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Phenolic Profile, Antioxidant Activity and Anti-proliferative Activity of Crabapple Fruits

Meiling Han[a], *Guo Li*[b], *Xiaowei Liu*[c], *Ai Li*[a], *Peiqi Mao*[a], *Pengyuan Liu*[a], *and Houhua Li*[a,*]

[a] *College of Landscape Architecture and Art, Northwest A&F University, Yangling, Shaanxi 712100, China*
[b] *Faculty of Natural Sciences, Institute of Horticultural Production Systems, Floriculture, Leibniz Universität Hannover, Hannover D-30419, Germany*
[c] *Novogene Bioinformatics Technology Co. Ltd, Beijing 100089, China*

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A B S T R A C T

To explore the potential of crabapples as functional food, polyphenols in crabapples and 'Fuji' apples were extracted, and the phenolic profile, total polyphenols, antioxidant activity and anti-proliferative activity against several human cancer cells were determined. The results indicated that crabapple extracts have more abundant phenols and higher total polyphenols (from 4.46 to 46.63 mg GAE·g^{-1} DW) compared to 'Fuji' apples. Crabapple extracts possessed higher antioxidant activity than apple by DPPH and ABTS analysis. All fruit extracts exhibited inhibitory effects on proliferation in different cancer cells; however, crabapple extracts performed significantly better, with half inhibitory concentration (IC_{50}) values varied from 48.34 μg·mL^{-1} to 974.81 μg·mL^{-1} for colon cancer cells SW480, 64.67–1 466.35 μg·mL^{-1} for stomach cancer cells BGC-803, 78.88–910.64 μg·mL^{-1} for esophageal cancer cells CaEs-17. Besides, the red crabapples had higher antioxidant activity and anti-proliferative activity than yellow fruits. These results showed that crabapples, especially red crabapples, have great potential as a healthy food, as they are rich in phenolic compounds with high antioxidant and anti-proliferative activities to cancer cells.

Keywords: Crabapple; Phenolic profile; Antioxidant activity; Anti-proliferative activity; Human cancer cell

1. Introduction

Many studies have suggested that the bioactive compounds of fruit and vegetable extracts are beneficial to human health and prolong life (Record et al., 2001). Polyphenols, also known as vegetable tannins, are found in many fruits and vegetables and have antioxidant activity (Valavanidis et al., 2009; Vieira et al., 2011), can lower blood pressure (Balasuriya and Rupasinghe, 2012), and exhibit anti-inflammatory (Andre et al., 2012), anti-obesity (Sergent et al., 2012), and anticarcinogenic activity (Seeram et al., 2004; Elansary and Mahmoud, 2015; Luo et al., 2017; Bahukhandi et al., 2018).

Multiple studies have focused on the antioxidant activity and anticancer activity of polyphenols in fruit and vegetable extracts. For instance, the association between phenolic compounds and antioxidant activity in different blueberry cultivars (Li et al., 2013) and mushroom (Liu et al., 2017) has been reported. Some researchers have demonstrated that the antioxidant activity of fruit extracts is closely associated with the anti-proliferative activity of several common cancer cells, including lung, breast, leukemia and colon (Jayaprakasha et al., 2007; Aneta et al., 2013; Luo et al., 2017). These studies indicated that phenolic compounds, antioxidant activity and anticancer activity are closely connected to one another.

Apples are important and popular fruits all over the world. Eating apples reduces the risk of cardiovascular disease, cancer, type II diabetes, and promotes weight loss (Kalinowska et al., 2014). Numerous published studies have extensively evaluated

* Corresponding author. Tel.: +86 15114800050
E-mail address: lihouhua73@163.com
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phenolic compounds and their function with respect to apple consumption. Both cultivar and organic or conventional agricultural practices have influenced polyphenolic profiles and the antioxidant activity of apples (Tsao et al., 2003). Kao et al. (2015) have studied the mechanism whereby apple polyphenols inhibit cancer cells.

Both crabapples and apples belong to the genus *Malus* (Rosaceae family) and have similar profiles of phenolic compounds (Chen et al., 2014). However, unlike apples, crabapples are typically used as ornamental plants. To our knowledge, most studies (Zhang et al., 2014, 2017) have focused on the ornamental features of crabapples, including flower and leaf colors. In some parts of China, crabapples are also used as a food source. However, only a few studies have focused on other, non-ornamental functions of crabapple. Li et al. (2014) reported different antioxidant activities among 10 wild crabapples varieties. Li et al. (2016) discovered that fruits extractions of five crabapples can lower hypercholesterolaemia. In a separate study (Qin et al., 2015), dihydrochalcone compounds isolated from crabapple leaves were shown to have anticancer effects. Our lab has previously demonstrated that crabapple fruits, especially red varieties, had more abundant phenolic compounds than apples (Wei et al., 2015).

