Correlation of Polyphenolics Content to Antioxidant Activity of *Forsythia Suspensa* Leaves

Su Tian[1] , Yan Wang[1] , and Kozo Nakamura[2]

[1] *Department of Nutrition and Food Hygiene, Hebei Medical University, Shijiazhuang, Hebei, China*
[2] *Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Nagano, Japan*
E-mail: sutian314@hebmu.edu.cn

Dried ripe fruit of *Forsythia suspensa* is widely used as traditional Chinese medicine including antifebrile, antiphlogistic, diuretic, drainage and analgesic for tumor and skin diseases. In this research, polyphenols in *Forsythia suspensa*, which is considered to participate in these physiological functions, were analyzed and the antioxidant activities were also investigated. *Forsythia suspensa* samples harvested in March 2008 - October 2008 were extracted with 60 % methanol and the polyphenol contents in the extract were investigated by the Folin-ciocalteu method, and the flavonoid contents were investigated by the $AlCl_3$ coloring method. Their anti-oxidization activities were estimated by DPPH radical scavenging activity and ABTS radical scavenging activity. As results, the polyphenol content and the flavonoid content in leaves were higher than those in the flower and the fruit, and DPPH radical scavenging activity and ABTS radical scavenging activity of the leaves were also higher. Changes of polyphenolic contents in the leaves accompanying growth was determined and it turned out that the leaves picked in March had highest polyphenol and flavonoid contents, and highest DPPH and ABTS radical scavenging activities. High correlativity was observed in the polyphenol and flavonoid content, and the radical scavenging activities of the leaves extract, and it was suggested to leaves of *Forsythia suspensa* that polyphenols with high antioxidant activity were contained.

Keywords: Polyphenol, Flavonoid, Antioxidant, *Forsythia suspensa*

Introduction

There is strong evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids and play an important role in the induction of a variety of diseases including aging, cancer, heart disease, diabetes, and nerodegeneration (Moskovitz, 2002).

Phenolic compounds are universally distributed in the plant as secondary products. Many of these phytochemicals such as flavonoids and other phenolics are known to have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (Boveris, 1998). And they have shown various biological effects including inhibition of low-density lipoprotein (LDL) oxidation, and antimicrobial and anticarcinogenic activities (Ryu, 2002).

Forsythia suspensa (Thunb.) Vahl is a well-known traditional Chinese medicine, named “Lianqiao” in Chinese. In general, the fruit of *Forsythia suspensa* is used for crude drug that had been widely used as an antipyretic, antidotal and anti-inflammatory agent for the treatment of infections, such as acute nephritis, erysipelas and ulcer (Xinyixueyuan, 1977). Recent interest on *Forsythia suspensa* has resulted from its inhibitory activity against elastase, resist hepatic injury and antiendotoxin, antioxidant,

and antiviral effects. A number of compounds have been isolated from the fruit of *Forsythia suspensa* including phenylethanoid glycosides, lignans, flavonoids, terpenes, and volatile oils. However, other parts of *Forsythia suspensa*, like leaves have been much less investigated. *Forsythia suspensa* leaves are generally soaked in hot water and drunk daily like tea in the Shanxi, Hebei provinces in China, especially in longevity village of Hebei. This suggests that *Forsythia suspensa* leaves can be useful to prevent agent and has better food safety. Moreover, *Forsythia suspensa* leaves possess of richer natural resource. However, only a few studies have been devoted to assess the antioxidative effect and active components of *Forsythia suspensa* leaves.

The purpose of this study was to determine total phenolics and total flavonoids content in flower, leaves collected in different growing period and fruit from *Forsythia suspensa* and to evaluate the contribution on antioxidation activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and 2,2'-azino-bis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTS) radical anion scavenging activity assay *in vitro*.

Materials and Methods

The *Forsythia suspensa* flower and leaves were collected between the top and 115 cm down in the schoolyard of Hebei Medical University, shijiazhuang, China in 21th day of every month during March 2008 to October 2008. The yellow flower and green leaves were dried in an air-draft oven at less than 35 °C for 24-36 h. Dried leaves were packed under vacuum and kept at -18 °C until analysis. The fruit of *Forsythia suspensa* used in this study was purchased from Lerentang Pharmaceutical Group Inc.

