwitasari and Ana Hidayati** 

KEY WORDS INDEX	244
DISCUSSION	246

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

# Antioxidant Properties and Phenolics Content of *Mikania scandens* L.(Wild)

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#### Abstract

*Mikania scandens*, belongs to the Asteraceae family, is a fast growing perennial creeper found in Malaysia. Traditionally, this plant has been used for treatments of diarrhoae, cancer and wound healing. The main objective of the study was to examine the antioxidant activities of *Mikania scandens* extracts. Whole plants were collected, dried and macerated sequentially in four different solvents, starting from Hexane (H), Ethyl acetate (EA), Ethanol (E) and Water (W). Ferric Reducing-Antioxidant Power (FRAP) and β-carotene bleaching methods were employed. Total phenolics and flavonoids content of the plant extracts were also determined. All *Mikania scandens* extracts, exhibited relatively good antioxidant activities in both assays. For the FRAP assay, the rank order of antioxidant activity is E > trolox = quercetin > W > EA > H. For the β-carotene bleaching assay, ethanol extracts remained the most potent extracts followed by H > EA > W. Ethanol extracts possessed the highest activities with potency as good as quercetin and trolox as exhibited in both assays. There is a positive correlation between phenolics content and antioxidant activity of the extracts based on the FRAP assay.

Key words: *Mikania scandens*, FRAP,  $\beta$ -carotene bleaching, Total phenolics content

#### Introduction

For centuries, plants have been used throughout the world to treat various diseases. In fact, approximately 60-80% of the world population still rely on traditional medicines derived from plants for treatment of ailments and about 50% of the drugs used in the clinical treatment are derived from plant sources (WHO, 2004; Zhang, X., 2004).

One interesting hyphothesis that many researchers is working on, is diseases purported to be caused by free radicals. It is thought that free radicals e.g reactive oxygen species are responsible for various degenerative diseases, e.g heart diseases, stroke, arteriosclerosis and cancer, as well as aging process (Willcox, *et al.*, 2004).

The aim of this study was to examine the antioxidant activities of *Mikania scandens* (L.) Wild. Asteraceae is the largest family after Orchidaceae, with almost 24,000 species (Steven, P.F., 2001). The member of the family are easily identified through their flowers. Most Asteraceae species grow well in moist soils and can be found along roads and foot trails (Stone, B.C., 1970; Cronquist, A., 1980; Holm, *et al.*, 1997; Rahman, *et al.*, 2008).

*Mikania scandens* is a herbaceous, perennial twinning herb with saggitate, hastate or cordate leaf bases. This plant is native to South America, useful for treatments of diarrhoae, cancer and wound healing (Hasan, *et al.*, 2009). The plant is reported to have antimicrobial, antiinflamatory, antipyretic, analgesic, ulcerprotective, and anticarcinogenic activities (Ysrael, *et al.*, 1990; Bishayee, A. and Chatterjee, M., 1994; Mosaddik, M.A., and Alam, K.M., 2000; Hasan, *et al.*, 2009). Three deterpenic acids known as kaurenic acid, butyryloxykaurenic acid and beta-sitosterin have also been isolated from this plant (Ghani, A., 2003). Mikania genus reduces growth and productivity of several crops such as oil palm, rubber, citrus, cassava, teak, eucalyptus, acacia, albizia, pineapple, and coconut in Malaysia (Sankaran, K.V., 2007). As a weed and in abundant supply, *Mikania scandens* could be exploited to be a powerful source of antioxidant agent.

#### Methodology

#### **Sample Preparation**

Whole plants of *Mikania scandens* (L.) Wild was collected from areas around Semenyih and Broga. The plant was dried at room temperature and ground into powder. The plant powder was soaked sequentially in four different solvents: Hexane, Ethyl acetate, Ethanol

72

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009 and Water. The solvents were evaporated using rotary evaporator under reduced pressure at 40°C. Dried extracts were kept at -20°C until tested.

