EFFECT OF EXTRACTION SOLVENTS ON BIOLOGICAL ACTIVITIES OF *MURRAYA PANICULATA* LEAVES IN COSMETIC APPLICATION

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ผลของตัวทำละลายที่ใช้ในการสกัดต่อฤทธิ์ทางชีวภาพของใบแก้วที่นำมาประยุกต์ใช้ทางเครื่องสำอาง

น.ส.สิริฤทัย เรือนใหม่

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Field of Study	Cosmetic Science
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สิริฤทัย เรือนใหม่ : ผลของตัวทำละลายที่ใช้ในการสกัดต่อฤทธิ์ทางชีวภาพของใบแก้วที่นำมาประยุกต์ใช้ทางเครื่องสำอาง. (EFFECT OF EXTRACTION SOLVENTS ON BIOLOGICAL ACTIVITIES OF *MURRAYA PANICULATA* LEAVES IN COSMETIC APPLICATION) อ.ที่ปรึกษาหลัก : รศ. ภญ. ดร.พรเพ็ญ วีระวัฒกานนท์, อ.ที่ปรึกษาร่วม : ผศ. ภญ. ร.ต.ท.หญิง ดร.วลัยศิริ ม่วงศิริ

วัตถุประสงค์ของงานวิจัยนี้เพื่อประเมินและเปรียบเทียบฤทธิ์ต้านอนุมูลอิสระและฤทธิ์การยับยั้งเอนไซม์ไทโรซิเนสของสารสกัดใบแก้วที่ได้จากการสกัดด้วยตัวทำละลายต่างชนิดเพื่อใช้ประโยชน์ทางด้านเครื่องสำอาง ผลแสดงว่าสารสกัดจากเอทานอล 75% มีฤทธิ์ต้านอนุมูลอิสระสูงที่สุด (IC50 = 1.43 มิลลิกรัม/มิลลิลิตร) ปริมาณสารประกอบฟีนอลลิครวมเท่ากับ 66.12 mg GAE/g และปริมาณฟลาโวนอยด์รวมเท่ากับ 7.83 mg QE/g สารสกัดทุกชนิดมีประสิทธิภาพในการยับยั้งเอนไซม์ไทโรซิเนสต่ำ สูตรสุดท้ายของโลชั่น MPE ประกอบไปด้วย MPE 1 %w/w ละลายในโพรพิลีนไกลคอล 50 %w/w กลีเซอรีน 20 %w/w และเอทานอล 7.5 %w/w จากการศึกษาความคงตัวของโลชัน MPE โดยการเก็บผลิตภัณฑ์ไว้ที่ 4 °C และ 40 °C เป็นเวลา 1 เดือน และมีการตรวจสอบปริมาณฟลาโวนอยด์ทั้งหมดในโลชันและฤทธิ์ต้านอนุมูลอิสระของโลชัน MPE ในระหว่างการเก็บรักษา ผลการศึกษาที่อุณหภูมิการเก็บรักษาทั้งสองแบบพบว่าไม่มีการลดลงของปริมาณฟลาโวนอยด์ และฤทธิ์ต้านอนุมูลอิสระจากโลชัน 1% MPE นั่นหมายความว่าโลชัน MPE นี้มีศักยภาพสูงพอที่จะสามารถพัฒนาเพื่อใช้ในเครื่องสำอาง

สาขาวิชา วิทยาศาสตร์เครื่องสำอาง ลายมือชื่อนิสิต .....

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ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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Siriruethai Ruanmai : EFFECT OF EXTRACTION SOLVENTS ON BIOLOGICAL ACTIVITIES OF *MURRAYA PANICULATA* LEAVES IN COSMETIC APPLICATION. Advisor: Assoc. Prof. PORNPEN WERAWATGANONE, Ph.D. Co-advisor: Asst. Prof. WALAISIRI MUANGSIRI, Ph.D.

The objective of this study was to evaluate and compare the antioxidant activity and tyrosinase inhibition activity of Murraya paniculata (Linn.) Jack leaves extract (MPE) obtained from different extraction solvents for the use of cosmetic purposes. The result showed that 75 % ethanolic extract presented the highest antioxidant activity (IC50 = 1.43 mg/mL), the total phenolic content of 66.12 mg GAE/g and the total flavonoids content of 7.83 mg QE/g. All of the obtained extracts had low tyrosinase inhibitory activity. The final MPE lotion contained 1 %w/w of 75 % ethanolic extract dissolved in 50 %w/w of propylene glycol, 20 %w/w of glycerin, and 7.5 %w/w of ethanol. Regarding stability study, the MPE lotion was stored at 4 °C and 40 °C for 1 month. Total flavonoid content remaining in the lotion and antioxidant activity of the MPE lotion were monitored during the storage. The results at both storage temperatures showed that no significant decrease of the total flavonoid content and antioxidant activity were observed from the 1 % MPE lotion. This meant that this MPE lotion had high potential to be developed for cosmetic purpose.

Field of Study: Cosmetic Science
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Student's Signature .....
Advisor's Signature .....
Co-advisor's Signature .....

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Siriruethai Ruanmai

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List of abbreviations

MPE	=	*Murraya paniculata* leaf extract
%w/w	=	Percent weight by weight
µg	=	Microgram
mg/mL	=	Milligram per milliliter
°C	=	Degree Celsius
nm	=	Nanometer
EC_{50}	=	The half maximal effective concentration
RBC	=	Red blood cell
IC_{50}	=	The half maximal inhibitory concentration
IU	=	International unit
GAE	=	Gallic equivalent
QE	=	Quercetin equivalent
DMSO	=	Dimethyl sulfoxide
ANOVA	=	Analysis of variance

CHAPTER I
INTRODUCTION

Murraya paniculata (Linn.) Jack (Orange jasmine) is a flowering plant in the family Rutaceae. This plant has grown widely in Southeast Asia and Australia. *Murraya paniculata* is very useful because it is used as an ornamental and a medicinal plant. From earlier research (Rohman & Riyanto, 2005; Sharker et al, 2009; Taher et al, 2015), it was found that an essential oil and an extract from *M. paniculata* contain many compounds providing pharmacological effects such as anti-inflammatory, antinociceptive, rheumatism, etc.

In-vivo and *in-vitro* studies showed that *M. paniculata* extract has a good antioxidant effect and also has a whitening effect owing to many components in *M. paniculata* extract. Therefore, *M. paniculata* is interesting to be studied and developed for cosmetic use. However, the difference in extraction processes such as method and solvent leads to the different types of compounds obtained in the extract. From literature reviews, there are various groups of antioxidants in *M. paniculata* leaf extract, including alkaloids, flavonoids, coumarins, terpenoids, and phenolic compounds. In each of the previous studies, different solvents were used in the extraction processes and components in the obtained extracts were different in each work.

This plant is widely available in Thailand. The reason for using leaf is that it is easy to collect, and the leaf is the part that must be cut off when the plant is decorated. Four solvents including methanol, ethanol, 50% ethanol and 75% ethanol were used to extract *M. paniculata* leaf. Then, antioxidant and tyrosinase inhibition activities of the obtained extract were evaluated. The tested sample

providing the strongest activities was further prepared in a form of topical viscous solution developed for cosmetic purpose.

This research aims to find a suitable solvent for *M. paniculata* leaf extraction study the antioxidant activity and tyrosinase inhibition activity of *M. paniculata* leaf extract and develop *M. paniculata* lotion for cosmetic use.

General Objectives

To study *M. paniculata* leaf extraction solvent and evaluate antioxidant and tyrosinase inhibition activities of the obtained extract for the use of cosmetic purpose.

Specific Objectives

The purposes of this study were as follows:

1. To compare the extraction efficiency from different solvents
2. To evaluate and compare the amount of total phenolic content and total flavonoid content in *M. paniculata* leaf extracts obtained from different solvent extraction processes
3. To compare antioxidant activity and tyrosinase inhibition of *M. paniculata* leaf extract obtained from different solvent extraction processes.
4. To formulate the lotion containing the extract of *M. paniculata* for cosmetic purpose

CHAPTER II

LITERATURE REVIEW

Orange Jasmine, the scientific name is *Murraya paniculata* (L.) Jack is a plant that belongs to the family Rutaceae, which is the same family as Citrus. In terms of botanical characteristics, *M. paniculata* is a bushy shrub or small tree usually growing 2-4 meters tall. Its alternately arranged leaves are once-compound with 3-9 glossy leaflets. The fragrant flowers are borne in clusters at the tips of the branches or in the upper leaf forks. The fruit is small egg-shaped or oval and turns from green to orange or bright red. *M. paniculata* is popularly grown as an ornamental plant in tropical regions. It can be found in Southern China, Taiwan, South-eastern Asia (i.e. Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia, Malaysia, and the Philippines), and northern Australia (i.e. the northern parts of the Northern Territory, far northern Queensland and northern Western Australia)

Figure 1 Morphology of Orange Jasmine

(https://keyserver.lucidcentral.org/weeds/data/media/HtmL/murraya_paniculata.htm)

Several parts of this plant are used in traditional medicine. For example, in Bangladesh, *M. paniculata* leaf is used to relieve pain (Sharker et al, 2009). In the Philippines, the leaf is used for the treatment of diarrhea and dysentery due to its astringent effect (Mondal et al, 2001). In India, *M. paniculata* root bark is sometimes used to relieve cough, hysteria and rheumatoid arthritis (Chatterjee et al., 2001).

Antioxidant activity of ethanolic extract from *M. paniculata* leaf has been reported at the IC_{50} value of 126.17 µg/mL (Rohman & Riyanto, 2005). Regarding ferric thiocyanate method, the ethanolic extract showed 67.77% antioxidant activity at the concentration of 500 µg/mL (Zhu et al, 2015).

Other than that, the leaf extract also presented antimicrobial and anti-inflammatory activities. *M. paniculata* extract could inhibit the growth of bacteria (Escherichia coli, Proteus mirabilis Samonella typhi, Enterobacter aerogenes and Shigella flexineri) at a concentration range of 300-500 mg and showed moderate antifungal activity at the concentration of 500 mg (Sundaram et al, 2011). *M. paniculata* extract revealed mild to moderate membrane stabilizing activity. The extract inhibited 30.20±0.75% and 60.83±1.45% heamolysis of RBCs under heat and hypotonic solution conditions, respectively (Akter Mita et al, 2013). Another research found that the ethanolic extract of *M. paniculata* showed a significant (P<0.001) anti-inflammatory activity against carrageenan-induced paw edema in rats (Rahman *et al.* 2010).

Chemical Constituents of *M. paniculata* (L.) Jack

M. paniculata has been studied for its bioactive compounds by several research groups. To date, various compounds are identified, ranging from indole alkaloids, phenols (Gautam et al, 2012b), flavonoids (Ferracin et al, 1998; Ito et al, 1990; Kinoshita & Firman, 1997), terpenoids (Li et al, 1988) to coumarins from leaf, fruit, flower and root bark. Besides, volatile and essential oils from the leaf of *M. paniculata* contain more than 60 types of compounds identified by chromatography

techniques. Moreover, phytochemical tests on the ethanolic extract of *M. paniculata* leaf showed the presence of alkaloids, tannins, saponins, gums, reducing sugar, flavonoids, and glycosides (Rahman *et al*, 2010).

Flavonoids providing antioxidant activity were isolated from *M. paniculata* extract such as 3',4',5,5',7,8-hexamethoxyflavone and 3,3',4',5,5',7,8-heptamethoxyflavone from the methanolic extract of *M. paniculata* leaf (Yang et al, 1984). Later 3,5,7,3',4',5'-hexamethoxyflavone was isolated from the flower (Wu et al, 1994). Another research group isolated polymethoxylated flavonoids (PMFs) from *M. paniculata* leaf (Zhang et al, 2011; Kinoshita & Firman, 1996; 1997) and ten flavonoids from pulp and peel of the fruit (Ferracin et al, 1998) and from branches (Zhang et al, 2013).

