Profile of Total Polyphenols, Flavonoids, and Antioxidant Activities of Different Solvent Extracts of *Forsythia suspensa* Leaves

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ABSTRACT— ***In this paper, the effects of different solvents (aether, ethyl acetate, acetone, ethanol, and methanol) on the content of total polyphenols and flavonoids, as well as antioxidant activities of extracts from Forsythia suspensa leaves were investigated. The results showed that the contents of total polyphenols and flavonoids were significantly affected by extraction solvents, and resulting in variation of antioxidant activities of Forsythia suspensa leaves. The methanol extract exhibited high antioxidant activities because it possessed the highest total polyphenols and flavonoids content. The acetone and ethanol extracts exhibited higher antioxidant activities, while other extracts had both lower the content of active compounds and bioactivities. These results indicated that selective extraction from Forsythia suspensa leaves, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity, which will be useful for the developing and application of Forsythia suspensa leaves as a new local source of bioactive compounds in foods and medicine industries.***

Keywords—*Forsythia suspensa* leaves, Extraction solvents, Polyphenols, Flavonoids, Antioxidant activity

1. INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism in living organisms. However, the excessive amounts of ROS and RNS are most responsible for the development of many diseases including carcinogenesis, atherosclerosis and heart diseases (Alexandrova & Bochev, 2005; Valko *et al*., 2007), and ROS is also one of the major causes of spoilage of foods containing significant amounts of polyunsaturated fatty acids (Jayaprakasha *et al*., 2003). For many years, a variety of different chemical and synthetic compounds has been made to prevent oxidation (Li *et al*., 2012). Recently, however, consumers have grown concerned about the side effects of synthetic chemicals and want safer materials for preventing and controlling oxidation (Tiwari *et al*., 2009; Delgado-Adámez *et al*., 2012).

Plants can be an excellent source of natural antioxidants and can be effectively used in the food industry as a source of dietary supplements or as natural antioxidants to preserve the quality and improve the shelf-life of food products (Tiwari *et al*., 2009; Voon *et al*., 2012). In addition, plants or their extracts can also be used as natural colorants of foodstuffs; as in most of the cases, they are believed to be safe, and non-toxic to humans (Burt 2004; Rymbai *et al*., 2011).

Forsythia suspensa (Thunb.) Vahl, a member of the Oleaceae, is a climbing plant widely distributed throughout China, Korea, Japan and many European countries. The fruit of this plant is a famous traditional Chinese medicine (lianqiao in Chinese) for gonorrhea, erysipedas, inflammation, pyrexia and ulcer (Cuellar *et al*., 1998; Ozaki *et al*., 2000; Li and Chen, 2005). Moreover, the leaves of *F. suspensa* contain abundant bioactive substances such as chlorogenic acid, forsythiaside, rutin, phillyrin and phillygenin and so on (Jiao *et al*., 2013), which can make it possess a wide range of biological activities, such as antioxidant activity, antibacterial activity, modulating blood lipids, anti-fatigue, anti-senile and anti-influenza activities (Huang *et al*., 2009; Xue & Yuan, 2009; Lu *et al*., 2012; Qu *et al*., 2012). Therefore, the leaves of *F. suspensa* are used as the succedaneum of the fruits in recent years due to their versatile health benefits (Qu *et al*., 2008). However, the content of bioactive substances is affected by genetic, cultural practices and climatic factors during the plant growth cycle, but the extraction yield is influenced by extraction methods (Aspé & Fernández 2011; Cheok *et al*., 2013) and extraction solvents (Alothman *et al*., 2009; Cheok *et al*., 2012) during extraction due to differences in the structure of these compounds and their physicochemical properties. So depending on the solvent used for extracting bioactive compounds, extracts obtained from the same plant may vary widely with respect to their concentration and activities (Alothman *et al*., 2009; Cheok *et al*., 2012). To the best of our knowledge, data on the leaves of *F. suspense* in this respect are still scarce. In order to assess the effect of solvent system on the content and bioactivities of bioactive substances from *F. suspensa* leaves, we compared the content of total phenolics,

flavonoids, and antioxidant activity of *F. suspensa* leaves under five extracting solvents. The expected results will be useful for the developing and application of *F. suspensa* leaves as a new local source of bioactive compounds for economic and health utilization.