Dried *Forsythia suspensa* flower, leaves, fruit were ground in an electric coffee grinder for 2 min. 0.5 g of the powder was defatted in petroleum ether (1:10, m/v) with agitation for 10 min and centrifuged at 3000 *g* for 10 min at 25 °C. The residue was defatted again using the same procedure. The defatted residue was air-dried under a fume hood to remove the residual petroleum ether. The defatted residue was extracted with 60% Methanol 10 ml by the ultrasound-assisted method (Tian, 2009) and filtered under vacuum. This procedure was repeated twice for the residue, and the filtrates were combined. The mixture was evaporated using a rotary evaporator under pressure at 40 °C. The phenolic concentrate was dissolved in 10 ml of methanol and stored at -18 °C until analyzed. Extraction was done in duplicate.

Content of total phenolics in each sample was determined by the Folin-Ciocalteu method (Singleton, 1965). The total phenolic content was expressed as gallic acid equivalents in grams per 100 grams of dry material. The content of total flavonoids was measured using a colorimetric assay developed by Zhishen et al. (Zhishen, 1999). Total flavonoids of *Forsythia suspensa* flower, leaves, and fruit were expressed as g of rutin equivalents pre 100 grams of dry material. Sample of each extraction were analyzed in triplicate.

The radical scavenging activity of *Forsythia suspensa* flower, leaves, and fruit against DPPH free radical was measured using the method of Brand-williams et al. (Brand-williams, 1995). 0.2 ml of the samples dissolved in methanol at vrious concentrations were added into 7.8 ml of 50 % acetone solution of DPPH (6.6×10^{-2} mM) and the mixture was then kept in a water bath at 25 °C in dark for 40 min. The absorbance of the sample was determined at 517 nm by spectrophotometer. The scavenging activity was measured as the decrease in absorbance of the DPPH expressed as a percentage of absorbance of a control DPPH solution without sample. All analyses were carried out in triplicate.

AAPH (1 mM) was mixed with ABTS (25 mM) as diammonium salt in phosphate buffer saline (PBS) solution (100 mM potassium phosphate buffer containing 150 mM

NaCl at pH 7.4). After the mixture was heated in a water bath at 68 °C for 13 min, the blue-green $ABTS^{\cdot-}$ solution was adjusted with fresh PBS solution to an absorbance of 0.580±0.020 at 734 nm. 300 μl of the sample solution added to 2 ml of the ABTS radical solution was incubated in a water bath at 37 °C for 10 min. The decrease of absorbance at 734 nm was measured at 10 min. A control consisted of 300 μl of 100 % methanol and 2 ml of $ABTS^{\cdot-}$ solution. The ABTS radical scavenging capacities of samples were expressed as mg of trolox equivalent antioxidant capacity/g. Samples of each extraction were analyzed in triplicate (Kim, 2002).

The data were expressed as the mean of three replicate determinations and standard deviation (SD). Statistical comparisons were made with Student-Newman-Keuls test (one-way analysis of variance). *P* values of < 0.05 were considered to be significant.

Results and Discussion

Total Phenolics and Total Flavonoids. Fig. 1 shows the amount of total phenolics and total flavonoids in the different plant parts of *Forsythia suspensa* with the seasonal variation. From the results, the same seasonal variation patterns of total phenolics and total flavonoids during the *Forsythia suspensa* growing term were found. Total flavonoids contents ranged from 2.79 g/100 g to 15.95 g/100 g expressed as rutin equivalent on a dry material. *Forsythia suspensa* leaf sample harvested at March 21st (21-Mar) exhibited the highest content of total flavonoids of 15.95 g rutin/100 g ($P < 0.01$), and the amount of total flavonoids in other leaves sample was very similar. The average content of total flavonoids in *Forsythia suspensa* leaves was 9.77 g rutin/100 g.

