International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 05-04-2019; Accepted: 07-05-2019
www.pharmacyjournal.in
Volume 4; Issue 4; July 2019; Page No. 00-00

Chemical composition and antioxidant capacity of three wild fruits

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Abstract
In this study, three wild fruits (*Chaenomeles speciosa* Nakai, *Malus halliana* Koehne, *Malus micromalus* Makino) were extracted with different concentrations of ethanol (50% ethanol, 70% ethanol, 95% ethanol), which provided rich resources of total phenols and total flavonoids. The results showed that the 50% ethanol extract of *Cs.n* had the highest total phenols content (287.89±14.42 mg/g) and total flavonoids content (44.63±2.32 mg/g). The *Cs.n* extract for 50% ethanol was demonstrated to have the highest DPPH scavenging ability (EC_{50} = 0.08±0.01 mg/mL), while its extract for 70% ethanol was ranked with the best ABTS scavenging ability (EC_{50} = 0.13±0.00 mg/mL). For reducing power assay, the *Cs.n* extract for 95% ethanol was found with the highest UV absorbance (0.927±0.00). Correlation analysis indicated that there was an extremely significant correlation between total phenols, total flavonoids and antioxidant activity.

Keywords: total phenols, total flavonoids, antioxidant activity assays

1. Introduction

Cellular metabolism, smoking, radiation and toxic chemicals etc. can cause the generation of reactive oxygen species, leading to pathological conditions in humans [1]. Antioxidants are to protect the cells from the damaging effects of reactive oxygen species [2]. Considering our health, there has been a tremendous trend in replacing the synthetic antioxidants with the natural ones nowadays. It has been demonstrated that many chemical compounds from natural fruits or vegetables possess more antioxidant potential than vitamin E or vitamin C, such as terpenoids, alkaloids, total flavonoids, total phenols and tannis [3, 4]. The fruits of *Malus prunifolia* are also a valuable source to be exploited [5]. As a common name of several plants of *Malus* and *Chaenomeles* [6, 7], *Malus prunifolia* has the unique pharmacological activities of oxidation resistance [2, 5, 8, 9], giddiness treating [10], α-glucosidase inhibition [11-13] and is an astringent by the Palliyar tribals [14]. Nowadays, the limited studies of *Malus prunifolia* were mostly concentrated on cultivation management [15-17]. For the researches of *Malus micromalus* Makino, only the volatile constituent has been studied [18]. There exists several articles on the exploration of leaves, full flowers and buds [5, 19], but few articles [20, 21] focusing on the fruit. Little research has been thoroughly done on these three kinds of fruits. The objective of this study was to evaluate and compare antioxidant properties of different solvents extracts of these three wild fruits, using 2, 2,-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-2-ethylbenz-othiazoline-6-sulfonate (ABTS) radical-scavenging and ferric-reducing antioxidant power (FRAP) assay. By measuring the total content of phenols and flavonoids, the correlation between these components and the radical scavenging capacities was also analyzed.

2. Materials and methods

2.1 Collection of Plant samples

The three kinds of fruits were collected in September from Southwest University of Science and Technology, Mianyang, Sichuan, China and were authenticated by the authority of Prof. Lin Ma from Engineering Research Center for Biomass Resource Utilization and Modification of Sichuan Province.

2.2 Extraction

The fresh fruits were cut and dried in the constant oven at 55°C for 48 h. Then, the dried fruits were crushed to powder (20 mesh). This extraction was carried out with Wei's method with some modification [22]. Ultrasonic extraction was conducted for 0.5 h with 50% ethanol, 70% ethanol and 95% ethanol respectively. This experiment was carried out by controlling the solid-liquid ratio (1:20), the ultrasonic power (150 W), and the temperature (50°C). Then, the filtrates were obtained and concentrated before being stored at room temperature.

2.3 Determination of total phenols

Total phenols content in the extracts was estimated based on Yang's [23] method with some modifications. Briefly, added 3 mL of 10% saturated sodium carbonate solution after the samples mixed with Folin-Ciocalteu. And the absorbance was read at 760 nm.