Antioxidant activity of *M. paniculata* extract

One of the important activities of this plant is the antioxidant activity. The antioxidant activity of ethanolic *M. paniculata* leaf extract was measured using DPPH method. The result showed that the IC_{50} value of *M. paniculata* leaf extract was 126.17 µg/mL, while the IC_{50} of vitamin E was 8.27 µg/mL (Rohman & Riyanto, 2005). In addition, the research indicated that the antioxidant activity was probably due to the flavonoid content in the extract. Acetone extract of *M. paniculata* leaf at a concentration of 100 µg/mL could inhibit lipoxygenase (LOX) up to 60% (Chen et al, 2009).

Antioxidant activity were evaluated when *M. paniculata* leaf was extracted using methanol. The results showed that reducing activity, lipid peroxidation inhibition and HO• scavenging activity of the methanolic extract were significantly higher than those of Trolox while other antioxidant indicators, *i.e.*, DPPH• scavenging, O^{2-}• scavenging, and H_2O_2 scavenging activities of the methanolic extract were comparable to those exhibited by Trolox (Table 1). These results indicate that the

deviation of the response to the indicators extract could be attributed to the different structures of constituent flavonoids in the extract when compared to the uniform structure of Trolox. (Zhu et al, 2015)

Table 1 Results of EC_{50} values of *M. paniculata* extract and Trolox

Antioxidative activity	**MPE (mg/mL)**	**Trolox (mg/mL)**
Reducing activity[b]	0.26±0.01[b]	0.70±0.01
Lipid peroxidation inhibition[b]	0.023±0.03[b]	0.052±0.02
DPPH radical scavenging activity	0.93±0.02	1.319±0.03
Superoxide anion radical scavenging activity	0.581±0.01	0.747±0.01
Hydroxyl radical scavenging activity[b]	0.302±0.05[b]	0.576±0.04
Hydrogen peroxide scavenging activity	0.470±0.02	0.583±0.02

[a] : EC_{50} values determined the concentration inhibited 50% of the scavenging activity

[b] : Statistically significant difference (α =0.05)

It was reported that after rats received the extracts of *M. paniculata* leaf at concentrations of 100, 200 and 400 mg/kg by oral for 14 days. Levels of blood glucose, cholesterol, triglyceride and, lipid level were significantly reduced. The amount of antioxidants was increased significantly in these rats. (Gautam et al, 2012b)

Phenolics and flavonoids in *M. paniculata* extract

Many researches indicated that the antioxidant activity is a result of phenolic and flavonoid compounds in extracts. From a study of Menezes et al, phenolic compounds and flavonoid compounds of hydro-alcoholic extract from *M. panaculata* leaf were isolated by HPLC-DAD analysis presenting that this extract contained 4 phenolic compounds and 6 flavonoids (Figure 2). Types and amount of phenolic and flavonoid compounds in the extract are shown in Table 2. (Menezes et al, 2014). Figure 3 and 4 show chemical structures of phenolic and flavonoid compounds in *M. paniculata* extract respectively.

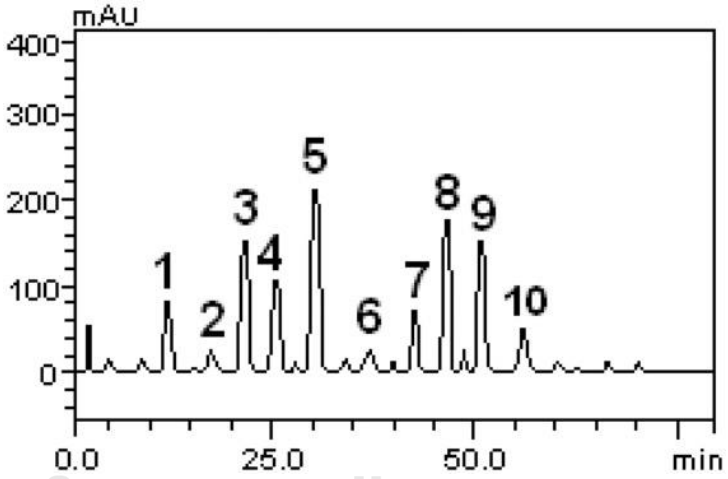
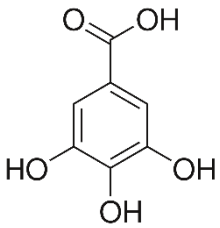


Figure 2 High performance liquid chromatography showing phenolic and flavonoid profile of M. paniculata. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), quercitrin (peak 8), quercetin (peak 9), and kaempferol (peak 10).

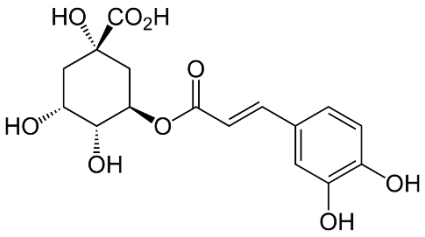
Table 2 Phenolic and flavonoid compositions of *M. paniculata*

Compound	*M. paniculata*		LOQ (µg/mL)	LOD (µg/mL)
	mg/g*	%		
Gallic acid	5.91±0.02	0.59	0.014	0.047
Catechin	1.83±0.01	0.18	0.032	0.105
Chlorogenic acid	9.56±0.03	0.95	0.005	0.016
Caffeic acid	6.70±0.02	0.67	0.027	0.089
Ellagic acid	13.41±0.01	1.34	0.026	0.085
Epicatechin	1.92±0.03	0.19	0.039	0.128
Rutin	5.37±0.04	0.53	0.011	0.034
Quercitrin	9.82±0.01	0.98	0.016	0.051
Quercetin	9.15±0.02	0.91	0.023	0.079
Kaempferol	3.29±0.01	0.32	0.009	0.027

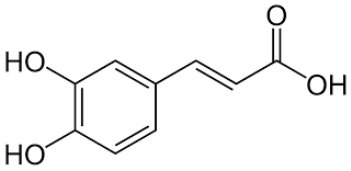
*Expressed as mean ± S.E.



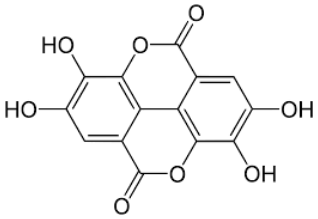
Gallic acid



Chlorogenic acid

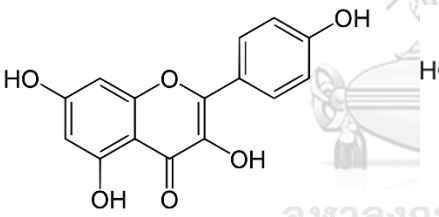


Caffeic acid

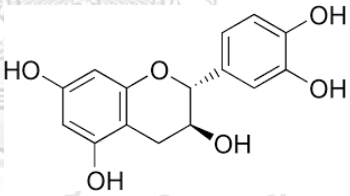


Ellagic acid

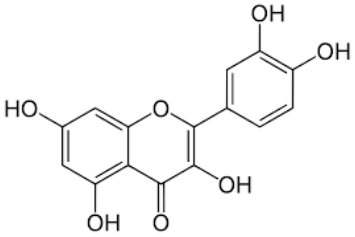
Figure 3 Structures of phenolic compounds in *M. paniculata* extract



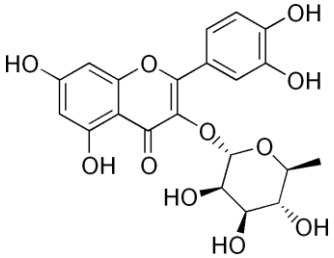
Kaempferol



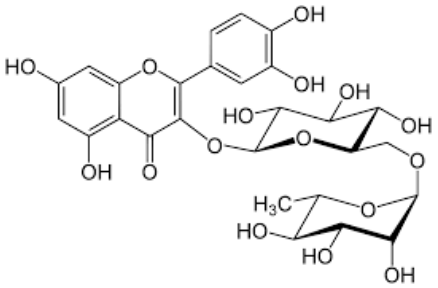
Catechin



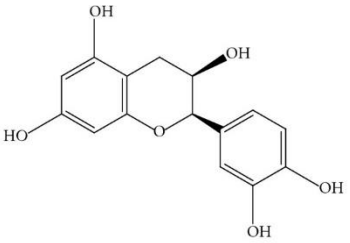
Quercetin



Quercitrin



Rutin



Epicatechin

Figure 4 Structures of flavonoids in *M. paniculata* extract

Tyrosinase inhibition activity of *M. paniculata*

Tyrosinase inhibition activity of *M. paniculata* is interesting. It was reported that the acetone extracts of *M. paniculata* at a concentration of 500 µg/mL could inhibit the enzyme tyrosinase at a concentration of 50 IU/mL up to 70% providing skin whitening effect. (Chen et al, 2009).

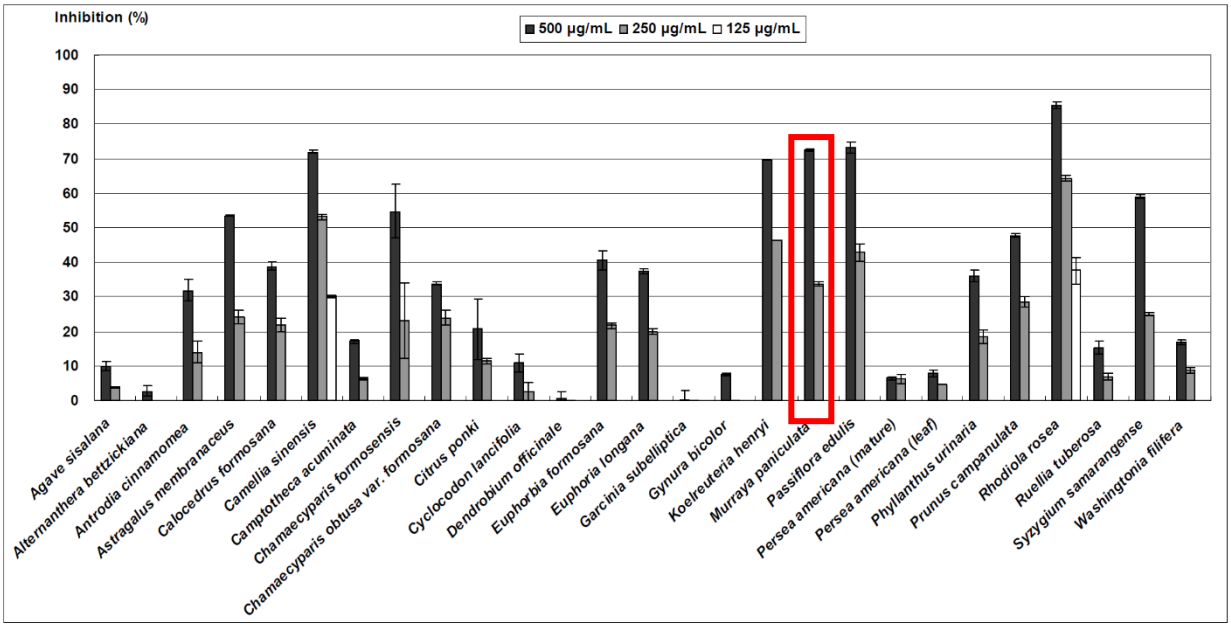


Figure 5 Tyrosinase Inhibition activity of *M. paniculata* acetone extract and other plants

It has been reported that the phenolic hydroxyl groups on the compounds are the higher antioxidant and anti-tyrosinase activities and the ethanolic extract of *M. paniculata* contained hydroxyl compounds (Zuo et al, 2018), (Menezes et al, 2014). Therefore, the ethanolic extract of *M. paniculata* may have the tyrosinase inhibition.