2. MATERIALS AND METHODS

2.1 Plant materials

The *F. suspensa* leaves were gathered from Huoshan, Shanxi on 12 June, 2014. They were dried with hot-air and then stored in polyethylene bags at 4 ^{o}C until analysis.

2.2 Preparation of extracts

Dried *F. suspensa* leaves were grounded and passed through a 40 mesh screen. Twenty-five grams of leaves were extracted three times with 250 mL solvents and shaking it with a rotary shaker set at 25°C for 1 h respectively. And then the homogenates was centrifuged at 4000 rpm/min at 4°C for 15 min. After centrifugation, the combined supernatants were dried under vacuum and stored at 4°C until analysis. Solvent used in our experiment contains methanol, ethanol, acetone, ethyl acetate and aether.

2.3 Determination of total phenolic content (TPC)

Total phenolic content was determined as described by Rebey et al. (2012) with slight modifications. An aliquot (0.1 mL) of diluted extracts, 2.8 mL of deionized water and 0.1 mL of 1.0M Folin-Ciocalteu reagent were mixed and stirred. After 8 min, 2 mL of 7.5% sodium carbonate solution was added and mixed thoroughly. The absorbance of the reaction mixtures was measured at 765 nm wavelength after incubation for 2 h at room temperature. Gallic acid was used for calibration of the standard curve and total phenolic content was expressed as milligram gallic acid equivalent per gram dried weight (mg GAE/g DW). All extracts were tested in triplicates.

2.4 Determination of total flavonoid content (TFC)

The level of total flavonoid was measured as described by Rebey et al. (2012) with some modifications. Briefly, an aliquot (1.0 mL) of diluted extracts and 0.3 mL of 5% $NaNO_2$ solution were mixed for 6 min. Then 0.3 mL 10% $Al(NO_3)_3$ was added and incubated for 6 min. Next, 4 mL of 4% NaOH was added. The final volume was adjusted to 10 mL with distilled water and mixed thoroughly. After 15 min, absorbance of the mixture was determined at 510 nm against the same mixture. Rutin was used for calibration of the standard curve and the content of flavonoids was expressed as milligram rutin equivalent per gram dried weight (mg RE/g DW). All samples were tested in three replications.

2.5 DPPH radical scavenging assay

DPPH radical scavenging assay was measured following the method of Xu et al. (2010) with some modifications. Extract was serially diluted to different concentrations and 0.5 mL of diluted extract mixed with 2.5 mL of 60 μmol/L DPPH solution dissolving in methanol. The mixture was shaken thoroughly and incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm. The scavenging rate of DPPH radical was calculated according to the formula given below: DPPH radical scavenging ability (%) = $[A_0-(A_1-A_2)]/A_0\times100$, where A_0 is the absorbance of the control in which methanol substitutes extract and A_1 is the result of the mixture of the extract and DPPH radicals while A_2 is the absorbance of the mixture of the extract and 2.5 mL methanol to eliminate the color effect of the extract. The DPPH radical scavenging activity of was measured by IC_{50} value which represents the effective concentration of the extract at which DPPH radical scavenging ability up to 50%.

2.5 ABTS radical scavenging assay

ABTS radical scavenging assay was determined according to the method of Xu et al. (2010). Briefly, a certain quality of ABTS and potassium persulfate was dissolved in water to keep the final concentrations of the two substances to be 7 mmol/L and 2.45 mmol/L respectively. The mixture was kept in the dark for 16~24 h to make the ABTS radical working solution and its absorbance at 734 nm was adjusted to 0.700±0.050. The ABTS radical scavenging ability was measured by adding 50 μL of diluted extract to 1.9 mL of ABTS radical working solution and the absorbance at 734 nm after 6 min was recorded. The scavenging rate and IC_{50} value were calculated using the equation described for DPPH assay.