2.4 Determination of total flavonoids

The total flavonoids of different fruit extracts were determined according to the method of Lv Peng [24] with Rutin being used as a standard. Firstly, different solvent extracts (0.1-0.5 mg/mL) were dissolved with 95% ethanol. Then 2 mL of the sample was placed in the tube and was

added 2 mL of the 5% NaOH instead.

2.5 DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts was measured by the method of Saltarelli R [25] with some modifications. The extracts were dissolved with ethanol and 2 mL of 0.08 mM DPPH solution. Percentage of inhibition of the DPPH radical was calculated according to the following equation (1). Results were expressed as a percentage of inhibition of the DPPH radical and EC_{50} values were calculated. The same procedure was repeated on Vitamin C (0.1-0.5 mg/mL).

$$\text{Scavenging rate } (\%) = \frac{A_0 - (A_i - A_j)}{A_0} \times 100\% \qquad (1)$$

Where A_0 is the absorbance of DPPH solution without a sample, A_i is the absorbance of the test sample mixed with DPPH, A_j is the absorbance of the sample without DPPH solution.

2.6 ABTS radical scavenging activity

The ABTS radical scavenging activity was measured by using ABTS radical cation decolorization assay [25]. The ABTS scavenging activity was calculated according to the equation(1) as well, where A_0 is the absorbance of $ABTS^+$ solution mixed with PBS, A_i is the absorbance of the test sample mixed with the $ABTS^+$ solution, and A_j is the absorbance of the sample mixed with PBS.

2.7 Reducing power assay

Reducing power assay was performed according to the method of Wang [12] with some modifications. The volume of 0.2 mol/L PBS, 1% potassium ferrocyanide solution and 10% trichloroacetic acid was changed to 0.5 mL. In like manner, 0.5 mL of the supernatants was mixed up with 0.5 mL of distilled water and 1 mL of 1% ferric trichloride solution for 10 min instead.

3. Results and discussion

3.1 Total phenols and total flavonoids content

The total phenols and the total flavonoids of different solvents from three kinds of fruits were showed in Fig. 1.

Contents of total phenols and total flavonoids from 50% ethanol extracts were much higher than the other two species, which suggested that 50% ethanol could serve as better extraction solvent for phenolic and flavonoids compound. Previous studies showed that the greater the polarity of the extraction solvent was, the higher the extraction rates of the phenolic compounds and the total phenol content would be [26]. The total phenols content of extracts from three cultivars ranged from 80.50 to 287.90 mg/g and total flavonoids content ranged from 12.80 to 44.63 mg/g, being the highest in 50% ethanol extract and the lowest in 95% ethanol extract. In addition the *Cs.n* with the highest flavonoids content (44.63±2.35 mg/g) had the highest phenolic content (287.90±14.42 mg/g). In recent studies, it has been reported that the contents of total phenols and total flavonoids from *Cs.n* were the highest among 14 species of crabapple fruits, which were followed by *Malus halliana* Koehne and *Malus micromalus* Makino [21]. The phenolic content and total flavonoids content of ethanol extracts in this study were much higher than the content of ethyl acetate extracts from the same three species reported by Li et al. [21] This could be as a result of the different geographical location of the plants[27] as well as the type of solvents [28].