Toxicity of *M. paniculata* extract

Regarding *in vivo* study, rats were orally administered with 2000 and 5000 mg/kg of *M. paniculata* extract. There were no abnormal toxicity signs (such as diarrhea, piloerection, and alteration in locomotor activity) or deaths during the 14 days of observation. No significant change was observed in the hematological data. (Menezes et al, 2014).

Extraction solvent

The extraction solvent is one of the factors that alters the efficiency of the extract (Sundaram et al, 2011). The *M. paniculata* leaf was extracted using 3 solvents; ethanol, hexane, and water. Antioxidant activity of the extracts was evaluated using Ferric thiocyanate method, in the concentration range of 100-500 µg/mL compared with the positive control (vitamin E). The order of potency from high to low is vitamin E > ethanolic extract > hexane extract > water extract.

In addition, extraction solvents also affected antibacterial activity of *M. paniculata*. The studied solvents; methanol, ethanol, petroleum ether, and 50% ethanol were used to extract *M. paniculata* leaf and their efficacy on bacterial growth inhibition was evaluated. Methanolic extract showed marked antibacterial activity. Phenolic and flavonoid contents were different in each extraction solvent. Methanolic extract contained the highest phenolic content followed by ethanol, petroleum ether and, hydro-alcoholic extracts (Gautam et al, 2012a).

Table 3 Total phenolic and flavonoid contents from different solvent extracts of *M. paniculata*

Extract	Solubility	Total phenolic content	Total flavonoid content
		µg of gallic acid equivalents (GAE)*	µg of quercetin acid equivalents (QE)*
Petroleum Ether	DMSO	13.5±0.96	3.38±1.89
Methanol	DMSO	24.8±0.64	2.11±0.23
Ethanol	DMSO	15.4±0.38	1.62±0.18
Hydro-alcoholic	DMSO	9.06±1.13	1.80±0.21

*mean±SE of 3 experiments in each group.

When methanolic *M. paniculata* leaf extract was fractionated using petroleum ether, carbon tetrachloride, chloroform, and water, antioxidant activities of the extracts correlated to their phenolic contents. A carbon tetrachloride fraction presented the highest phenolic content and strongest antioxidant activity. While the water fraction extract had the lowest phenolic content and lowest antioxidant activity (Akter Mita et al, 2013).

Regarding these researches, types of extraction solvents affect the contents of active ingredients in the extracts leading to the difference of efficacy of the extracts. These differences were due to the polarity of the solvents. Figure 6 presents class and order of polarity of various solvents. Thus, a proper solvent for *M. paniculata* leaf should be evaluated.

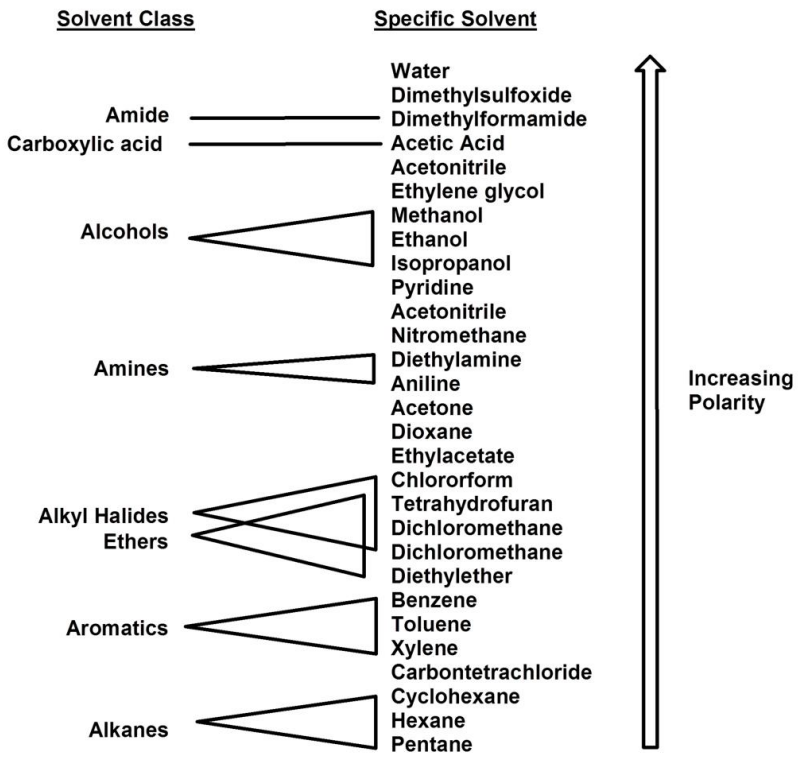


Figure 6 Polarity of solvents

(https://www.aceorganicchem.com/Elite/organic-chem-15-organic-solvents-likes-dissolve-likes/)

CHAPTER III

MATERIALS AND METHODS

Materials

1. Standard material
   - Quercetin, lot no. LRAB7760, Sigma
   - Gallic acid, lot no. BCBW7890, Sigma
   - Kojic acid, Sigma
   - Trolox, lot no. S33986-236, Sigma.
   - Tyrosinase enzyme, lot no. SLBM7158V, Sigma
2. Chemical reagent
   - Folin-Cioalteu reagent, Merk
   - 2,2-diphenyl-1-picrylhydrazyl (DPPH), Sigma
   - Methanol AR grade, RCI Labscan
   - Ethanol AR grade, RCI Labscan
   - Dimethyl Sulfoxide (DMSO), RCI Labscan
   - Aluminum Chloride ($AlCl_3$), Merk
   - Sodium Carbonate (Na_2CO_3), Ajax Finechem
   - L-dopa, Sigma
   - Glycerin USP grade
   - Phenoxyethanol
   - Propylene glycol USP grade
   - Potassium hydrogen phosphate (K_2HPO_4), Merk
   - Potassium dihydrogen phosphate (KH_2PO_4), Merk
   - Sodium hydroxide, Merk

3. Analytical instrument
   - Analytical balance, XS105, Mettler Toledo, Switzerland.
   - pH meter, Mettler toledo
   - UV-Visible spectrophotometer, UV-1601, Shimadzu, Japan.
   - Rotary evaporator, R-200, Buchi, German.
   - Microplate reader, cariostar, BMG Labtech, Germany.

Method

1.) Plant material and extraction

M. paniculata leaf was collected from the botanical garden at faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand in February 2018. This plant material was washed with fresh water, and then dried at 30±2 °C for several days until the plant weight did not change. The dried plant was ground using a blender. The powdered plant was passed through a 40-meshes sieve and stored at room temperature (Zhu et al, 2015). *M. paniculata* leaf was extracted using 4 different solvents; methanol, ethanol, 50% ethanol or 75% ethanol. By maceration method, twenty grams of powdered plant was soaked in 60 mL of the solvent at room temperature for 3 days and filtered through a Whatman Number 5. The plant was extracted three times with the fresh solvent at each time. The filtrates from the three extraction times were combined and then concentrated using a rotary evaporator under vacuum at 40°C. *M. paniculata* leaf extract (MPE) was stored in a refrigerator at -20 °C and protected from light until further use. The extraction process was performed using the different solvents. The experiment was run triplicate for each solvent.

The percent yield of the extract was calculated using equation 1.

$$\%\text{yield of extract} = \frac{\text{weight of extract (g)}}{\text{weight of the dried plant (g)}} \times 100 \quad \text{----------} \quad \text{Equation 1}$$

2) Characterization

2.1) Determination of total phenolic content

Total phenolic content of the extract was determined using Folin – Ciocalteau's reagent (FC) (Stankovic, 2011). The extract solution was prepared at a concentration of 1 mg/mL in 50% DMSO in water. The reaction mixture was prepared by mixing 0.5 mL of extract solution, 2.5 mL of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5% Na_2CO_3. Blank, containing 0.5 mL of 50% DMSO, 2.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL of 7.5% of Na_2CO_3, was concomitantly prepared. The samples were allowed to stand at room temperature for 60 minutes. Absorbance of the mixture was determined using a spectrophotometer at λ_{max} = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of the absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid in methanol in the concentration range of 10 - 100 ug/mL and the calibration line was constructed. Linear regression of absorbance vs gallic acid concentration was determined based on a formula Y = ax+ b.

The phenolic content in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract) using the calibration line and equation 2.

$$T = C \times \frac{V}{M} \quad \text{----------} \quad \text{Equation 2}$$

T : Total phenolic content in mg/g of the extracts as GAE

C : the concentration of gallic acid established from the calibration curve in mg/mL

V : the volume of the extract solution in mL

M : the weight of the extract in g

2.2) Determination of total flavonoid content

The content of flavonoids in the *M. paniculata* leaf extract (MPE) was determined using aluminum chloride ($AlCl_3$) colorimetric method (Ordoñez et al, 2006). The extract solution at a concentration of 1 mg/mL was prepared in 50% DMSO in water. The tested samples contained 1 mL of the extract solution and 1 mL of 2% $AlCl_3$ solution dissolved in methanol. The tested samples were incubated for an hour at room temperature. The absorbance of tested samples was determined using a UV-spectrophotometer at λ_{max} = 415 nm against a blank solution consisting of the extract solution (1 mL) and methanol (1 mL) without aluminum chloride. The tested samples were prepared in triplicate for each analysis and the mean value of absorbance was calculated. The same procedure was repeated for the standard solution of quercetin in place of the extract solution and the calibration line between the absorbance vs quercetin concentration was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/mL) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of quercetin equivalent (mg of QE/g of extract).

The total flavonoid content can be calculated as a quercetin equivalent (QE) by the following equation:

$$T = C \times \frac{V}{M} \qquad \text{---------- Equation 3}$$

T : the total flavonoid content in mg/g of the extracts as QE

C : the concentration of quercetin established from the calibration curve in mg/mL

V : the volume of the extract solution in mL

M : the weight of the extract in g

2.3) Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) assay)

The antioxidant activity of the plant extract was evaluated using the standard method DPPH radical scavenging activity. The radical scavenging activity of the *M. Paniculata* leaf extraction (MPE) was determined according to the method modified from Zhu *et al.* (2015). The MPE at various concentrations (1.0-10.0 mg/mL) was prepared using the extraction solvents. A solution of DPPH reagent (0.1 mM) was prepared by dissolving 3.94 mg of DPPH in 100 mL methanol. DPPH solution (2.7 mL) was added in the extract solution (0.3 mL) and then incubated at room temperature in the dark for 30 min. UV absorbance at λ_{max} = 517 nm of the sample mixture was measured. If the extract provided antioxidant activity, the colour changed from purple to yellow. Blank was concomitantly prepared, containing 0.3 mL of the extract solution and 2.7 mL of methanol. The solution without the extract was used as the negative control, while Trolox was added in place of the extract being used as the positive control. Radical scavenging activity was calculated using equation 4. Due to the color of extract, the absorbance of each sample was subtracted by the absorbance of control before percent scavenging effect calculation (Equation 4).

$$\text{DPPH• scavenging effect (\%)} = 1 - \frac{A_{sample} - A_{MPEcontrol}}{A_{negative\ control}} \times 100 \quad \text{----- Equation 4}$$

A_{sample} : Absorbance of extract + DPPH

$A_{negative\ control}$: Absorbance of DPPH + methanol without extract

$A_{MPE\ control}$: Absorbance of extract + methanol (blank)

Percent scavenging effect and MPE concentration profiles from various extraction solvents were plotted. Then a linear equation of the relationship between the concentration and percent scavenging effect was obtained from each solvent. The IC_{50} value was calculated from the extract concentration at 50% scavenging effect. The efficiency of the extract obtained from different solvents was compared using IC_{50} values.