The total phenolics content in *Forsythia suspensa* showed the same tendency of varieties with total flavonoids. 21-Mar leaf sample had the highest total phenolics content, and the average content of total phenolics in *Forsythia suspensa* leaves was 3.17 g gallic acid/100 g. In the different plant parts *Forsythia suspensa* grown, total flavonoids and total phenolics in leaves were better than those in flower and fruit; the content of total flavonoids and total phenolics in the fruit decreased significantly compared with the flower and leaves. The results are in agreement with those reported in the literature (Guerra, 2008).

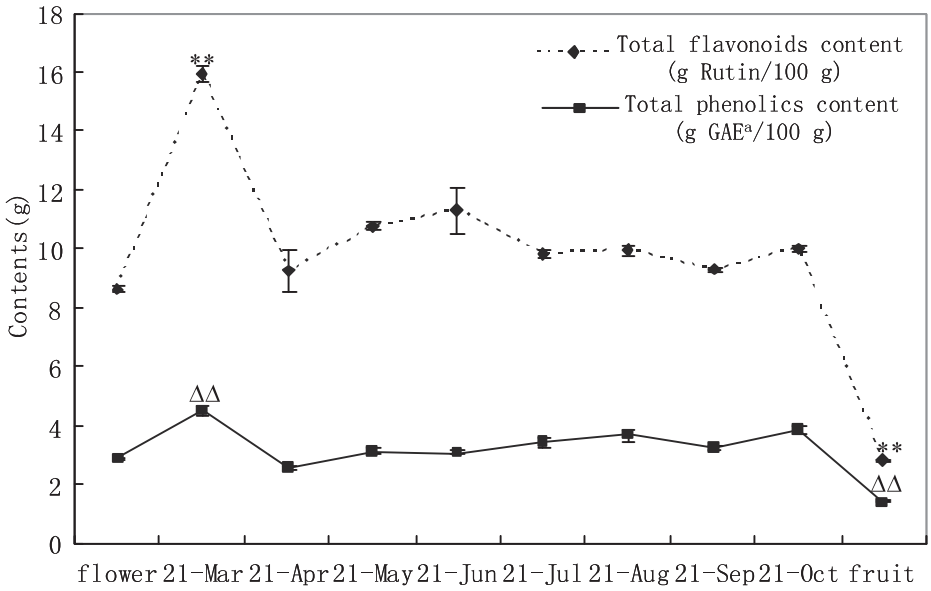

Fig. 1 Schematic presentation of the seasonal variation of total flavonoids and total phenolics in different plant parts of *Forsythia suspensa.* [a] GAE stands for gallic acid equiv. ** $P < 0.05$. ΔΔ $P < 0.05$.

Forsythia suspensa is an agricultural product, and as such, it is influenced by countless variables during its cultivation. These variables include geographical location, rainfall, temperature, elevation, sun exposure, soil composition, and many more. The total flavonoids and total phenolics contents in *Forsythia suspensa* collected in cooler months (March) were significantly higher ($P < 0.01$) than those collected in warmer and rainy months (from July to September). This difference may be due to rainfall and temperature difference, because Heck et al. reported that rain and temperature were negatively associated with the concentration of phenolic compounds in mate teas (*Ilex paraguariensis*), this means that as the amount of rainfall increased, also, as temperature increased, the level of polyphenols decreased (Heck, 2008). In addition, it has been shown that the biosynthesis of phenolic compounds can be effectively induced by sunlight (Harbowy, 1997). *Forsythia suspensa* leaves collected on March had the highest total flavonoids and total phenolics contents because during the germination and development of shoots, *Forsythia suspensa* fresh leaf are exposed to full sun and a much greater concentration of UV light. The light that is not absorbed by the leaves to produce energy is then able to generate free radicals and induce cellular damage. To protect against this, the plant produces antioxidant compounds.