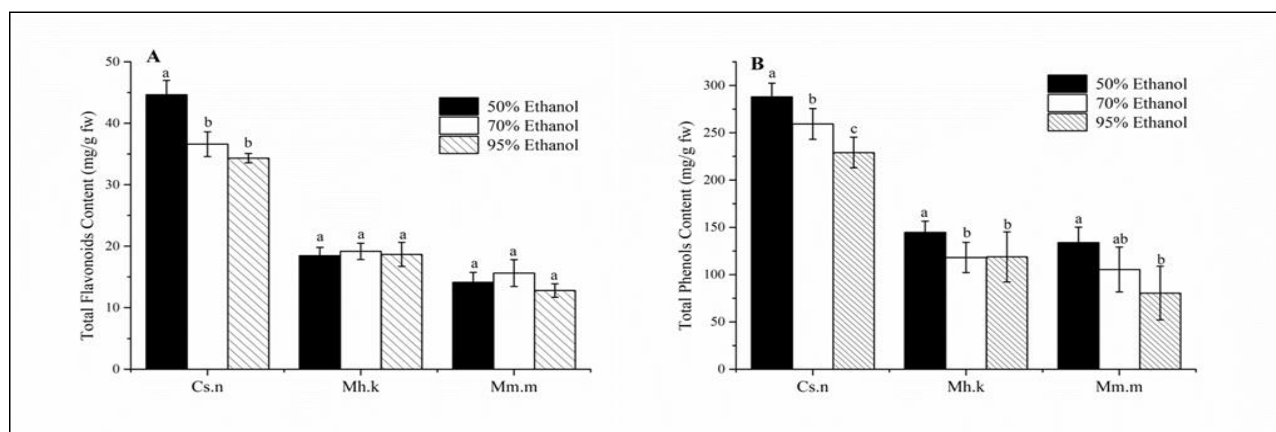

Fig 1: Total flavonoids content (A) and total phenols content (B) of three fruits with different Ethanol. Vertical bars show standard deviation of means from three replicates. Values with different letters from the same kind of fruit are significantly different ($p<0.05$). *Cs.n* stands for *Chaenomeles speciosa (Sweet)* Nakai extracted with three ethanol concentrations. *Mh.k* stands for *Malus halliana* Koehne extracted with three ethanol concentrations. *Mm.m* stands for *Malus micromalus* Makino extracted with three ethanol concentrations.

3.2 DPPH radical scavenging activity

DPPH assay is frequently used to measure radical scavenging activity of natural compounds [29]. The DPPH scavenging activities of different solvent extracts from three kinds of fruits were presented in Fig.2. It showed the DPPH radical scavenging activity increased with concentration of the extracts, which was in accordance with Motalleb G [8]. At 0.5 mg/mL, the highest scavenging rate for *Mm.m* was 53.52%, but the scavenging rate for *Cs.n* extracts reached the same level at 0.1 mg/mL. Highest scavenging was observed in *Cs.n* extract with 50% ethanol, which EC_{50} value was 0.08±0.01 mg/mL. *Mm.m* extract with 70% ethanol had the low EC_{50} value (0.48±0.02 mg/mL) (Table 1). The lower the EC_{50} value of a sample demonstrated the stronger antioxidant activity. In conclusion, the DPPH scavenging activities order of the extracts was: *Cs.n* > *Mh.k* >*Mm.m*, 50% ethanol > 70% ethanol > 95% ethanol overall. Furthermore, the linear correlation between DPPH

scavenging activities and total phenolic as well as flavonoids contents in the extracts of three kinds of fruits were investigated (Table 2). As was shown in Table 2, significant positive correlations (R^2 = 0.887, R^2 = 0.908) were separately observed between total phenolic also flavonoids contents and EC_{50} values for DPPH, suggesting the contribution of total phenols and total flavonoids to these antioxidant assays [29].