2.4) Tyrosinase inhibition activity

The tyrosinase inhibitory activity was measured using tyrosine as the substrate, according to the method developed by (Chen et al, 2009). The MPE at various concentrations (1.0-10.0 mg/mL) was prepared using 50% DMSO. The sample mixture was prepared by mixing mushroom tyrosinase aqueous solution (40 µL, 150 IU/mL), phosphate buffer (pH 6.8, 120 µL) and extract solutions (40 µL). Negative control was concomitantly prepared, containing phosphate buffer (pH 6.8, 160 µL) and mushroom tyrosinase aqueous solution (40 µL, 150 IU/mL). The mixture was preincubated at 37 ℃ for 5 minutes, and then mixed with 80 µL of 0.1 mg/mL L-Dopa. Kojic acid was used in place of the extract solution as a positive control. The mixture was also preincubated at 37 ℃ for 5 minutes, and then mixed with 80 µL of 0.1 mg/mL L-Dopa.

All mixtures were incubated at 37℃ for 30 minutes. The amount of dopachrome was measured from the absorbance at λ_{max} = 475 nm using a microplate spectrophotometer. The percent inhibition of tyrosinase activity was calculated using equation 5. Due to the color of extract solution, the absorbance of each sample was subtracted by that of extract solution before the percent inhibit calculation (Equation 5). Kojic acid was used as positive control.

$$\% \text{ inhibition} = 1 - \frac{A_{sample} - A_{MPEcontrol}}{A_{negative\ control}} \times 100 \quad \text{---------- Equation 5}$$

$A_{negative\ control}$: absorbance at 475 nm without the extract solution.

A_{sample} : absorbance at 475 nm with the extract solution.

$A_{MPEcontrol}$: absorbance of MPE without tyrosinase

The IC_{50} value was calculated from linear regression analysis and denoted the concentration of sample required to inhibit 50% of tyrosinase activity.

3.) Formulation of *M. paniculata* lotion

3.1) Solvent screening for the preparation

M. paniculata extract was dissolved in solvent combinations presenting in table 4. Appearance of the solutions was evaluated. Transparency of the formulas was compared. Clear solution without any precipitation was selected.

Table 4 Formula of solvent combinations

	formula 1	formula 2	formula 3	formula 4
Ingredient	**%w/w**	**%w/w**	**%w/w**	**%w/w**
M. paniculata extract	1	1	1	1
Propylene glycol	50	0	25	50
Glycerin	0	20	10	20
Ethanol	7.5	7.5	7.5	7.5
Phenoxyethanol	0.5	0.5	0.5	0.5
Purify water to	100	100	100	100

Stability of the extract in the formulas was measured using the amount of flavonoid content remaining at days 0, 10, 15 and, 30.

The solvent combination providing clear solution and the highest flavonoid remaining was selected for preparing MPE lotion in 3.2)

3.2 Preparation of MPE lotion

If necessary, a thickener might be added in the selected solvent combination from 3.1). Slightly viscous clear lotion was expected. After that, antioxidant activity of the formula was evaluated using DPPH method as presented in 2.3). The result was interpreted in term of %DPPH inhibition.

3.3 Stability of MPE lotion

The MPE lotion was stored at a temperature of 4 °C and 40 °C and protected from light for 1 month. The flavonoid remaining in the formulas and antioxidant activity at days 0, 10, 15 and, 30 were evaluated using $AlCl_3$ method colorimetric as in 2.2) and DPPH method as in 2.3) respectively. For antioxidant activity, the formula was prepared at three different concentrations; initial concentration, dilution in 5-fold (5X) and 10-fold (10X) of the initial concentration. The result was interpreted in %DPPH inhibition at days 0, 10, 15 and, 30 by considering the decrease of antioxidant activity.

4. Statistics analysis

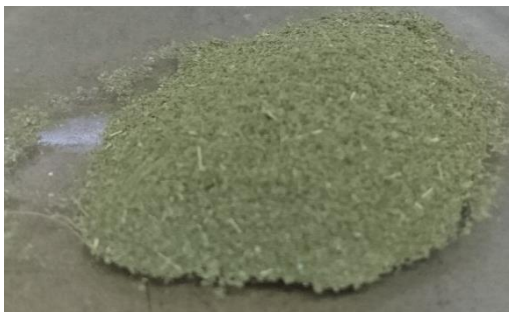
The data results from the 3 repeated experiments were shown as the mean value ± Standard deviation (SD). Statistical significance was determined by a one-way analysis of variance (ANOVA) followed by Turkey and Dunnett's multiple comparisons test. P values less than 0.05 were considered significant.

CHAPTER IV

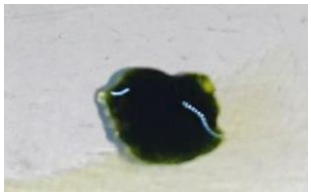
RESULTS AND DISCUSSIONS

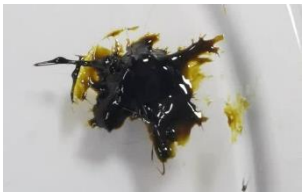
1.) Plant material and extraction

The consideration of solvent selection for use in this study was based on safety of the obtained extract. because the residue of some organic solvents may be toxic when used in cosmetic preparations. Therefore, ethanol and hydroalcoholic solvent were used in this study where water could adjust polarity in hydroalcoholic solvent. by mixing with water in various ratios. Methanol was chosen because it was reported to be superior to other solvents in solvent extraction process. When powder of dried *M. paniculata* leaf was macerated in different solvents, the solvents turned dark green. After solvent evaporation, all four *M. paniculata* extracts (MPE) turned to be viscous liquid. the appearance of methanolic MPE and ethanolic MPE were deep green viscous liquid appearance. The extract from 50% ethanol and 75% ethanol were green-yellow viscous liquid. The obtained MPEs were presented in figure 7. The difference in extract color may be due to compounds in the extract. The green color in methanolic MPE and ethanolic MPE may be from chlorophyll. The color of 50% ethanolic MPE and 75% ethanolic MPE were yellowish-green, probably, due to flavonoid substances, such as ellagic acid, rutin, etc. Thus, further characterization was needed.



a)





b) c) d) e)

Figure 7 Appearance of a) dried plant, b) methanolic MPE, c) ethanolic MPE, d) 50% Ethanolic MPE, e) 75% Ethanolic MPE

Table 5 The yields of *M. paniculata* leaf extract from various solvents.

Extraction solvent	% Yield of extract
Ethanol	8.60 ± 0.07
Methanol	15.39 ± 1.07
50% ethanol	15.25 ± 0.46
75% ethanol	18.49 ± 1.22

The yields of extract obtained from 20 g of dried plant material using different solvents were presented in Table 5. The highest yield was obtained using 75% ethanol as the extraction solvent. In this research, polarity of these extraction solvents was different causing different extraction yield. It probably implied that *M. paniculata* leaf contained semi-polar compounds and could be effectively extracted using 75% ethanol. These results were consistent with a previous study in 2017

which found that MPE obtained from ethanol provided the highest yield of extract when compared to petroleum ether (non-polar), chloroform (low polarity) and water (polar) (Sharma et al, 2017).

2.) Characterization

Table 6 Total phenolic content and total flavonoid content of *M. paniculata* leaf extract from various extraction solvents.

Extraction solvent	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)
Ethanol	50.79 ± 0.48	3.23 ± 0.18
Methanol	47.74 ± 0.66	3.61 ± 0.14
50% ethanol	49.38 ± 0.58	3.45 ± 0.19
75% ethanol	66.12 ± 7.30	7.83 ± 1.07

2.1 Total phenolics content

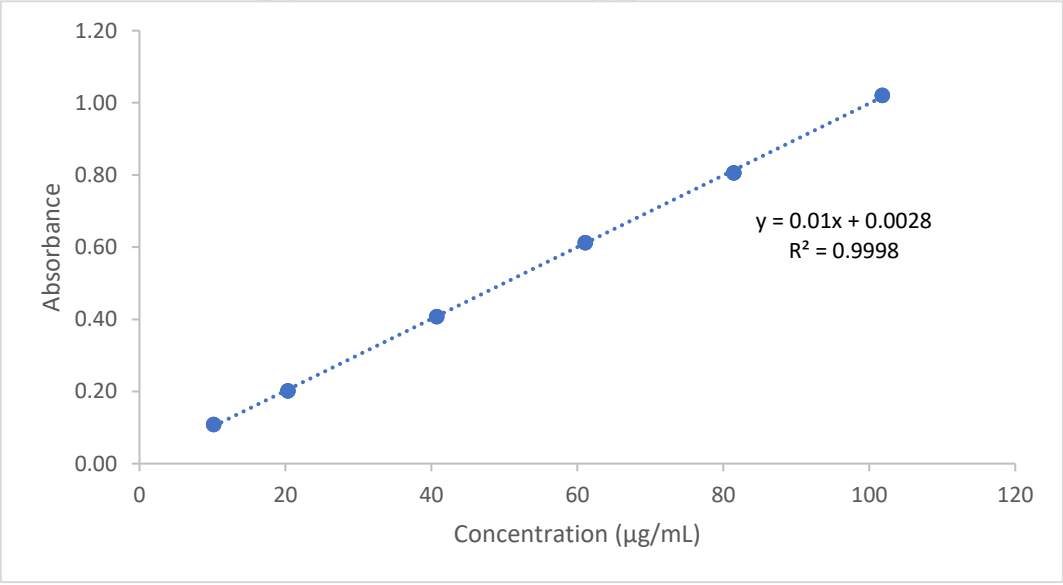


Figure 8 Standard curve of gallic acid

The total phenolic contents in the extracts using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalent. The calibration curve from various concentrations of the standard gallic acid yielded the following equation: y = 0.001x + 0.0028 (R^2= 0.9998) as shown in Figure 8. The total phenolic content of MPE was calculated based on the above equation and reported as gallic acid equivalents (Table 6). Among four extraction solvents, 75% ethanolic showed the highest amount of phenolic content (57.90 ± 0.05 mg GAE/g) and the lowest total phenolic content was obtained from methanolic solvent extract.

2.2 Total flavonoid content

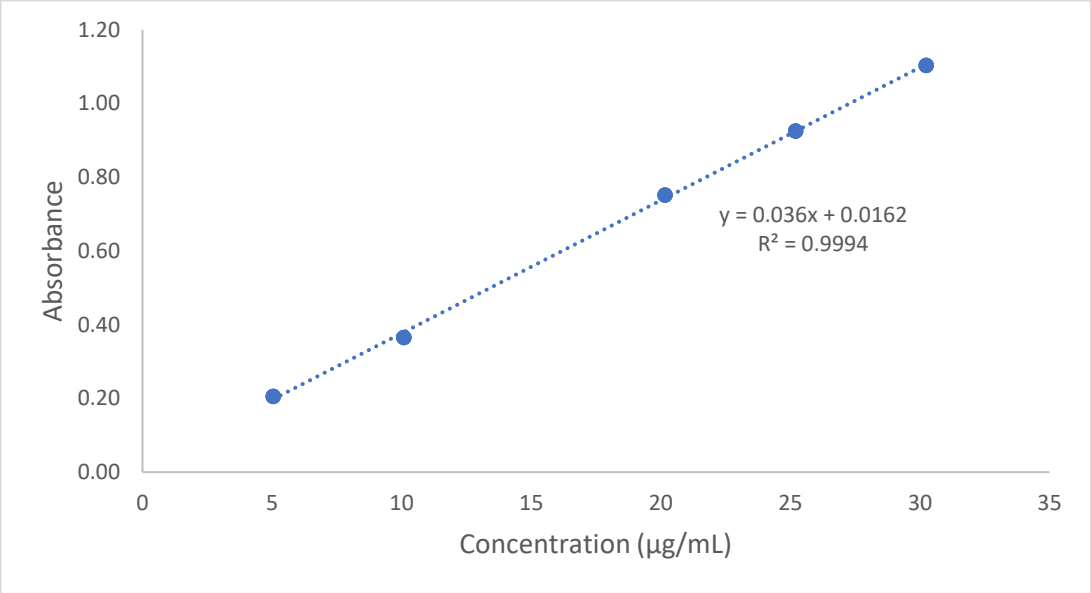


Figure 9 Standard curve of quercetin

The concentration of flavonoids in MPE from different extraction solvents was determined using spectrophotometric method. The calibration curve from various concentrations of the standard quercetin yielded the following equation: y = 0.036x + 0.0162 (R^2= 0.9994) as presented in Figure 9. The total flavonoid content of MPE was calculated and reported as quercetin equivalents (Table 6). The highest flavonoid content was obtained from 75% ethanolic solvent extract (6.74 ± 0.03 mg QE/g)

The flavonoid content in the MPE also depended on properties of the extraction solvents, i.e. the polarity of solvent in the extract process. From the result, 75% ethanol solvent extraction provided the highest contents of phenolic and flavonoids compounds. Hence, 75% ethanol was the most suitable solvent for phenolic and flavonoid extractions from *M. paniculata* leaf.

