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Polyphenol and Flavonoids Content and Antioxidant Activity of Different Solvent Extracts from *Artemisia argyi*

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Authors' contributions

This work was carried out in collaboration among all authors. Author DQW designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JY and JX managed the analyses of the study. Author JX managed the literature searches. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

The effects of extraction solvents (methanol, ethanol, acetone, petroleum ether and n-hexane) on the content of total polyphenols and flavonoids, as well as antioxidant activities of *Artemisia argyi* were investigated. The results showed that, the ethanol extract had the highest total polyphenols and flavonoids content, and it exhibited stronger antioxidant activities, followed by methanol and acetone extracts. Correlation analysis revealed that the content of phytochemicals was well correlated with antioxidant activities of extracts from *Artemisia argyi*, which indicates that different solvents had a great influence on the level of total polyphenols, flavonoids and antioxidant activities of extracts. Therefore, selective extraction from *Artemisia argyi*, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity, which will be useful for the developing and application of *Artemisia argyi*.

Keywords: Artemisia argyi; extraction solvent; polyphenols; flavonoid; antioxidant activity.

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1. INTRODUCTION

Artemisia argyi (*A. argyi*) is known in Chinese as "Aicao" and in Japanese as "Gaiyou" and belongs to the Arteraceae family that comprises over 500 species [1], which is mainly distributed in Asia, Europe and North America [2]. As a traditional medicinal and edible plant, ancient Chinese commonly pick the buds and leafs of *A. argyi* consumed as a food supplement or a flavoring and colorant for the Chinese dish Qingtuan [3]. *A. argyi* also contains many active compounds such as volatile oil [1], flavonoids [4], phenols, organic acids [4], polysaccharides [5], terpenoids [6] and glycosides [4,7-9]. Several Studies have reported that *A. argyi* extracts possess various biological activ ities, such as antioxidant [8], anti-inflammatory [10], antibacterial, anti-tumor [11], hemostatic, anti-hypertensive effects, immunoregulatory, anti-asthmatic, oncogene inhibitory and anti-osteoporotic activities [7,8,12,13]. Extract solvents is one of the most important factors affecting the chemical composition and biological activity of plant extracts [14,15]. Therefore, the objective of this work was to investigate the content of total polyphenols, flavonoids and antioxidant activity of different solvent extracts (methanol, ethanol, acetone, petroleum ether, n-hexane) from *Artemisia argyi.*

2. MATERIALS AND METHODS

2.1 Preparation of Extracts

Fresh leaves of *artemisiae argyi* were collected around the fifth day of the fifth lunar month in 2019 from Linfen (Shanxi Province) and were dried at 40°C. The dried leaves (100 g) were grounded and were extracted with 250 mL solvents (methanol, ethanol, acetone, petroleum ether, n-hexane) and kept in a shaker at 25°C for 1 h. And then the mixture was centrifuged at 4000 rpm/min for 15 min at 4°C. The precipitation was extracted with 250 mL solvent once again and mixed supernatants. The extracts were dried under vacuum and stored at 4°C until analysis.

2.2 Determination of Polyphenolic Content

Polyphenolic content of extracts was determined according to method described by Bettaieb et al. with some modifications [14]. Briefly, an aliquot (0.1 mL) extract was mixed with 2.8 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent (1.0 mol/L), and then the solution was mixed and incubated at room temperature (25°C) for eight minutes. Following that, 2 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added and shaken thoroughly. The mixture was incubated for 2 h in the dark at room temperature (25°C) and the absorbance was determined at 765 nm. 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.

2.3 Determination of Flavonoid Content

An aliquot (1 mL) extract was mixed with 0.3 mL of 3% $NaNO_3$ solution and incubated for 6 min. After that, 0.3 mL of 10% $Al(NO_3)_3$ was added and the solution was kept at room temperature (25°C) for 6 min. Finally, mix the solution with 4 mL of 4% NaOH solution and add water to 10 mL. After 20 minute of incubation, the absorbance of the mixture at 510 nm was measured. Rutin was used for calibration of the standard curve, and flavonoid content was expressed as milligram rutin equivalents per gram dried weight.

2.4 DPPH Radical Scavenging Assay

DPPH radical scavenging assay was measured the method described by Xu et al. with some modifications [16]. 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 (25°C) for 30 min, and the absorbance was measured at 517 nm. Trolox was used for calibration of the standard curve and the DPPH radical scavenging activity of sample was expressed as milligram Trolox equivalent per gram dried weight (mg Trolox/g).

2.5 ABTS Radical Scavenging Assay

ABTS radical scavenging assay was determined according to the method described by Xu et al [16]. 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. Trolox was used for

calibration of the standard curve and the ABTS radical scavenging activity of sample was expressed as milligram Trolox equivalent per gram dried weight (mg Trolox/g).

2.6 Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing ability was determined as described by Xu et al [16]. Briefly, the FRAP reagent was freshly prepared from 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-Tri(2-pyridyl)-s-triazine (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°C in a water bath prior to use. Then 0.1 mL sample solution was mixed with 1.8 mL FRAP reagent and 3.1 mL ultra-pure water. The absorption of the reaction mixture was measured at 593 nm after incubation for 30 min at 37°C. The standard curve was constructed using $FeSO_4$ solution (100-1000 μM), and FRAP value was expressed as micromoles Fe(II) per gram DW.