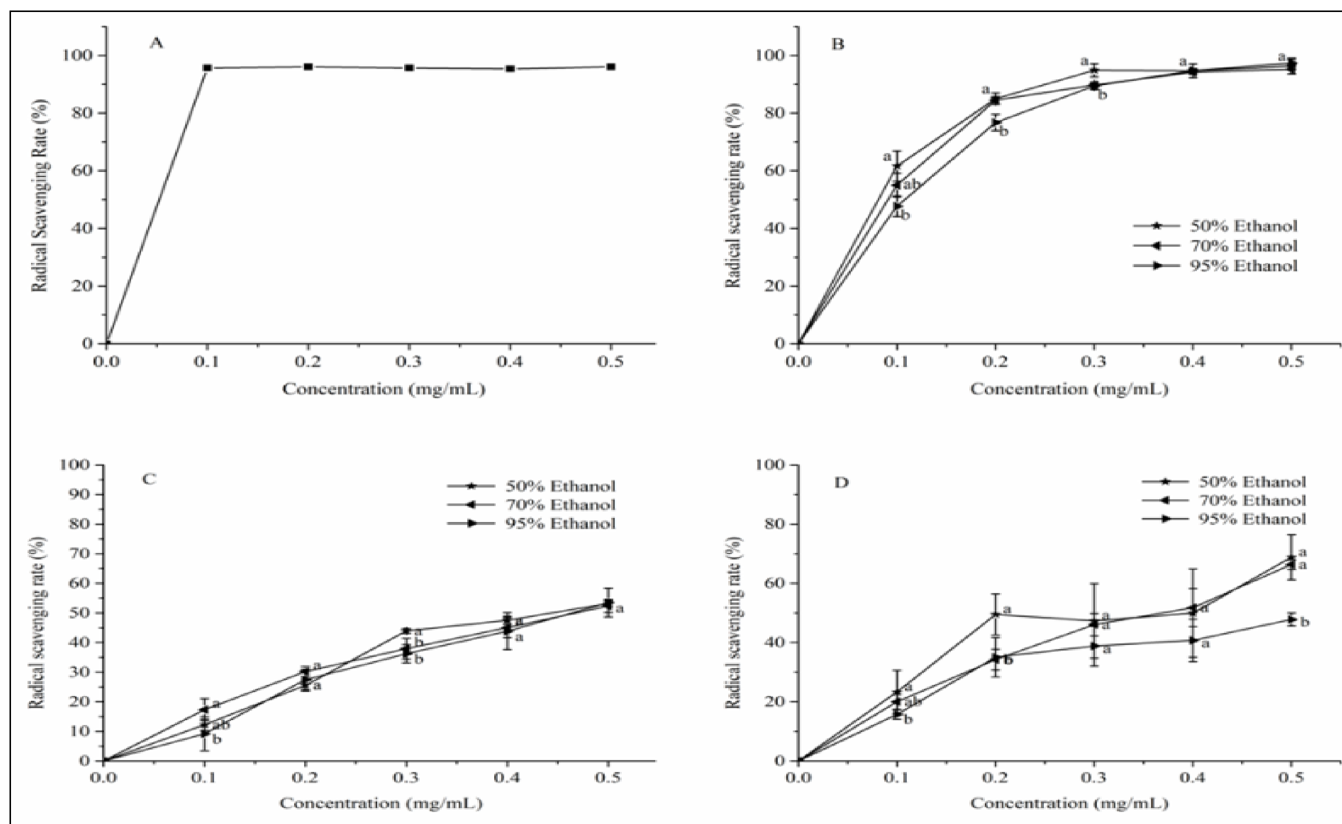

Fig 2: The DPPH radical scavenging activity of three fruits extracted with different ethanol. Vertical bars show standard deviation of means from three replicates. Values with different letters from the same kind of fruit are significantly different (p<0.05). (A) stands for VC. (B) stands for *Chaenomeles speciosa (Sweet)* Nakai extracted with three ethanol concentrations. (C) stands for *Malus micromalus* Makino extracted with three ethanol concentrations. (D) stands for *Malus halliana* Koehne extracted with three ethanol concentrations.

3.3 ABTS assay

The ABTS scavenging activity of different solvent extracts from three kinds of fruits was showed in Fig.3. In comparison with the DPPH assay, the antioxidant activity order of 50% ethanol and 70% ethanol extracts for *Cs. n* was a little different, with the highest ABTS radical scavenging rate 99.96% at 0.5 mg/mL extracted from 70% ethanol. During concentration from 0.1 mg/mL to 0.3 mg/mL, the gradient of the curves of percentage of inhibition versus concentration for *Cs.n* is steeper than for the other two species, indicating that in this concentration the anti-radical activity increased rapidly with concentration [30]. The EC_{50} values for ABTS were somewhat different from the EC_{50} values for DPPH. That proved the inferior capacities for them to scavenge free radical. The EC_{50} for *Malus micromalus* Makino extract with 95% ethanol, which was read at 0.47±0.02 mg/mL, was not in accordance with the radical scavenging rate that was the lowest. It could be that the radical scavenging capacity increased slowly after certain concentration. As shown in Table 2, significant positive correlations (R^2 = 0.887, R^2 = 0.864) were separately observed between total phenolic also flavonoids contents and EC_{50} values for ABTS, suggesting the contribution of total phenols and total flavonoids to these antioxidant assays [29].