2.3 Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) assay)

Table 7 DPPH scavenging activity of various extracts of *M. paniculata* leaf

Extraction solvent	**IC_{50} (mg/mL)**
Ethanol	2.35 ± 0.19
Methanol	2.10 ± 0.13
50% ethanol	2.26 ± 0.32
75% ethanol	1.43 ± 0.14
Trolox (control)	0.06 ± 0.01

The antioxidant activity of four different extracts from *M. paniculata* leaf was determined and expressed in terms of IC_{50} values (mg/mL) compared with the standard substance (Trolox) (Table 7). Trolox was a positive control because Trolox is a polar derivative of vitamin E. Both Trolox and flavonoids contain a Chromanol ring structure providing antioxidant activity. The mechanism of the flavonoids and Trolox may be similar. The IC_{50} values of extracts were found to be within the range of 1.58 to 2.96 mg/mL where the highest free radical scavenging activity was demonstrated by 75% ethanolic extract (IC_{50}= 1.58 ± 0.15 mg/mL). The difference in IC_{50} values of DPPH• scavenging activities of 50% ethanolic, methanolic and, ethanolic extracts were statistically insignificant. The result implied that more active compounds could be extracted by 75% ethanol. However, antioxidant activity of all MPE was still less potent than Trolox, the positive control. From the result, the extract was obtained from 75% Ethanol was chosen to prepare MPE lotion in the next step because the extract had the highest antioxidant activity.

Correlation of antioxidant activity VS total phenolic content/total flavonoid content was determined. The extract performing the highest antioxidant activity had the highest contents of phenolic compounds and flavonoids. Both phenolic compounds and flavonoids provide strong antioxidant activity. Antioxidant activity of phenolic compounds and flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al, 2009; Stankovic, 2011; TOSUN et al, 2009). The purpose of this part was to compare the correlations between total phenolic content/total flavonoid content and antioxidant activity.

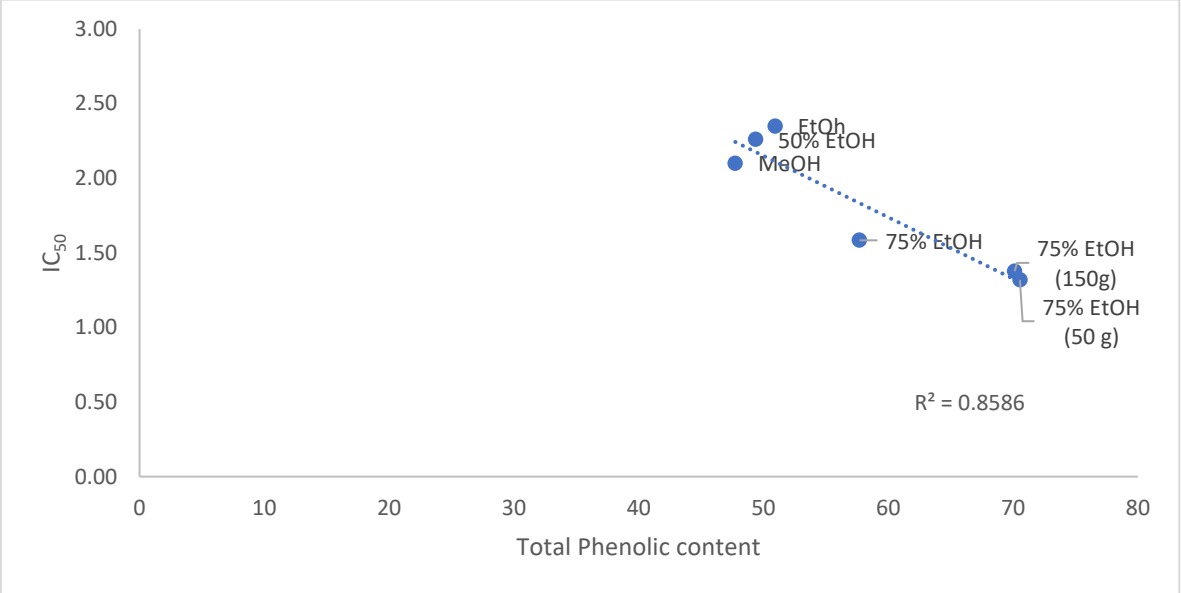


Figure 10 Linear correlation between total phenolic contents and antioxidant activity of the different extracts.

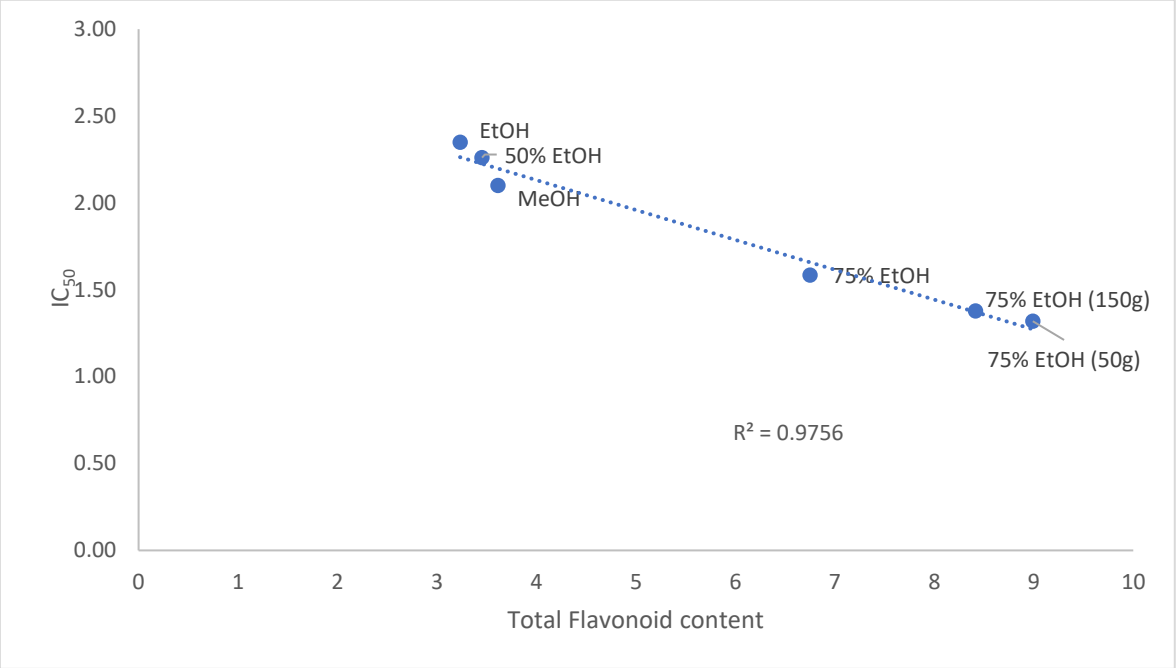


Figure 11 Linear correlation between total flavonoids contents and antioxidant activity of the different extracts.

Regarding the linear correlations, the coefficient of determination (R^2) obtained from total phenolic content and total flavonoid content was 0.8586 and 0.9769, respectively (Figure 10 and 11). The plot of total flavonoid content presented a better correlation. It could be explained that some phenolic compounds did not express antioxidant activity.

1.4 Tyrosinase inhibition activity

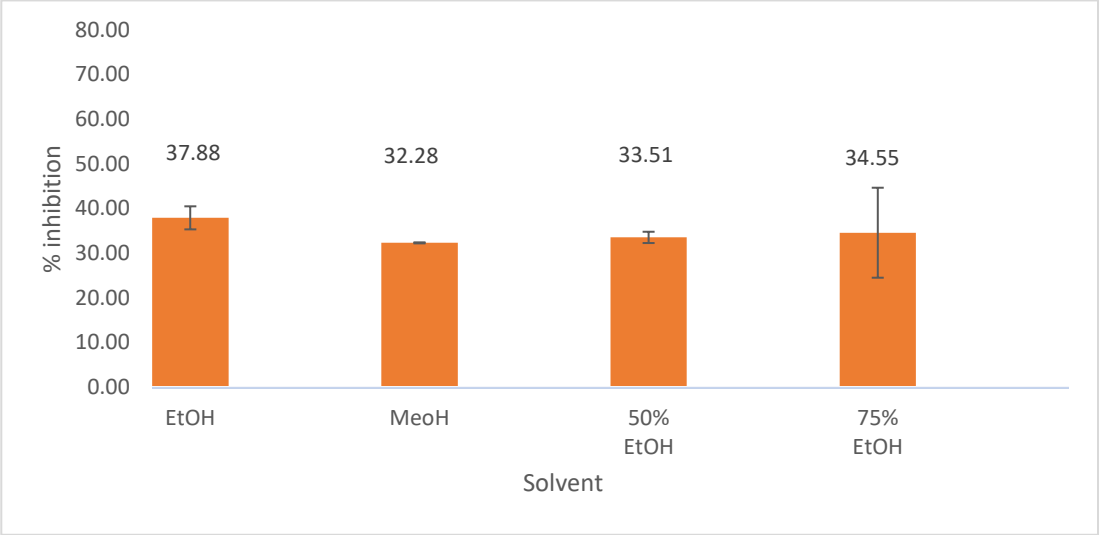


Figure 12 Tyrosinase Inhibition activity of *M. paniculata* leaf from different extraction solvents.

All extracts were evaluated for tyrosinase inhibitory activity. The IC_{50} value of tyrosinase inhibition could not be analyzed due to the low activity of the extracts. Thereby, the results were expressed as %inhibition at 1 mg/mL of each extract instead of IC_{50} value because this concentration was the maximum concentration that the extract could be prepared without precipitation. The result showed that all the extracts provided the tyrosinase inhibition activity less than 40% at 1 mg/mL while kojic acid, a positive control, had an IC_{50} value of 0.28 mg/mL.

These results were inconsistent with a previous study which found that MPE at a concentration of 500 µg/mL could inhibit the enzyme tyrosinase up to 70% (Chen et al, 2009). It is possible that our study used the more polar solvents than the previous study (acetone). Moreover, a different source of plant materials, resulted in the different efficacy.

The results indicated that MPE extract from the highest polar solvent did not present the highest extract yield or highest antioxidant activity. This study had shown

that 75% ethanol was the optimal solvent condition for *M. Paniculata* leaf since the highest yield and antioxidant activity were obtained from this condition.

3.) Formulation of *M. paniculata* lotion

3.1) Solvent screening for the preparation

M. paniculata extract obtained from 75% ethanolic extraction was used for the preparation. The MPE lotion was prepared at 1% MPE because the concentration at 1 %w/w was the highest concentration that MPE could dissolve in the formula and this concentration was 7 times of the IC50 value of the extract based on antioxidant activity.