DPPH and ABTS Scavenging Activity. DPPH is a stable free radical that shows a maximum absorption at 517 nm. When DPPH encounters proton donating substances such an antioxidant and a radical species, the absorbance at 517 nm disappears because the DPPH radical is scavenged. On the basis of this principle, the radical scavenging effect of each sample was measured and the results are presented in Table 1. The percentage of scavenging activity was plotted against the sample concentration to obtain the IC_{50}, defined as the concentration of sample necessary to cause 50 % inhibition. The IC_{50} of *Forsythia suspensa* flower, fruit on DPPH scavenging activity was 53.17 μg/ml, 168.61 μg/ml, respectively and the average IC_{50} of *Forsythia suspensa* leaves was 51.75 μg/ml. Similarly to the result of total flavonoids and total phenolics, flower and leaves had stronger DPPH radical scavenging activities ($P < 0.01$). The strongest activity was shown in the *Forsythia suspensa* leaf sample of 21-Mar, its antioxidant activity in scavenging DPPH was about 4 times higher than that of *Forsythia suspensa* fruit. The IC_{50} (37.12 μg/ml) of *Forsythia suspensa* leaves collected on March found for the DPPH assay is lower than that previously reported for this radical species under the same assay conditions, revealing a higher antioxidant potential possibly duo to the higher content of polyphenolic compounds.

The total antioxidant capacity of *Forsythia suspensa* determined by ABTS radical scavenging activity is shown in Table 1. *Forsythia suspensa* leaves sample collected on March showed the highest antioxidant capacity with 33.34 ± 4.85 g of Trolox equivalent ($P < 0.01$). Followed by flower, other leaves sample, there were few significant differences among them. The average amount of the antioxidant capacity in *Forsythia suspensa* leaves was 17.30 g Trolox/100 g, which is higher than that of *Forsythia suspensa* fruit's 7.21 g Trolox/100 g.

Our data confirm that *Forsythia suspensa* leaves have a higher antioxidant capacity and that considerable variation in antioxidant capacity exists among different plant parts and different growing season of *Forsythia suspensa*. Moreover, this study showed that *Forsythia suspensa* leaves exhibited a significantly higher antioxidant activity while the germination and development of shoots began. It is likely that plants living in the stressful environment produced more ROS, which activates the synthesis of antioxidant or other radical-scavenging systems to prevent cell death (Niggeweg, 2004).

Table 1 DPPH scavenging activity and antioxidant capacity of *Forsythia suspensa*[a]
[a] Means and standard deviations are indicated. Values for each radical scavenging activity followed by the same letter were not significantly different ($p < 0.05$).

Sample	DPPH scavenging activity IC_{50}（μg/ml）	ABTS scavenging capacity (g Trolox/100 g)
flower	53.17 ± 4.48 b	16.38 ± 1.32 b
leaf		
21-Mar	37.12 ± 0.63 a	33.34 ± 4.85 a
21-Apr	52.88 ± 5.39 b	13.96 ± 0.92 b
21-May	58.82 ± 1.69 b	14.04 ± 1.59 b
21-Jun	51.79 ± 3.26 b	16.65 ± 2.19 b
21-Jul	53.01 ± 0.90 b	15.55 ± 1.64 b
21-Aug	54.79 ± 2.09 b	17.06 ± 1.54 b
21-Sep	53.81 ± 5.54 b	13.18 ± 1.35 b
21-Oct	51.81 ± 1.67 b	14.59 ± 1.53 b
fruit	168.61 ± 31.65 c	7.21 ± 0.60 c

Relationships between Phenolics and antioxidant activity. To evaluate the correlation between total flavonoids, total phenolics and DPPH scavenging activity, a new parameter: Antiradical Efficiency (AE) was introduced. AE was defined as AE = 1 / EC_{50} (Sánchez-Moreno, 1998). The relationships between total flavonoids, total phenolics with AE, antioxidant capacity by ABTS scavenging activity were shown in Fig. 2. There was good linear relationship between the amount of total flavonoids and DPPH scavenging activity, antioxidant capacity ($r^2 = 0.8932$, $r^2 = 0.7796$, respectively). The amount of total phenolics and AE, antioxidant capacity also showed good correlation ($r^2 = 0.7730$, $r^2 = 0.5749$, respectively). The results prompt that polyphenolics, especially flanovoids may play a major role in the antioxidant capacity in *Forsythia suspensa*. However, the relationship between total flavonoids, total phenolics and antioxidant capacity by ABTS scavenging activity was less pronounced than the highly linear relationship observed between AE, it could be explained by the instability of ABTS radical solution during experiment.