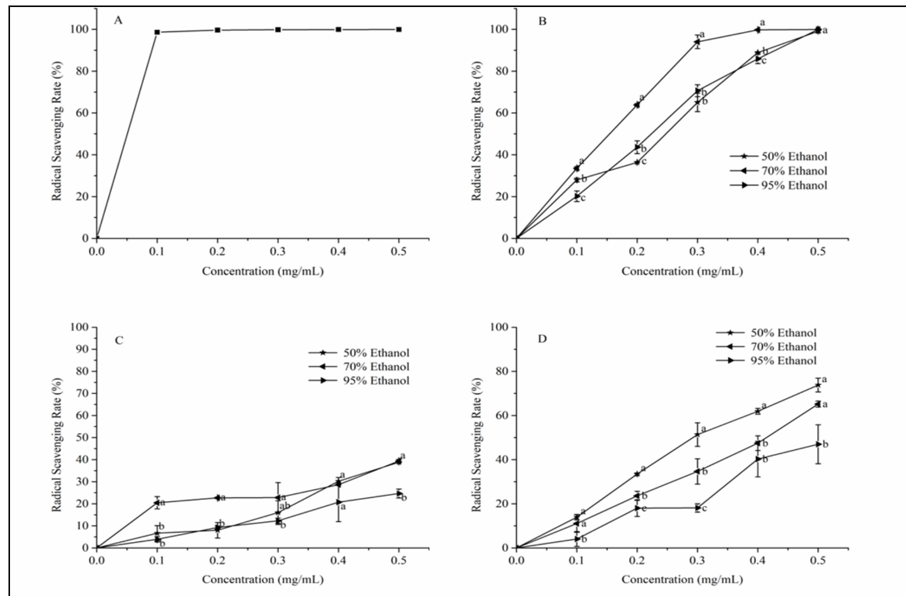

Fig 3: The ABTS radical scavenging activity of three fruits extracted with different ethanol. Vertical bars show standard deviation of means from three replicates. Values with different letters from the same kind of fruit are significantly different (p<0.05). (A) stands for VC. (B) stands for *Chaenomeles speciosa (Sweet)* Nakai extracted with three ethanol concentrations. (C) stands for *Malus micromalus* Makino extracted with three ethanol concentrations. (D) stands for *Malus halliana* Koehne extracted with three ethanol concentrations.

3.4 Reducing power assay

The UV absorbance for all extracts was showed in Fig.4. The higher value of UV absorbance was, the better reducing power would be. The UV absorbance of extracts against reducing power was dependent on concentration. Nevertheless, the *Cs.n* extract for 95% ethanol was the best in this analytic system, in contrast with the results from Fig.2 or Fig.3. The antioxidant activities for different *Mm.m* extracts were so close to each other. The gradients increased slowly and remained constant for *Cs.n* after 0.1 mg/mL. The UV absorbance for *Cs.n* extract with 70% ethanol decreased after 0.1 mg/mL while for *Mm.m* extract with 50% ethanol after 0.3 mg/mL, this could because that the reducing power method is not stable for long time experiment.

The correlation between the content of bioactive compounds (total flavonoids, total phenols) and antioxidant activity was showed in Table 2. There was significant correlation between the chemical compounds with the radical scavenging activity. The results revealed that both chemicals are important groups in representing the antioxidant activity. The result was in agreement with the conclusion of Shim S M [31], who suggested that strong correlation between antioxidant activity and total phenolic content (R^2 = 0.927). Also, the good linear correlations obtained between phenolic con-centration and antioxidant capacity determined by the DPPH and ABTS assays from Piluzza G [32] suggested that phenolic content could be used as an indicator of antioxidant properties of the examined plant species.