#### Antioxidant Assay

### Ferric-Reducing Antioxidant Power

The FRAP method adopted was based on Benzie & Strain (1996) with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM Acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O in a 10:1:1 ratio. Briefly, 180 µl of FRAP reagent was mixed with 20 µl of test sample, so the final dilution of the test sample in the reaction mixture was 1/10. Readings were taken at 90 minutes (at 600 nm) instead of 4 minutes using spectrophotometer (Dynex MRX-Revelation). We have chosen to take the readings at 90 min because some polyphenols take a longer time to react and a longer reaction times is required for detection of any antioxidant activity (Phipps, *et al.*, 2007). Fe(II) concentrations in the range 1 µM – 125 µM/I (FeSO<sub>4</sub>.7H<sub>2</sub>O) were used as standard. Trolox and quercetin were used as a positive control with the same concentration range as the plant extracts.

Readings were obtained from three independent experiments, each performed with triplicate measurements. FRAP value was calculated as Ferrous Equivalents, the concentration of trolox/quercetin or extracts which produced an absorbance value equal to that of 1 mM Fe<sub>2</sub>SO<sub>4</sub>.

#### Inhibition of β-carotene Bleaching

β-carotene bleaching assay was conducted according to Barreira, *et al* (2008) with some modifications. A solution of β-carotene was prepared by dissolving two mg of β-carotene in 10 ml chloroform. Two ml of this solution was pipetted into 100 ml round-bottom flask. After the chloroform was removed at 40°C under vacuum, 40 mg of linoleic acid, 400 mg of tween 80, and 100 ml of distilled water were added to the flask with vigorous shaking. 96-wells microtiter plate was used for this method, that giving better reproducibility and higher sample throughput (Tsao,*et al.*, 2003). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 490 nm using spectrophotometer (Dynex MRX-Revelation). Absorbance readings were recorded at 20 min intervals for 240 minutes. A blank, devoid of β-carotene, was prepared for background subtraction. % Antioxidant activity (AA) was calculated using the following equation: % AA = ((DR contol-DR sample) / DR control) x 100, where DR is degradation rate of sample (DR = ln (initial absorbance (470 nm) at time zero) / (absorbance at 240 minutes) / t (time in minutes)). EC<sub>50</sub> of the plant extracts or controls (quercetin and trolox) were calculated from the graph of antioxidant activity percentage against concentration.

#### **Total Phenolics Content**

Total Phenolics content of *Mikania scandens* was determined using Folin-Ciocalteau assay, as described by Slinkard and Singleton (1977). Basically 20  $\mu$ l of diluted *Mikania scandens* extracts were mixed with 1,58 ml of water and 100  $\mu$ l of Folin-Ciocalteau's reagent. After standing for 5 minutes at room temperature, 300  $\mu$ l of sodium carbonate (20% w/v) were added. The solutions were mixed and allowed to stand 30 minutes at 40°C. Changes in absorbance were determined at 765 nm against the blank (the "0 ml" solution) using a UV-Vis Spectrophotometer (Biochrom Libra S12). Gallic acid (50, 100, 150, 250 mg/ml) were used as a calibration standard curve. Results were expressed on fresh weight basis of g gallic acid equivalents/g of sample.

#### **Total Flavonoids Content**

Estimation of Total flavonoids content in the plant extracts was carried out using the method, described by Froehlicher, *et al.* (2009). A series of methanolic dilution of Quercetin was prepared and used as a standard curve. Flavonoids amount in the plant extracts were expressed in mg of quercetin/g of plant extracts.

#### **Results and Discussions**

Research in recent years has implied the role of oxidative and free-radical mediated reactions in degenerative processes in ageing and progression of diseases such as cancer,

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

coronary heart disease and other neurodegenerative disorders such as Alzheimer's disease (Ames, B.N., 1983; Gey, K.F., 1990; Ames, *et al.*, 1993; Harman, D., 1995; Diaz, *et al.*, 1997). There is an increasing interest in discovering natural plant products that possess potential anti-ageing or cytoprotective properties as preventative agents against the degenerative processes.

Antioxidant can be chemically grouped into two groups, based on their solubility, hydrophilicity and lipophilicity (Huang, *et al.*, 2002). FRAP assay is usually employed to test for hydrophilic antioxidants and  $\beta$ -carotene bleaching method for lipophilic molecules. Two different assays were used for the study to anticipate the possibility that *Mikania scandens* containing both types of antioxidants.

Results obtained in this present study revealed that the reducing ability of the extracts were in the range of 1.14 - 13 mg/ml (Table 1). The activity of all the plant extracts was comparable to quercetin and trolox and the rank order antioxidant activity is E > trolox = quercetin > W > EA > H extracts. Ethanol extracts possessed the highest reducing ability with potency as good as quercetin and trolox (Table 1).