2.6 Ferric Reducing Antioxidant Power (FRAP) Assay.

The reducing ability was determined by using the ferric reducing antioxidant power (FRAP) assay described by Xu et al. (2010). Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 ^{o}C in a water bath prior to use. Then 0.1 mL of extracts was mixed with 1.8 mL of FRAP reagent and 3.1 mL ultrapure water. The absorption of the reaction mixture was measured at 593 nm after

incubation for 30 min at 37 ^{o}C. A standard curve was constructed using $FeSO_4$ solution (100-1000 μM). FRAP value was expressed as micromoles Fe(II) per gram extracts.

2.7 Statistical analysis

All results are expressed as mean ± SD (n=3). One-way analysis of variance (ANOVA) and Duncan's test were performed with significant level being considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Contents of total polyphenols and flavonoids

The levels of total polyphenols and flavonoids in different extracts from the leaves of *F. suspense* were given in Table 1. Results showed that total phenolic contents of different extracts varied considerably and ranged from 0.26 to 5.28 mg GAE/g DW, respectively. The methanol extract had the highest polyphenol content, followed by acetone, ethanol, ethyl acetate and aether. The extraction of flavonoids was also influenced significantly by extracting solvent ($P < 0.05$), and its contents varied from 0.19 to 27.89 mg RE/g DW, respectively for aether and methanol. With regard to flavonoid content, solvents could be sequenced in the following decreasing order: methanol>hexane>ethanol>ethyl acetate>aether. However, acetone and ethanol extracts had no significant difference ($p<0.05$) in the TPC and TFC. Generally speaking, differences in the content of total polyphenols and flavonoids from different solvent extracts may be come from differences in the polarity of solvents. Besides, the levels of total polyphenols and flavonoids also could possibly be influenced by dielectric constant, chemical structure of organic solvents (Cheok *et al*., 2012) as well as chemical properties of plant phytochemicals (Jayaprakasha *et al*., 2003) and growing season of *F. suspense* leaves (Jiao *et al*., 2013).

Table 1: The contents of total polyphenols and flavonoids of different solvent extracts

	TPC (mg GAE/g, DW)	TFC (mg RE/g, DW)
Methanol	5.28±0.30 a	27.98±2.35 a
Ethanol	2.16±0.12 b	5.86±0.55 b
Acetone	2.88±0.22 b	6.16±0.47 b
Ethyl acetate	0.31±0.05 c	1.12±0.22 c
Aether	0.26±0.02 c	0.19±0.05 d

Values are represented as mean ± standard deviation of triplicates; Different letters within a column indicate statistically significant differences between the means at P < 0.05 for the solvents.

3.2 Effects of different solvents on DPPH and ABTS radicals scavenging abilities

Due to the differences in the compositions and contents of total polyphenols and flavonoids, the extracts showed the different antioxidant abilities. DPPH and ABTS radicals scavenging abilities of the extracts diluted to series of concentrations has been tested in our study and IC_{50} value of each sample could be calculated through regression equation. The lower IC_{50} value represents higher scavenging abilities. The Table 2 showed us that there were significant differences of DPPH radical scavenging abilities of different solvent extracts. Consistent with the results in phytochemical test, IC_{50} values of methanol and acetone extracts were 1.63 mg/mL and 1.86 mg/mL respectively, indicating that they owned the highest DPPH radical scavenging ability, followed by ethanol and ethyl acetate extracts, and aether extracts had a poor DPPH radical scavenging ability, of which IC_{50} values was 9.13 mg/mL. All of these three extracts possessed high ABTS radical scavenging abilities in that their phytochemical components and contents might have some equivalence regarding to ABTS radical scavenging ability. Similar to the results of DPPH, IC_{50} values of methanol and acetone extracts were 9.02 mg/mL and 9.04 mg/mL respectively, exhibiting the highest ABTS radical scavenging ability, followed by ethanol and ethyl acetate extracts, the lowest for aether extracts. In addition, DPPH andABTS cation radicals scavenging activity of each extract increased dose-dependently at concentrations, which may be attributable to its hydrogen-donating ability thereby inhibiting the propagation of radical chain reactions and other biological oxidants (Terao *et al*., 1993; Jin *et al*., 2005)

Table 2: The scavenging activity of different extracts on DPPH and ABTS radicals

	Extracts	Regression equation	Correlation coefficient (R^2)	IC_{50}/mg·mL^{-1}
DPPH	Methanol	y=29.867x＋1.3999	0.9981	1.63

