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Determination of flavonoid content and antioxidant activity from ferns by ultrasonic extraction

Jutarat Tawekijpokai*, Jindarat Pimsamarn

Department of Chemical Engineering, Faculty of Engineering,
King Mongkut's University of Technology Thonburi, Bangkok, 10140, Thailand
*e-mail: am_juju@hotmail.com

Abstract – Flavonoid is a group of phenolic compounds commonly found in plants, which has antioxidant property. The objective of this research is to determine flavonoid content and antioxidant activity from ferns such as *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. by ultrasonic extraction with different solvents including acetone, ethanol and ethyl acetate. The extracts were analyzed for flavonoid content by aluminium chloride colorimetric method and antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. As a result, ethanol extraction of all ferns showed the highest content of flavonoid content and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. The flavonoid content of *D. esculentum (Retz.) Swartz*., *M. crenata Presl*. and *D. querciforia (L.) J. Sm*. were 19.974, 11.202 and 7.573 mg QE / g dried, respectively. The percentage inhibition values of *D. esculentum (Retz.) Swartz*., *M. crenata Presl*. and *D. querciforia (L.) J. Sm*. were 24.590, 27.644 and 13.326, respectively. A correlation between flavonoid content and antioxidant activity was observed with the significant correlation coefficient 0.326.

Keyword*: Ferns, flavonoid contents, antioxidant activity, DPPH assay, ultrasonic extraction*

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1. Introduction

Conventional extraction such as heating, boiling or refluxing can be used to extract flavonoids. However, the disadvantages are the loss of flavonoids due to hydrolysis, oxidation and ionization during extraction as well as the long extraction time [1]. Nowadays, the technique of ultrasonic extraction has attracted much attention due to simple, rapid, efficient and low cost. Ultrasonic extraction from medicine herb is based on the interaction among chemical compounds present in the plant with extracting media. Thus, ultrasound exerts a mechanical effect, allowing greater penetration of solvent into the tissue of fiber, increasing the contact surface area between fiber and liquid phase [2].

Flavonoids are polyphenols abundantly found in fruits, vegetables, and herbs. Flavonoids are important components of a healthy diet because of the antioxidant activity. It is suggested that flavonoids may prevent diseases such as cancer and inhibit low density lipoprotein (LDL) oxidation induced by free radicals. Flavonoids have been reported to have negative correlation with incidence of coronary heart disease. Furthermore, flavonoids have anti-bacterial, anti-viral, anti-tumor, anti-inflammatory and antiallergenic effect [3]. Several methods for the determination of flavonoids in medicinal plants have been reported, including thin-layer chromatography, high performance liquid chromatography, capillary electrophoresis, and spectrophotometry. Among all these methods, only spectrophotometry can be used to determinate the total amount of the flavonoids. UV–visible spectrophotometry is the most extensively used method to determine the flavonoid content in plant [4].

Herbs have antioxidant property. The variety of herbs have antioxidant property more than some vitamins. This reason, herbs were brought to make medicines in order to use as a supplement to health. The objective of this research is to determine flavonoid content and antioxidant activity from ferns such as *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. by ultrasonic extraction with different solvents

2. Methods

2.1. Preparation of raw material

Fresh leaves of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. were washed, dried in hot air oven at 50 ^{o}C for 8 hrs., powdered with a mechanical grinder and stored in desicator.

2.2. Ultrasonic extraction

4 g of powdered leaves of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. was extracted with acetone, ethanol and ethyl acetate 200 ml. (1:50) by ultrasonic at 40 ^{o}C for 20 min. Each of the extract was filtered through Whatman NO.4 filter paper and evaporated with rotary evaporator at 45 ^{o}C. The extracts so obtained were weighed and stored in a brown bottle at 4 ^{o}C until further use.

2.3. Determination of the flavonoid content

The flavonoid content was determined by aluminium chloride colorimetric method. 0.5 ml. of the extracts were mixed with 1.5 ml. of 95% ethanol, 0.1 ml. of 10% aluminium chloride, 0.1 ml. of 1M potassium acetate and 2.8 ml. of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. with UV-visible spectrophotometer. The experimental was repeated triplicate. The standard curve was prepared using 30, 60, 90, 120, 150 mg /L solutions of quercetin in ethanol. The flavonoid content was expressed in terms of quercetin equivalent (mg QE /g of dry extract).