Our results suggest that *Forsythia suspensa* leaves can be considered as a valuable source of antioxidant products. It is necessary to analysis antioxidant compounds and to determine each polyphenolic changed with growing term in the future.

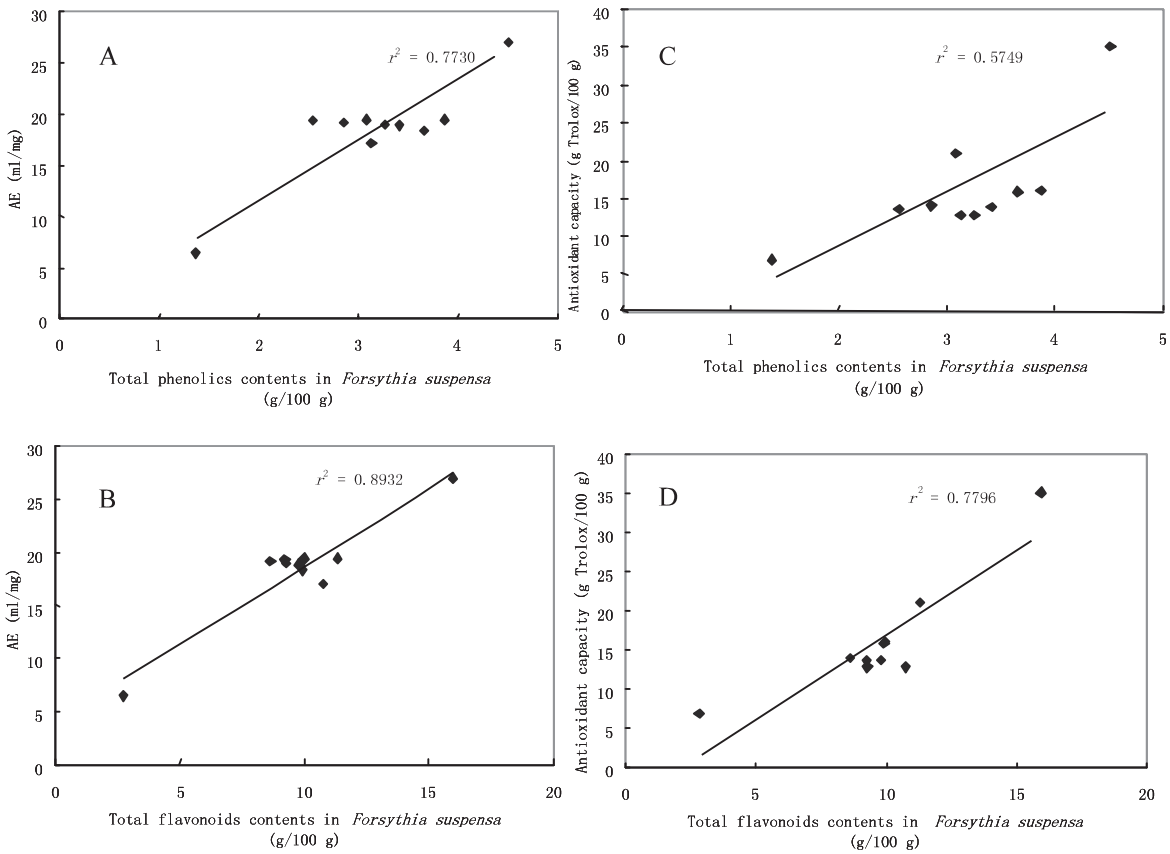

Fig. 2 Correlation of total phenolics, total flavonoids and antioxidant capacity by DPPH, ABTS scavenging activity.

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