Glycerin and propylene glycol were used as co-solvents which were used at different concentrations in each formula. Formula 1 contained propylene glycol as a co-solvent at a concentration of 50 %w/w because this concentration is the highest concentration that can be used without irritation (Fiume, et al. 2012). Formula 2 contained glycerin at a concentration of 20 %w/w because it is the highest concentration that can be used without irritation (Wilma et al. 2014). Formula 3 were a combination of glycerin (10 %w/w) and propylene glycol (25 %w/w) which are a half of the maximum concentrations. Lastly, formula 4 were combination of glycerin and propylene glycol with the maximum concentration of both co-solvents.

Phenoxyethanol was used as a preservative in the formulas, although these formulas contained ethanol and propylene glycol at a high concentration and this might lead to have self-preserve property. However, phenoxyethanol was added to reduce the risk of microbial contamination and also broaden spectrum of antimicrobial activity because the formula contained a reasonable amount of natural extract, which might facilitate the growth of microorganisms. The appearance of all 4 formulas are following;

Formulation 1: yellowish-green solution, precipitation and low viscosity.

Formulation 2: deep yellowish-green, obvious precipitation and low viscosity.

Formulation 3: yellowish green solution, rather obvious precipitation and low viscosity.

Formulation 4: yellowish-green solution, clear, no precipitation and slight viscous.

Regarding the ability to dissolve MPE in formula 1-4, clear solution of 1% MPE was received only from formula 4. Precipitation was found in formula 1-3. Thus, formula 4 was carried on in next study.

Figure 13 Appearance of MPE in different solution

3.2 Preparation of MPE lotion

The final formula of MPE lotion could be prepared as Presented in Table 8. The selected lotion was clear, and slightly viscous. The drop of lotion did not freely flow when it was on skin and it was applied easily without stickiness and remaining color after applied. Then no thickener was added in this formula.

Table 8 MPE lotion formula

Ingredient	%w/w
M. paniculata extract	1
Propylene glycol	50
Glycerin	20
Ethanol	7.5
Phenoxyethanol	0.5
Purify water to	100

Figure 14 Appearance of MPE lotion

The appearance of the MPE lotion was a clear solution, yellowish-green color, no precipitation, low viscosity and light odor of the extract. The pH value of the formula was 6.61, which is in a stable flavonoid pH range (5 - 7) (Srivastava & Gupta, 2009).

The antioxidant activity of MPE lotion was evaluated. The result showed that the DPPH scavenging activity of MPE lotion presented 92.91 ± 0.27 % inhibition compared to the formula without MPE where the inhibition was 0%.

This lotion provided antioxidant activity as aspired. The site of action is at the viable epidermis layer. Since the active ingredients in MPE are semi-polar compounds, they have poor permeability through the stratum corneum. The formula contained a high concentration of propylene glycol which may increase the

permeability of the active compounds. However, the skin permeation of this formula should be studied in the future.

3.3 Stability of MPE lotion

Stability studies on MPE lotion was performed in different temperature. Temperature plays an important role and increasing temperature had an effect on flavonoid degradation (Sharma et al, 2015). In this study, the MPE lotion was stored in 4°C and 40°C for 30 days.

a) Store at 4 °C b) store at 40 °C

Figure 15 Appearance of MPE lotion after 1 month

After 1 month of storage, the appearance of MPE lotion did not change. As shown in Figure 15, MPE was still clear without precipitation.

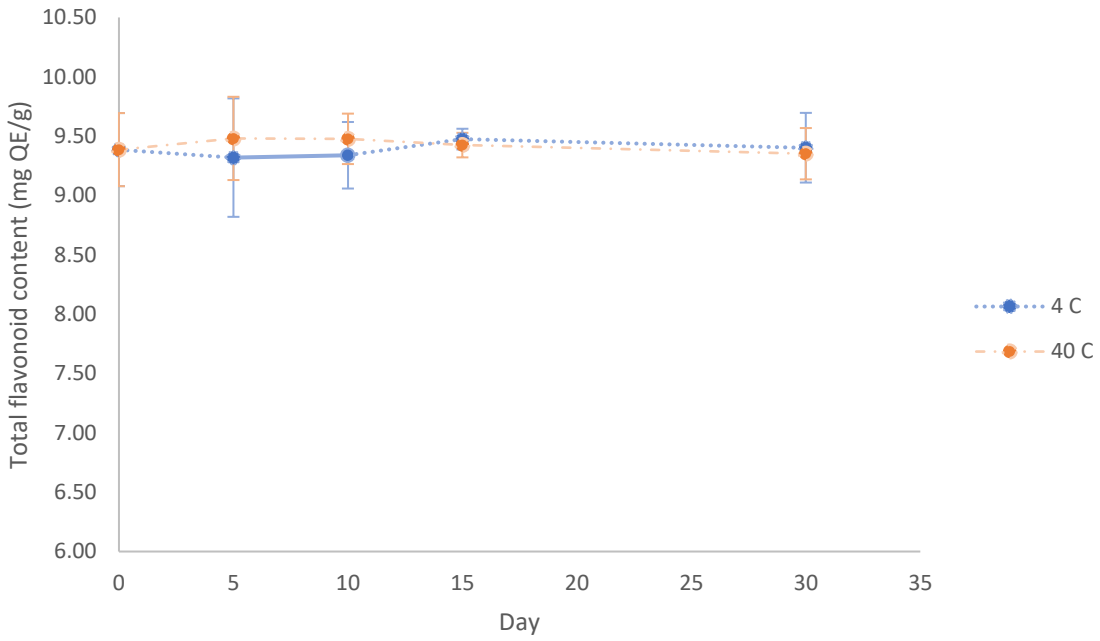
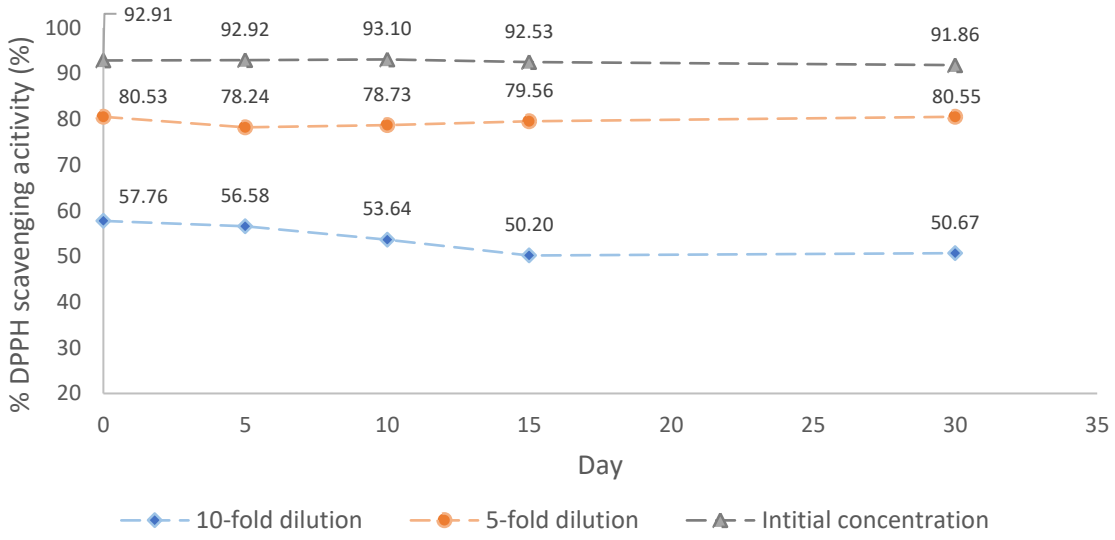


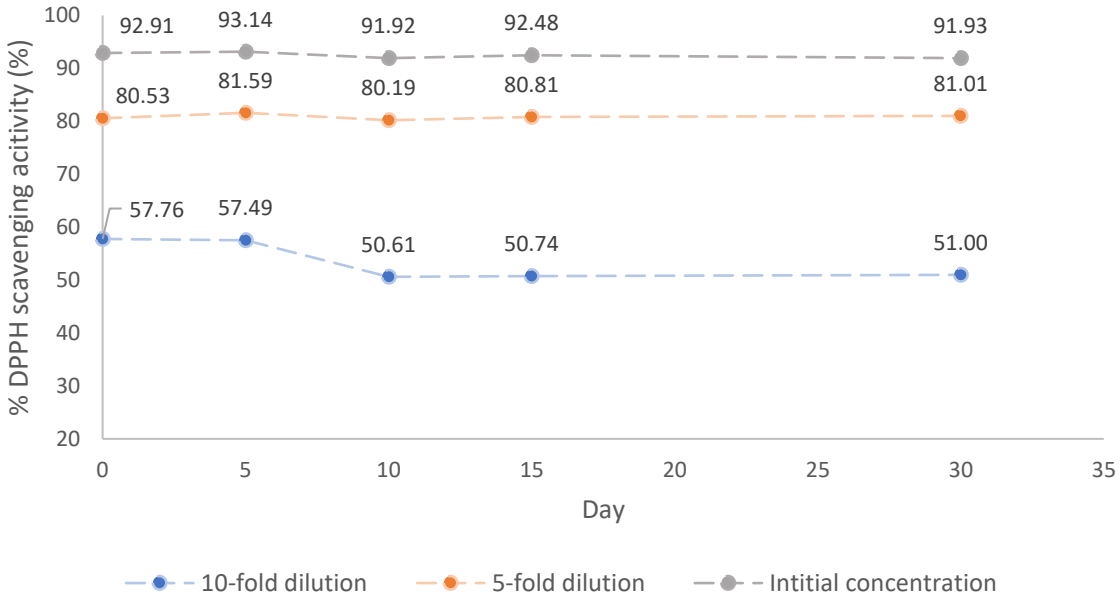
Figure 16 Stability of flavonoid in MPE lotion

The stability of MPE lotion was monitored using the total flavonoid content that remains in formula and antioxidant activity during storage.

The total flavonoid content vs time profile is presented in Figure 16. No significant change of the flavonoid content was observed in both storage temperatures by comparing at the same temperature on different days (ANOVA and Dunnett's test). If means that flavonoids in the MPE lotion did not degrade to be other compounds. In the extraction process, the solvent evaporation temperature was 40 °C. Therefore, the remaining compound could withstand at 40 °C.



a) storage at 4 ℃

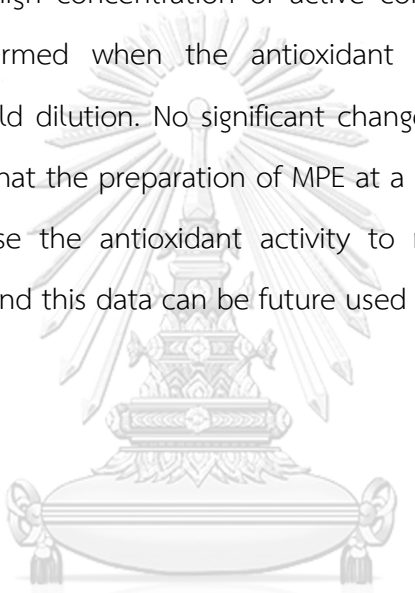


b) storage at 40 ℃

Figure 17 Antioxidant activity of MPE lotion

Antioxidant activity of MPE lotion was determined at initial concentration and after dilution. The Initial concentration of MPE in the lotion was 1 %w/w which was

approximately 7 times of IC_{50} (DPPH scavenging activity). Thus, above 90% inhibition was expected and this activity did not significantly change during storage in both storage conditions (ANOVA and Dunnett's test). Then the antioxidant activity of MPE lotion was measured again after dilution. The slight decrease of antioxidant activity was observed at 10-fold dilution after 10 days at 40 °C. This result implied that there was change of the active ingredients in MPE lotion. However, the antioxidant activity of MPE lotion at initial concentration did not change during storage due to the compensation of the high concentration of active compounds in MPE lotion. This explanation was confirmed when the antioxidant activity of MPE lotion was measured again at 5-fold dilution. No significant change of the activity presented at this dilution. It means that the preparation of MPE at a concentration of 1 %w/w was strong enough to cause the antioxidant activity to remain the same under the accelerated condition and this data can be future used in cosmetic preparations.