2.4. Determination of antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. 0.1 ml. of the extracts were mixed with 3.9 ml. of DPPH in ethanol (0.1 mM). Subsequently, the mixture was immediately vortexed and incubated in the dark for 30 min. The absorbance of mixture was measured at 517 nm with UV-visible spectrophotometer and compared with vitamin C. The experimental was repeated triplicate. The percentage of inhibition was calculated using the following formula:

$$\%\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

when;

$A_{control}$ = absorbance of control (solvent + DPPH)
A_{sample} = absorbance of sample (sample + DPPH)

3. Results

In this study, the determination of flavonoid content and antioxidant activity from ferns by ultrasonic extraction divided into 3 parts extraction yield, flavonoid content and antioxidant activity by DPPH method.

3.1. Extraction yields

From Table 1. showed that extraction yield of all ferns were extracted from ethanol higher than ethyl acetate and acetone. As a result of the extraction efficiency depends on the polarity of the solvent used for extraction. This extraction yields were higher than extraction yield of *Teucrium chamaerdys* L. *var. glanduliferum Haussk*. leaves were extracted with ethyl acetate and acetone [5].

Table 1. Extraction yield of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*

	% Extraction yield		
	Diplazium esculentum (Retz.) Swartz.	Marsilea crenata Presl.	Drynaria querciforia (L.) J. Sm.
ethanol	4.004	2.252	8.846
ethyl acetate	2.052	1.025	4.509
acetone	3.699	2.070	6.947

3.2. Flavonoid content

From Fig.1. showed the flavonoid content of the extracts in term of quercetin equivalent (the standard curve equation: y = 0.0054x - 0.0079, r^2 = 0.9994). Ethanol extraction have the highest flavonoid content, which is content of *D. esculentum (Retz.) Swartz*., *M. crenata Presl*. and *D. querciforia (L.) J. Sm*. were 19.974, 11.202 and 7.573 mg QE/g dried, respectively.

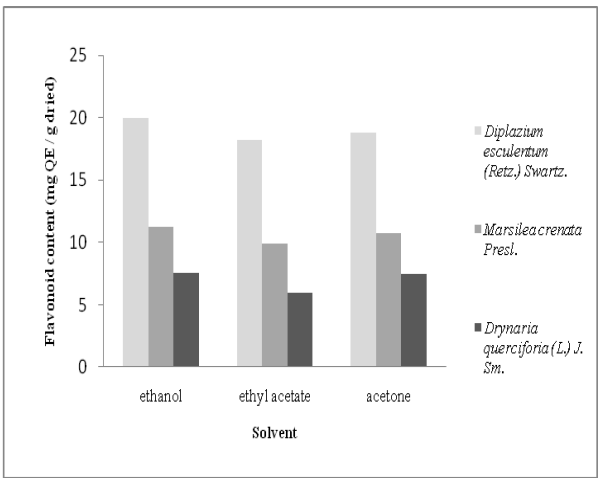

Fig.1. Flavonoid content of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm.*

3.3. Antioxidant activity by DPPH method

The antioxidant activity was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Ethanol extraction have the highest percentage inhibition values of *D. esculentum (Retz.) Swartz*., *M. crenata Presl*. and *D. querciforia (L.) J. Sm*. were 24.590, 27.644 and 13.326, respectively (Fig. 2.). Moreover, a correlation between flavonoid content and antioxidant activity of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. with ethanol extraction were observed with the significant correlation coefficient 0.326 (Fig. 3.).