3. RESULTS AND DISCUSSION

3.1 Contents of Total Polyphenols and Flavonoids

The contents of phytochemicals of different solvent extracts were showed in Fig. 1. Depending on the solvent used, the total polyphenol content ranged from 6.58 to 25.6 mg GAE/g. Ethanol extract had the highest polyphenol content, followed by methanol, acetone, the lowest for petroleum ether and n-hexane. However, no significant difference in total phenolic content was found between Petroleum ether and n-hexane extracts. The range of flavonoid content in different solvent extracts was 12.6-40.2 mg Re/g and their order corresponded to the same order in the polyphenol content. 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. However, there was not a one-to-one relationship between the polarity of solvent and the content of phytochemicals. These differences in the content of total phenolics and flavonoids from different extracts may be come from differences in the polarity, chemical structure, dielectric constant of solvents [17], which can influence the extraction efficiency and compositions of extracts.

3.2 DPPH and ABTS Radicals Scavenging Abilities of Different Extracts

Due to the differences in the compositions and contents of the phytochemicals, the extracts showed the different antioxidant abilities. Table 2 showed that there were significant differences of DPPH radical scavenging abilities of different solvent extracts. Consistent with the results in phytochemical test, ethanol extract owned the highest DPPH radical scavenging ability, followed by methanol and acetone extracts. Petroleum ether and n-hexane extracts had lowest DPPH radical scavenging ability, of which were 0.63 and 0.86 mg Trolox/g respectively. But there was no significant difference between petroleum ether and n-hexane extracts. The results of ABTS radical scavenging abilities were similar to that of DPPH assay. The difference was that there was no significant differences between methanol and ethanol extracts.

3.3 Ferric Reducing Antioxidant Power (FRAP)

Table 1 also showed that different extracts from *Artemisia argyi* exhibited different reducing power. There was a significant difference ($p < 0.05$) in the reducing power among different extracts, and ranged from 16.7 to 55.6 μmol Fe(II)/g DW for n-hexane, petroleum ether, acetone, ethanol and methanol extracts, respectively. As observed in the DPPH and ABTS, the reducing power of methanol extracts was the highest, followed by ethanol, acetone, and petroleum ether extracts, the lowest for hexane extracts. The results suggested that extracts from *Artemisia argyi* had a potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction [16].

3.4 Correlation among Total Phenolics, Flavonoids and Antioxidant Activities

To further investigate their interrelationship, the correlation between the active compounds and biological activities were established, and correlation coefficients (R) are shown in Table 2. In this study, the DPPH and ABTS were highly and positively correlated to the content of both total phenolics and flavonoids ($R \geq 0.893$), indicating total phenolics and flavonoids are the main constituents contributing to the antioxidant

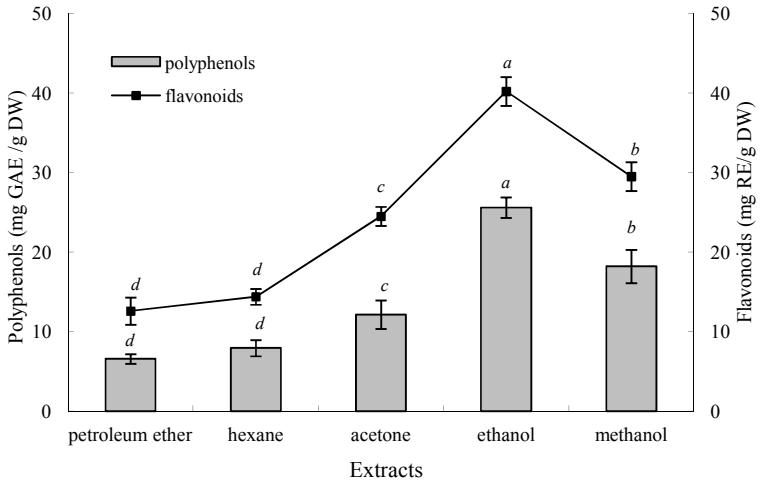

Fig. 1. The contents of total phenolics and flavonoids of extracts from *Artemisia argyi*

Table 1. The antioxidant activities of different extracts from *Artemisia argyi*

	DPPH (mg Trolox/g)	ABTS (mg Trolox /g)	FRAP (μmol Fe(II)/g)
Petroleum ether	0.63±0.06d	2.88±0.63c	18.6±1.3d
n-Hexane	0.86±0.16d	2.55±0.52c	16.7±1.4 d
Acetone	2.76±0.42c	6.84±0.73 b	31.5±1.9 c
Ethanol	6.93±0.44a	14.25±1.42a	48.5±2.2 b
Methanol	4.35±0.52b	12.72±1.57 a	55.6±3.1 a

Table 2. Correlation analysis of polyphenols, flavonoids and antioxidant activities

	Phenolics	Flavonoids	Antioxidant activities		
			DPPH	ABTS	FRAP
Phenolics	1				
Flavonoids	0.964*	1			
DPPH	0.992**	0.988*	1		
ABTS	0.930	0.893	0.935	1	
FRAP	0.557	0.501	0.568	0.821	1

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

and antibacterial activities of extracts, which was supported by previous reports studied on some cereals or plants [18,19]. However, FRAP was moderately correlated to the content of phenolics and flavonoids.

4. CONCLUSIONS

Plant polyphenols and flavonoids have been considered as one of the most widely distributed secondary metabolites which contains various complicated compounds. Based on the principle of the dissolution in the similar material structure, different solvent extracts differ in its active constituents as well as their contents due to the different polarity and solubility of extracting solvents. Our findings indicated that ethanol extract had the highest content of polyphenol and flavonoid among all of five solvent extracts. Consistent with results described above, ethanol extract showed the highest antioxidant abilities in DPPH, ABTS radicals scavenging and FRAP assays. These results indicated that selective extraction from *Artemisia argyi*, by an appropriate solvent, is important for the development and utilization of *Artemisia argyi*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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