CHAPTER V
CONCLUSION

Murraya paniculata leaf was extract using 4 solvents; methanol, ethanol, 50% ethanol, and 75% ethanol.

The appearance of all *M. paniculata extract* (MPE) was viscous liquid and green odor. Methanolic MPE and ethanolic MPE were deep green. 75% ethanolic MPE and 50% ethanolic MPE were green-yellow. Results showed that MPE obtained from 75% ethanol had the highest %yield of extraction at 18.49% and also contained the highest total phenolic content and flavonoid content compared to other MPEs. The lowest yield was obtained using ethanol.

Regarding the DPPH assay, 75%ethanolic MPE provided the highest antioxidant activity. The difference in IC_{50} values of DPPH radical scavenging activities of 50% ethanolic, methanolic and, ethanolic extracts were statistically insignificant. In the study of tyrosinase inhibition activity, all of MPEs had low activity.

In the extracts, flavonoids had more potential to contribute antioxidant activity than phenolic compounds based on the linear correlation of these compounds and their antioxidant activity. The obtained MPE lotion contained 1% w/w of 75% ethanolic MPE 50% w/w of propylene glycol, 20% w/w of glycerin, and 7.5% w/w of ethanol.

Finally, the stability test of MPE lotion found that flavonoids content remained constant in this formula when stored at 4 ℃ and 40 ℃ for 1 month and there was no significant change of the antioxidant activity at the concentration of 1 %w/w.

Future studies, a longer period of stability study and preparation in other formulas should be investigated. In addition, the studies on *in vivo* efficacy and safety of the product are required.

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APPENDIX

Comparison of %yield of extraction

ANOVA

%yield of extract

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	127.660	3	42.553	88.941	.000
Within Groups	3.828	8	.478		
Total	131.488	11			

Multiple Comparisons

Dependent Variable: %yield of extract

Tukey HSD

(I) Solvent	(J) Solvent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	Methanol	-6.7897004*	.5647695	.000	-8.598291	-4.981110
	50% EtOH	-6.6505850*	.5647695	.000	-8.459176	-4.841994
	75%EtOH	-8.5522407*	.5647695	.000	-10.360831	-6.743650
Methanol	Ethanol	6.7897004*	.5647695	.000	4.981110	8.598291
	50% EtOH	.1391154	.5647695	.994	-1.669475	1.947706
	75%EtOH	-1.7625403	.5647695	.056	-3.571131	.046050
50% EtOH	Ethanol	6.6505850*	.5647695	.000	4.841994	8.459176
	Methanol	-.1391154	.5647695	.994	-1.947706	1.669475
	75%EtOH	-1.9016557*	.5647695	.040	-3.710246	-.093065
75%EtOH	Ethanol	8.5522407*	.5647695	.000	6.743650	10.360831
	Methanol	1.7625403	.5647695	.056	-.046050	3.571131
	50% EtOH	1.9016557*	.5647695	.040	.093065	3.710246

*. The mean difference is significant at the 0.05 level.

Comparison of total phenolic contents

ANOVA

Total phenolic content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	172.501	3	57.500	185.871	.000
Within Groups	2.475	8	.309		
Total	174.976	11			

Multiple Comparisons

Dependent Variable: Total phenolic content

Tukey HSD

(I) Solvent	(J) Solvent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	Methanol	3.0513492*	.4541331	.001	1.597055	4.505643
	50% EtOH	1.4141715	.4541331	.057	-.040122	2.868465
	75%EtOH	-6.9048729*	.4541331	.000	-8.359167	-5.450579
Methanol	Ethanol	-3.0513492*	.4541331	.001	-4.505643	-1.597055
	50% EtOH	-1.6371777*	.4541331	.029	-3.091472	-.182884
	75%EtOH	-9.9562221*	.4541331	.000	-11.410516	-8.501928
50% EtOH	Ethanol	-1.4141715	.4541331	.057	-2.868465	.040122
	Methanol	1.6371777*	.4541331	.029	.182884	3.091472
	75%EtOH	-8.3190445*	.4541331	.000	-9.773338	-6.864751
75%EtOH	Ethanol	6.9048729*	.4541331	.000	5.450579	8.359167
	Methanol	9.9562221*	.4541331	.000	8.501928	11.410516
	50% EtOH	8.3190445*	.4541331	.000	6.864751	9.773338

*. The mean difference is significant at the 0.05 level.

Comparison of total flavonoid contents

ANOVA

Total flavonoid content

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.977	3	8.326	377.656	.000
Within Groups	.176	8	.022		
Total	25.153	11			

Multiple Comparisons

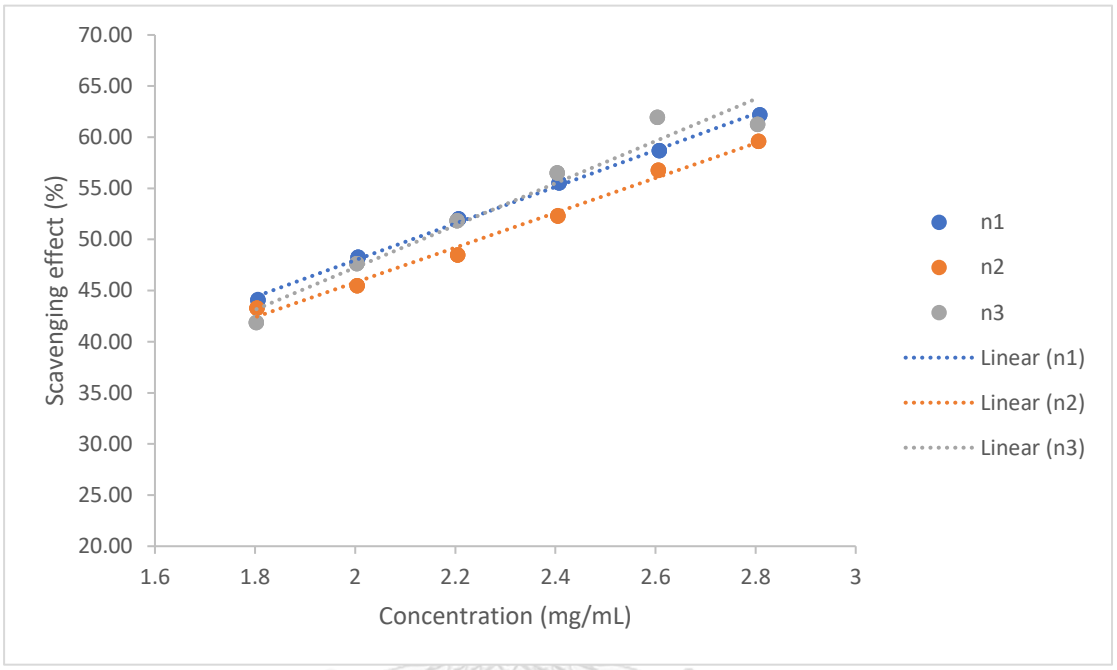
Dependent Variable: Total flavonoid content

Tukey HSD

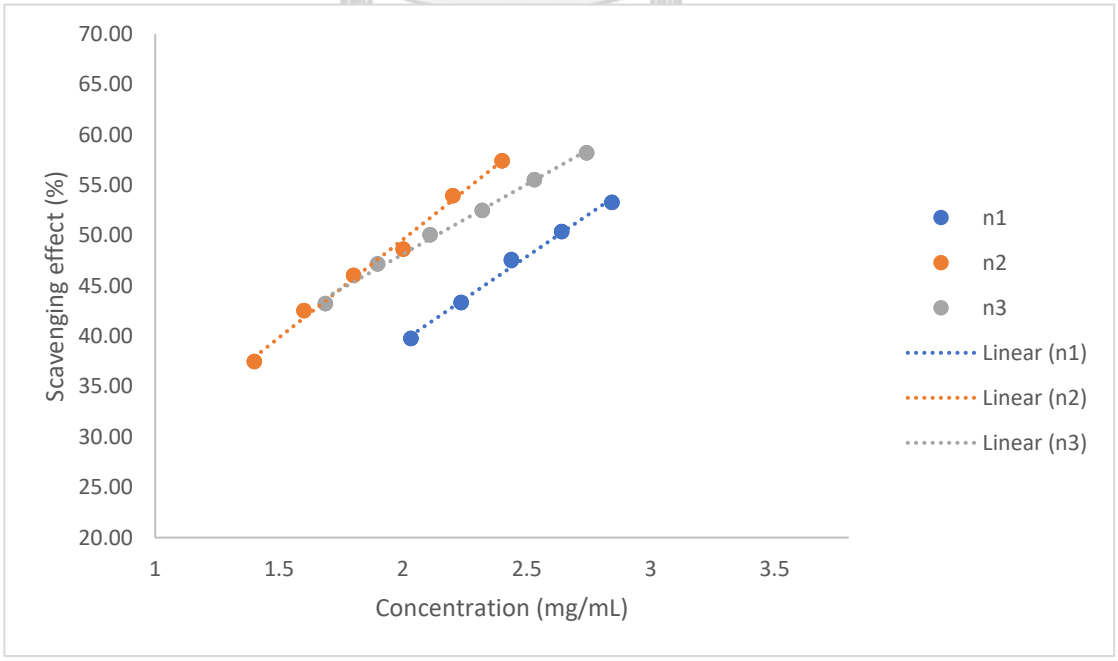
(I) Solvent	(J) Solvent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	Methanol	-.3812011	.1212316	.054	-.769427	.007025
	50% EtOH	-.2207796	.1212316	.331	-.609006	.167447
	75%EtOH	-3.5177667*	.1212316	.000	-3.905993	-3.129541
Methanol	Ethanol	.3812011	.1212316	.054	-.007025	.769427
	50% EtOH	.1604215	.1212316	.575	-.227805	.548648
	75%EtOH	-3.1365656*	.1212316	.000	-3.524792	-2.748339
50% EtOH	Ethanol	.2207796	.1212316	.331	-.167447	.609006
	Methanol	-.1604215	.1212316	.575	-.548648	.227805
	75%EtOH	-3.2969871*	.1212316	.000	-3.685213	-2.908761
75%EtOH	Ethanol	3.5177667*	.1212316	.000	3.129541	3.905993
	Methanol	3.1365656*	.1212316	.000	2.748339	3.524792
	50% EtOH	3.2969871*	.1212316	.000	2.908761	3.685213

*. The mean difference is significant at the 0.05 level.