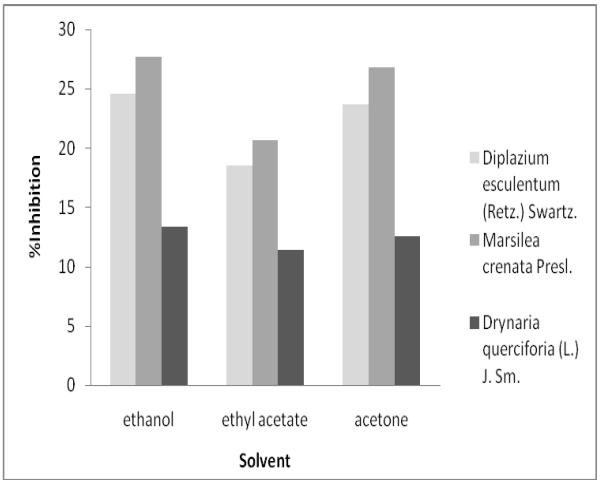

Fig.2. DPPH asay of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm.*

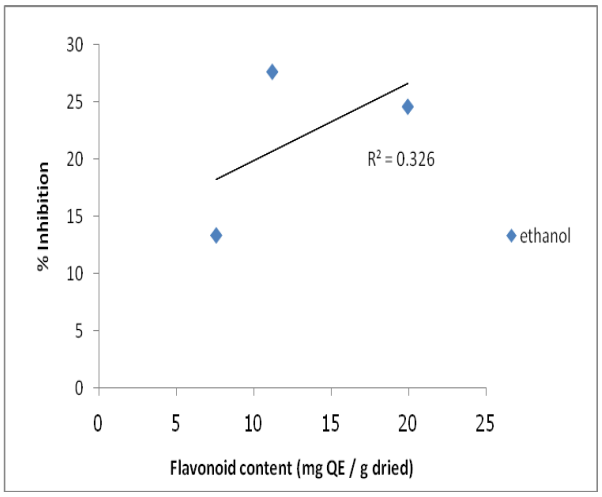

Fig. 3. Correlation between the flavonoid content and antioxidant activity of *Diplazium esculentum (Retz.) Swartz*., *Marsilea crenata Presl*. and *Drynaria querciforia (L.) J. Sm*. extracts

4. Conclusions

The type of solvents affect to the flavonoid content and antioxidant activity from ferns. Ethanol extraction of all ferns have the flavonoid content and antioxidant activity higher than acetone and ethyl acetate.

References

[1] Yan-ling Wang, Guang-Sheng Xi, Yong-Chun Zheng and Fu-Sheng Miao, Microwave-assisted extraction of flavonoids from Chinese herb *Radix puerariae (Ge Gen)*, Department of

Chinese Medicine, Jilin Agricultural Science and Technology College. China, January 27(2010).

[2] Shuang Li, Ronghua Zhu, Ming Zhong, Yuping Zhang, Kelong Huang, Xu Zhi and Shuting Fu, Effects of ultrasonic-assistant extraction parameters on total flavones yield of *Selaginella doederleinii* and its antioxidant activity, Journal of Medicinal Plants Research Vol. 4(17), pp. 1743-1750, September 4 (2010**).**

[3] Subramani Sellappan and Casimir C. Akoh. 2002. Flavonoids and antioxidant activity of Georgia grown Vidalia onions. Journal of Agricultural and Food Chemistry, 50(19): 5338-5342.

[4] Hongbin Zhu & Yuzhi Wang & Yuxuan Liu & Yalin Xia & Tian Tang, Analysis of flavonoids in *Portulaca oleracea* L. by UV–vis spectrophotometry with comparative study on different extraction technologies, Food Anal. Methods (2010) 3:90–97.

[5] Milan S. Stankovic, Marina Topuzovic, Slavica Solujic and Vladimir Mihailovic, Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaredys* L. var. *glanduliferum Haussk*, Journal of medicinal plants research Vol. 4(20), pp. 2092-2098, October 18 (2010).

[6] Chia-chi Chang, Estimation of total flavonoid content in propolis by two complementary colorimrtric methods, *Journal of food and drug analysis*, Vol. 10.NO. 3, 2002, page178-182.

[7] Ongard, C. and Dara, P., Antioxidant activity study of stachytarpheta indica vahl, Agricultural Sci. J. 41(3/1)(Suppl.): 329-332 (2010).

[8] Hariram V Bhaskar and Natarajan Balakrishnan, In vitro antioxidant property of Laticiferous plant species from western ghats tamilnadu, India, *International journal of health research*, June 2009; 2(20: 163-170 (e228p59-66).

[9] Ramya Premanath and N. Lakshmidevi, Studies on antioxidant activity of *Tinospora cordifolia* (Miers.) leaves using in vitro models, *Journal of American science*, 2010; 6(10).

[10] Selamassakul, O., Laohakunjit, N. and Kerdchoechuen, O., Antioxidant capacity of extracts from *Gracilaria fisheri*, Agricultural Sci. J. 40(3)(Suppl.): 25-28 (2009).