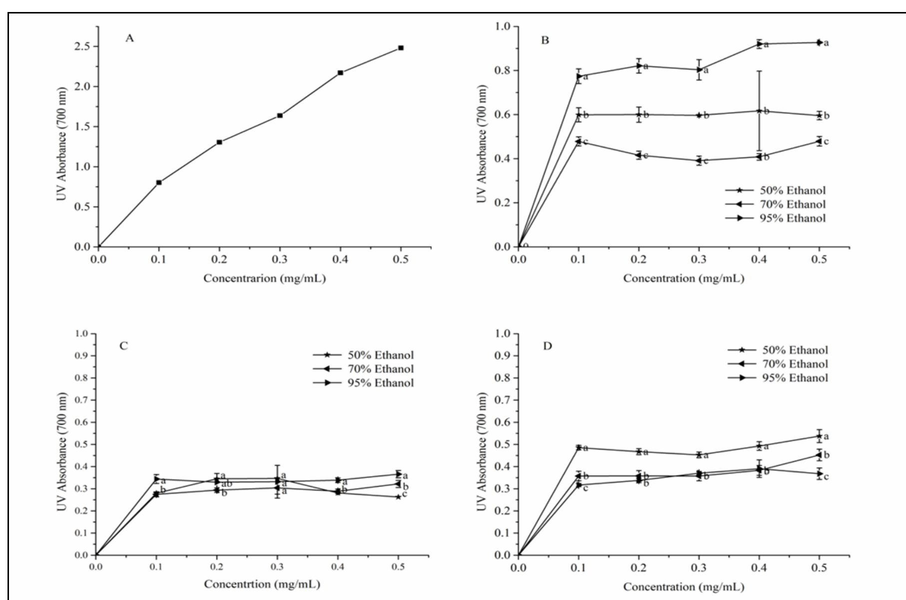

Fig 4: The UV absorbance of three fruits extracted with different ethanol. Vertical bars show standard deviation of means from three replicates. Values with different letters from the same kind of fruit are significantly different (p<0.05). (A) stands for VC. (B) stands for *Chaenomeles speciosa (Sweet)* Nakai extracted with three ethanol concentrations. (C) stands for *Malus micromalus* Makino extracted with three ethanol concentrations. (D) stands for *Malus halliana* Koehne extracted with three ethanol concentrations.

Table 1: EC_{50} of DPPH RSC (Radical Scavenging Capacities) and ABTS RSC of extracts from three fruits. Data was from three repetitions, with mean± standard deviation. Values with different letters in each column are significantly different (p<0.05).

Extracts	EC_{50} of DPPH RSC (mg/mL)			EC_{50} of ABTS RSC (mg/mL)		
	50% Ethanol	70% Ethanol	95% Ethanol	50% Ethanol	70% Ethanol	95% Ethanol
Cs.n	$0.08±0.01^{c}$	$0.09±0.01^{c}$	$0.11±0.01^{b}$	$0.20±0.01^{c}$	$0.13±0.00^{c}$	$0.20±0.00^{b}$
Mh.k.	$0.30±0.06^{b}$	$0.33±0.02^{b}$	$0.50±0.13^{a}$	$0.28±0.01^{b}$	$0.40±0.02^{b}$	$0.56±0.12^{a}$
Mm.m	$0.42±0.01^{a}$	$0.48±0.02^{a}$	$0.47±0.02^{a}$	$0.52±0.02^{a}$	$0.48±0.19^{a}$	$0.47±0.02^{a}$

Table 2: The correlation between the content of bioactive compounds (total phenols, total flavonoids) and antioxidant activity at the concentration 0.5 mg/mL, *, p<0.05; **, p<0.01.

Antioxidant active components	Radical Scavenging Activity		
	DPPH	ABTS	Reducing Power
Total flavonoids	0.908**	0.864**	0.655**
Total phenols	0.887**	0.877**	0.603**

4. Conclusion

The results indicated that *Cs.n* ethanol extracts had higher antioxidant scavenging activity compared with other extracts (50% ethanol DPPH RSC EC_{50} = 0.08±0.01 mg/mL, 70% ethanol ABTS RSC EC_{50} = 0.13±0.00 mg/mL, UV absorbance = 0.927± 0.00). Thus, the fruit of *Chaenomeles speciosa* Nakai might be potential and valuable for further research in food and chemistry industries. This study also supported the view that the antioxidant ability of extracts can be predicted by total phenolic and flavonoid content and illustrated that total flavonoids, total phenols content and antioxidant activity were various based on the type of plants and solvents used for extraction.

5. Acknowledgements

This work was supported by Innovative practice project by school of life science and engineering, Southwest University of Science and Technology (Grant No. smcx1708).

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