In the  $\beta$ -carotene bleaching test,  $\beta$ -carotene undergoes rapid discoloration in the absence of antioxidant (Cao, *et al.*, 2009). The rank order of potency observed in the  $\beta$ -carotene bleaching assay was quercetin > trolox > E > H > EA > W (Table 1). In this assay, Hexane extracts appeared to be more active when compared to both Ethyl acetate and Water extracts. It is tought that Hexane extracts comprised mainly lipophilic compounds would dissolve in the lipid phase of the reaction media thus exhibiting a good antioxidant activity.

 Table 1. Antioxidant activities, total phenolics and flavonoids content of Mikania
 scandens

 extracts
 scandens

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	FRAP [Ferrous Equivalent (mg/ml)]	β-carotene bleaching assay [*EC <sub>50</sub> (µg/ml)]	Total phenolics content [Gallic acid Equivalent (g/g)]	Total flavonoids content [Quercetin Equivalent (mg/g)]
Hexane (H)	$13.00 \pm 0.04$	$10.00 \pm 0.64$	$106.67 \pm 0.00$	3.33 ± 0.03
Ethyl acetate (EA)	2.57 ± 0.01	26.67 ± 0.29	441.56 ± 0.01	$2.46 \pm 0.03$
Ethanol (E)	$1.14 \pm 0.03$	$5.87 \pm 0.59$	$1066.67 \pm 0.02$	$2.12 \pm 0.01$
Water (W)	$2.17 \pm 0.03$	$46.87 \pm 0.17$	$347.56 \pm 0.02$	$1.43 \pm 0.00$
Quercetin	$1.31 \pm 0.02$	$1.09 \pm 0.30$	-	, <b>-</b> ", , , , , , , , , , , , , , , , , , ,
Trolox	$1.14 \pm 0.02$	$1.68 \pm 0.21$	-	-

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean  $\pm$  SD.

\*  $EC_{50}$  represents the effective concentration at 50% of total antioxidant activity.

Polyphenols are bioactive compounds believed to be involved in the defence process against harmful oxidative damage, by donating hydrogen to highly reactive radicals (Fresco, *et al.*, 2006; Lapornik, *et al.*, 2005). Expressed in Gallic Acid Equivalent (g/g), Total phenolics content of *Mikania scandens* extracts were in the order of E > EA > W > H. A direct relationship has been found between the content of total phenolics and antioxidant capacity of plants (Ferreira, *et al.*, 2007; Robards, *et al.*, 1999). Data on the phenolics content in our study give a positive correlation with the antioxidant activity measured by the FRAP assay (see Figure 1). Phenolics compounds are usually found in the water or ethanol extracts (Waksmundzka-Hajnos, 2008).

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009



Figure 1. Correlation between FRAP Antioxidant activity and total phenolics content of *Mikania scandens* extracts

Flavonoids are a subgroup of these polyphenolic compounds that possess strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions (Bors, *et al.*, 1990). Several studies have highlighted that flavonoids can act as a good antioxidants (Lewis, 1999; Vijayakumar *et al.*, 2008, Vinson, 1995). Total flavonoids content found in *Mikania scandens* extracts were in the range of 1 -3 mg/g quercetin equivalent. In plants, flavonoid aglycones (flavonoid without attached sugars), occurs in variety and tend to be more soluble in non polar organic solvents (Waksmundzka-Hajnos, 2008). Our data showed that the highest quantity of flavonoids was found in the Hexane fractions. This may suggest that lipid soluble flavonoids contributed to the antioxidant activity of Hexane in the  $\beta$ -carotene assay.

#### Conclusion

The results of this present study demostrated that *Mikania scandens* crude extracts possessed appreciable antioxidant activity. There was a positive correlation between antioxidant activity in FRAP assay and total phenolics content and possibly weak relationship between lipid soluble flavonoids and the antioxidant activities found in the  $\beta$ -carotene assay. Further research to investigate the cytoprotective properties of *Mikania scandens* extract is warranted.

#### Acknowledgement

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Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

Sumi Wijaya, et al.

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76

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8 2

Proceeding International Conference on Pharmacy and Advanced Pharmaceutical Sciences Yogyakarta, Indonesia, 2009

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