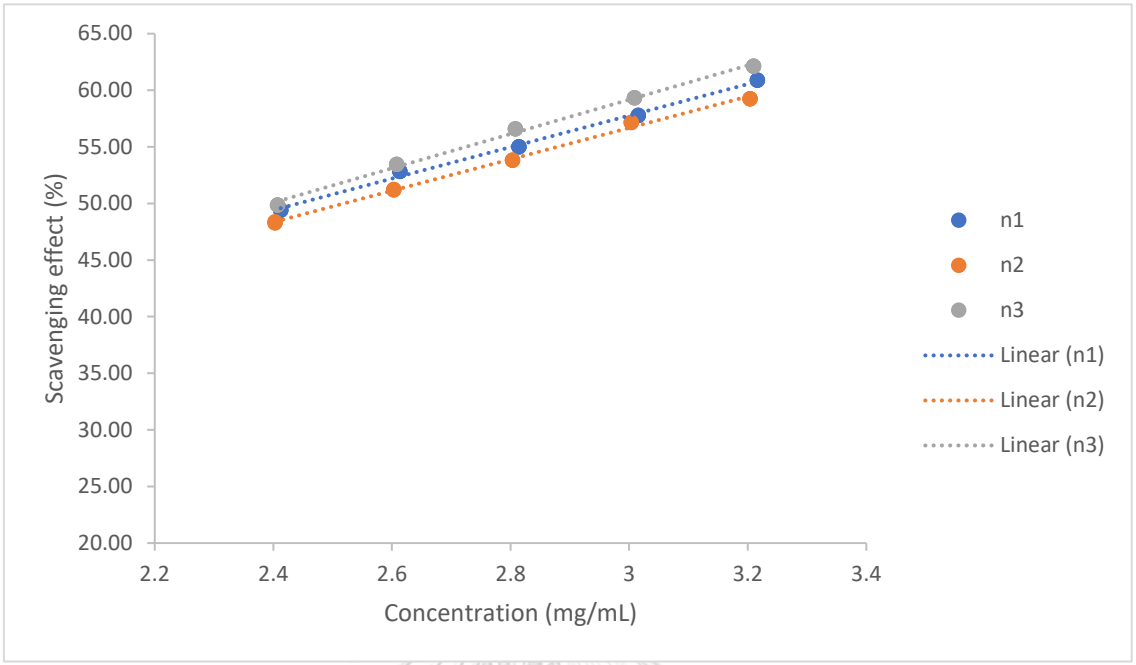
Profiles of percent scavenging activity vs MPE concentration from different solvents



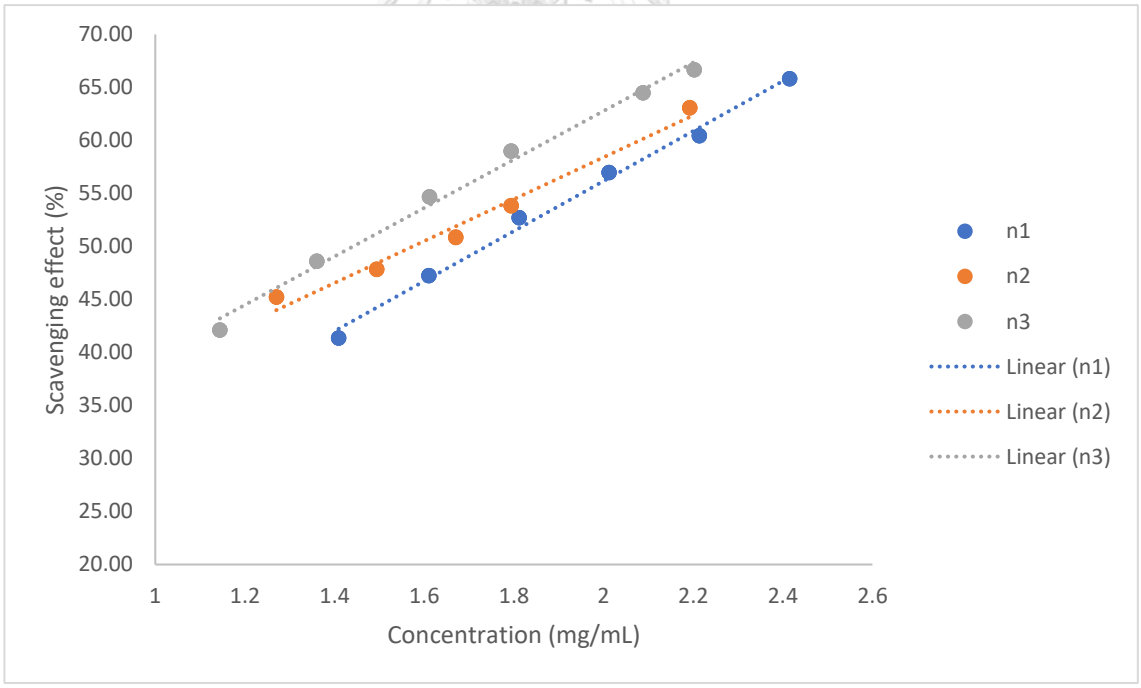
Methanol MPE



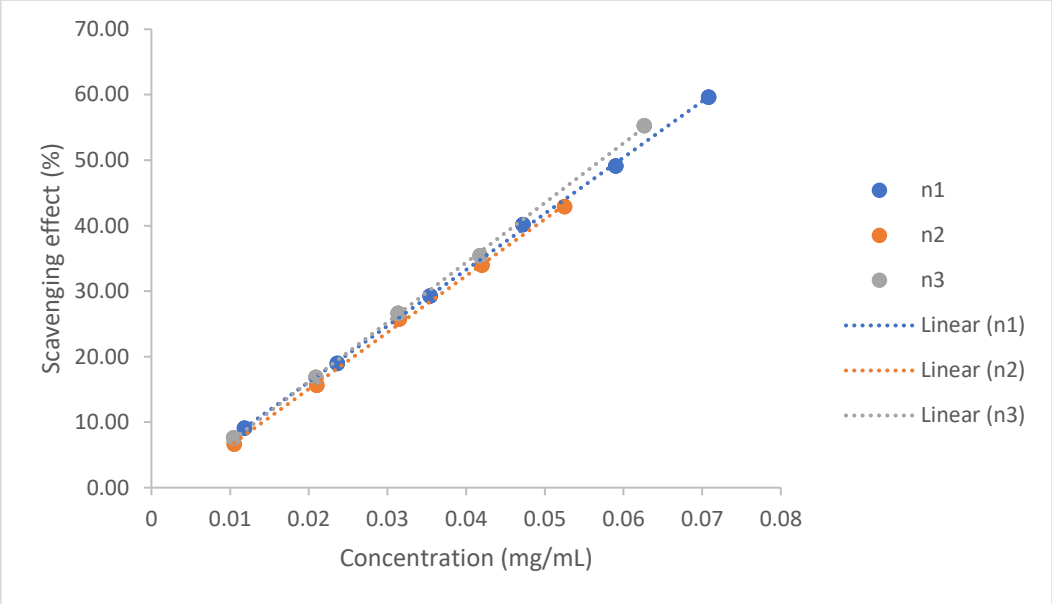
50% Ethanol MPE



Ethanol MPE



75% Ethanol MPE



Trolox

Comparison of DPPH scavenging activity

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total flavonoid at 4 C	Between Groups	.045	4	.011	.108	.977
	Within Groups	1.033	10	.103		
	Total	1.077	14			
Total flavonoid at 40 C	Between Groups	.036	4	.009	.141	.963
	Within Groups	.641	10	.064		
	Total	.677	14			

Multiple Comparisons

Dependent Variable: EC50 (linear)

Tukey HSD

(I) Solvent	(J) Solvent	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	Methanol	.2481667	.1548365	.527	-.261413	.757746
	50% EtOH	.0881333	.1548365	.977	-.421446	.597713
	75%EtOH	.7648333*	.1548365	.004	.255254	1.274413
	Trolox	2.2895000*	.1548365	.000	1.779920	2.799080
Methanol	Ethanol	-.2481667	.1548365	.527	-.757746	.261413
	50% EtOH	-.1600333	.1548365	.835	-.669613	.349546
	75%EtOH	.5166667*	.1548365	.047	.007087	1.026246
	Trolox	2.0413333*	.1548365	.000	1.531754	2.550913
50% EtOH	Ethanol	-.0881333	.1548365	.977	-.597713	.421446
	Methanol	.1600333	.1548365	.835	-.349546	.669613
	75%EtOH	.6767000*	.1548365	.010	.167120	1.186280
	Trolox	2.2013667*	.1548365	.000	1.691787	2.710946
75%EtOH	Ethanol	-.7648333*	.1548365	.004	-1.274413	-.255254
	Methanol	-.5166667*	.1548365	.047	-1.026246	-.007087
	50% EtOH	-.6767000*	.1548365	.010	-1.186280	-.167120
	Trolox	1.5246667*	.1548365	.000	1.015087	2.034246
Trolox	Ethanol	-2.2895000*	.1548365	.000	-2.799080	-1.779920
	Methanol	-2.0413333*	.1548365	.000	-2.550913	-1.531754
	50% EtOH	-2.2013667*	.1548365	.000	-2.710946	-1.691787
	75%EtOH	-1.5246667*	.1548365	.000	-2.034246	-1.015087

*. The mean difference is significant at the 0.05 level.

Stability of flavonoid in MPE lotion

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Day of stability	(J) Day of stability	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Total flavonoid at 4 C	day 5	day 0	-.06737	.26239	.997	-.8258	.6911
	day 10	day 0	-.04763	.26239	.999	-.8061	.7108
	day 15	day 0	.08877	.26239	.990	-.6697	.8472
	day 30	day 0	.01600	.26239	1.000	-.7424	.7744
Total flavonoid at 40 C	day 5	day 0	.08830	.20667	.978	-.5091	.6857
	day 10	day 0	.09070	.20667	.975	-.5067	.6881
	day 15	day 0	.03813	.20667	.999	-.5592	.6355
	day 30	day 0	-.03463	.20667	.999	-.6320	.5627

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Stability of DPPH scavenging activity of MPE lotion for 1 month

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
int. conc at 4c	Between Groups	1.671	4	.418	6.710	.007
	Within Groups	.622	10	.062		
	Total	2.293	14			
5 fold at 4c	Between Groups	12.496	4	3.124	20.447	.000
	Within Groups	1.528	10	.153		
	Total	14.023	14			
10 fold at 4c	Between Groups	138.432	4	34.608	514.387	.000
	Within Groups	.673	10	.067		
	Total	139.105	14			
int. conc at 40c	Between Groups	3.088	4	.772	5.446	.014
	Within Groups	1.418	10	.142		
	Total	4.506	14			
5 fold at 40c	Between Groups	3.376	4	.844	9.533	.002
	Within Groups	.885	10	.089		
	Total	4.262	14			
10 fold at 40c	Between Groups	168.851	4	42.213	371.155	.000
	Within Groups	1.137	10	.114		
	Total	169.988	14			

Multiple Comparisons

Dunnett t (2-sided)[a]

Dependent Variable	(I) Day of stability	(J) Day of stability	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
int. conc at 4c	day 5	day 0	.42667	.20370	.178	-.1621	1.0155
	day 10	day 0	.54667	.20370	.070	-.0421	1.1355
	day 15	day 0	.04000	.20370	.999	-.5488	.6288
	day 30	day 0	-.39000	.20370	.233	-.9788	.1988
5 fold at 4c	day 5	day 0	-2.28333*	.31914	.000	-3.2058	-1.3609
	day 10	day 0	-1.68667*	.31914	.001	-2.6091	-.7642
	day 15	day 0	-.97000*	.31914	.039	-1.8925	-.0475
	day 30	day 0	.02333	.31914	1.000	-.8991	.9458
10 fold at 4c	day 5	day 0	-1.17667*	.21179	.001	-1.7888	-.5645
	day 10	day 0	-4.11667*	.21179	.000	-4.7288	-3.5045
	day 15	day 0	-7.56000*	.21179	.000	-8.1722	-6.9478
	day 30	day 0	-7.08000*	.21179	.000	-7.6922	-6.4678
int. conc at 40c	day 5	day 0	.65333	.30743	.170	-.2353	1.5420
	day 10	day 0	-.57667	.30743	.247	-1.4653	.3120
	day 15	day 0	-.01667	.30743	1.000	-.9053	.8720
	day 30	day 0	-.56667	.30743	.259	-1.4553	.3220
5 fold at 40c	day 5	day 0	1.06667*	.24295	.005	.3644	1.7689
	day 10	day 0	-.34000	.24295	.470	-1.0423	.3623
	day 15	day 0	.27333	.24295	.639	-.4289	.9756
	day 30	day 0	.49333	.24295	.196	-.2089	1.1956
10 fold at 40c	day 5	day 0	-.26333	.27536	.746	-1.0593	.5326
	day 10	day 0	-7.14667*	.27536	.000	-7.9426	-6.3507
	day 15	day 0	-7.01667*	.27536	.000	-7.8126	-6.2207
	day 30	day 0	-6.75667*	.27536	.000	-7.5526	-5.9607

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

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