	Ethanol	y=17.269x＋9.4379	0.9885	2.35
	Acetone	y=23.973x＋5.3285	0.9993	1.86
	Ethyl acetate	y=18.115x－2.11	0.9925	2.88
	Aether	y=4.3653x＋10.126	0.9993	9.13
	Methanol	y=4.7238x＋7.3914	0.9976	9.02
	Ethanol	y=3.4516x＋2.7452	0.991	13.69
ABTS	Acetone	y=4.7801x＋6.2989	0.9942	9.14
	Ethyl acetate	y=1.9824x＋11.322	0.9944	19.51
	Aether	y=0.65619x＋12.266	0.9829	57.50

3.2 Ferric Reducing Antioxidant Power (FRAP)

Figure 1 showed that different extracts of the leaves of *F. suspense* exhibited different ferric reducing power, but there was a significant difference ($p < 0.05$) in the reducing power among different extracts. The reducing power of extracts ranged from 19.4 to 355.1 μmol Fe(II)/g DW. As observed in the DPPH and ABTS radical-scavenging capacity measurements, the reducing power of methanol extracts was the highest, and much higher than that of other extracts, while the lowest for aether extracts. The result suggested that extracts of *F. suspense* leaves had a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

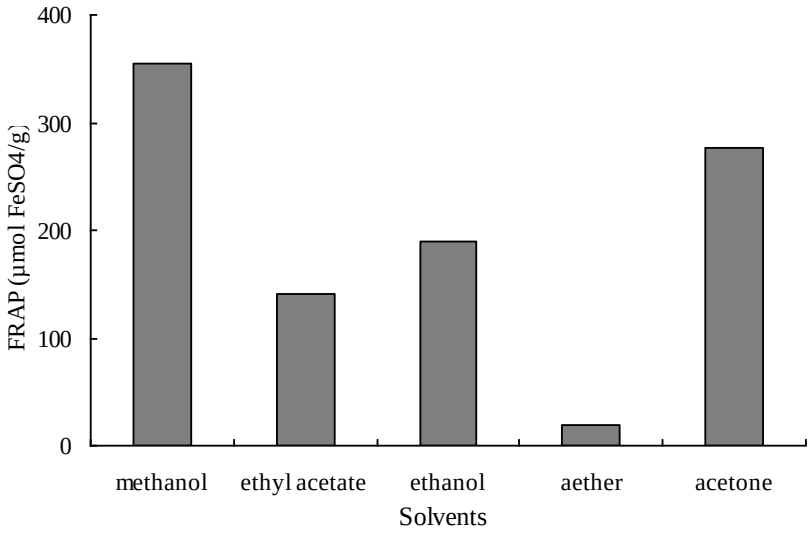

Figure 1 Ferric reducing power (FRAP) of the different extracts

In this study, the DPPH, ABTS, FRAP were highly correlated to the content of both total polyphenols and flavonoids (**Table 3**, $R \geq 0.9601$), indicating polyphenols and flavonoids are the main constituents contributing to the antioxidant activities of extracts, which was supported by previous reports studied on some cereals or plants (Alothman *et al*., 2009; Rebey *et al*., 2012). The variation of the total antioxidant activity as affected by the extracting solvent used has been reported in many previous studies (Zieliński *et al*., 2000; Tian *et al*., 2009).

Table 3: Correlation analysis among phytochemicals and antioxidant activities of different extracts

	antioxidant activities		
	DPPH	ABTS	FRAP
Polyphenols	0.9681**	0.9981**	0.9706**
Flavonoids	0.9782**	0.9724**	0.9601**

*Values are correlation coefficient R. [a] Significantly different: ** P < 0.01, * P < 0.05*

4. CONCLUSION

The extracting solvents significantly affected total polyphenols, flavonoids content as well as antioxidant activities of the leaves of *F. suspense*. In our study, the methanol extract from the leaves of *F. suspense* possessed the highest content of total polyphenols and the strongest antioxidant activity. The acetone and ethanol extracts had the highest content of flavonoids and anthocyanin respectively and also exhibited higher antioxidant activities, while other extracts had both lower the content of active compounds and bioactivities. These results indicated that selective extraction from natural sources, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity.

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