Malus pumila 'Fuji' ('Fuji' apples), the dominant apple cultivar in China, has been shown to possess antioxidant and antiproliferative activity (Luo et al., 2016). To explore the potential of crabapples as functional food, 3 different colors of crabapple as well as 'Fuji' apples were used in our experiments. Fruit extracts were obtained, and the following parameters were determined: phenolic profile, total polyphenols, antioxidant activity and whether an inhibitory effect existed with respect to several different human cancer cells.

2. Materials and methods

2.1. Plant materials

Three crabapple varieties were chosen. Fruit of *Malus* 'Red splendor' is red, while the fruits of *M. micromalus* 'Haihongguo' and *M. micromalus* are yellow. The apple variety *M. pumila* 'Fuji' was used for comparison.

All plant materials were grown in the crabapple germplasm nursery of Northwest A&F University, Yangling, China. Fruits with similar maturity were harvested from late August to early October 2016. Afterwards, all the fruits were washed, cut into slices, and stored at –4 °C for less than 12 h. Samples were frozen at –80 °C for 12 h before vacuum freeze-drying for 72 h. After being comminuted into powder, samples were stored in airtight containers until use.

2.2. Chemicals and reagents

Methanol, acetic acid, acetonitrile (HPLC grade), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazine (DPPH), Dimethyl sulfoxide (DMSO), and 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) were purchased from Sigma Chemical Co. (St. Louis, USA). Fetal bovine serum (FBS) and RPMI 1640 medium were obtained from Gibco (New York, USA). The phenolic compound standards were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). EnoGeneCell^{TM} Counting Kit-8 was provided by EnoGene Biotech Co., Ltd. (Nanjing, China). Paclitaxel was obtained from Sichuan Taiji Pharmaceutical Co., Ltd. (Chengdu, China). Human colon cancer SW480, stomach cancer BGC-803 and esophageal cancer CaEs-17 cell lines were provided by Nanjing OGpharma Co., Ltd. (Nanjing, China). All other chemicals were analytical grade.

2.3. Preparation of fruit extracts

The extraction method was adopted from Bi et al. (2014) with slight modifications. Each powder sample was weighed to 0.3 g, and polyphenolic compound extraction was performed with methanol, formic acid and water (70:2:28 in volume ratio) at 4 °C for 24 h. Extracts were then centrifuged (10 000 × *g* for 10 min), and extraction solution was added to the supernatant until the total volume was 15 mL, which was then passed through a 0.22 μm syringe filter. The pH of extracts was determined by ST2011 pH meter (Ohaus Corporation, Shanghai, China) and stored at 4 °C for phenolic profiling, and measurements of total polyphenols and antioxidant activity were determined.

A total of 200 g of powder was extracted from each sample with 1 000 mL methanol for 12 h for 3 times. Supernatants were combined and concentrated by rotary flash evaporation. After concentration, samples were stored at –20 °C for evaluating the inhibition of cancer cell proliferation in vitro. All experiments with the extracts were repeated 3 times.

2.4. Identification of the phenolic compounds by HPLC

Phenolic compounds were analyzed by a HP1200 Liquid Chromatograph equipped with a diode array detector (Agilent Technology, Palo Alto, USA) and an Inertsil ODS-3 column (5.0 μm particle size, 4.6 mm × 250 mm) (GL Sciences Inc., Japan) preceded by an Inertsil ODS-3 Guard Column (5.0 μm, 4.0 mm × 10 mm) as previously described by Bi et al. (2014). HPLC separation was performed using a linear gradient of A (10% formic acid dissolved in water) and B (10% formic acid and 1.36% water in acetonitrile) at 30 °C at a flow rate of 1.0 mL·min^{-1}. The solvent gradient used was as follows: 0 min, 95% A, 5% B; 25 min, 85% A, 15% B; 42 min, 78% A, 22% B; 60 min, 64% A, 35% B; and 65 min, 95% A, 5% B. The post-run time was 10 min. Identification of phenolic compounds was carried out by comparing retention times and UV spectral data with authentic standards. The concentrations of phenolic compounds were carried out using calibration curves of the standards.

2.5. Determination of total polyphenols

Total polyphenols of extracts were determined by the method of Singleton, with minor modifications (Singleton and Joseph, 1965). Briefly, 0.85 mL of the Folin–Ciocalteu reagent was added to 0.5 mL of sample and incubated at room temperature for 5 min. Then, 0.25 mL of 20% sodium carbonate solution was added to the mixture. After 30 min incubation in the dark, the absorbance of the mixture was measured at 760 nm using a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan). Total polyphenol content was calculated by comparing with a standard curve of gallic acid, and results were expressed as milligrams of gallic acid equivalents (GAE) per g dry weight (mg GAE·g^{-1} DW).

2.6. Quantification of antioxidant activity

The DPPH radical scavenging capacity of the extracts was determined by a modified protocol (Gulcin et al., 2010). A total of 5 μL of the extract was added to 2 mL of 62.5 μmol·L^{-1} DPPH•ethanol solution. The absorbance of the reaction mixture was measured immediately at 517 nm, designated A1. After incubation for 30 min in the dark, the absorbance of the mixture was recorded at 517 nm, designated A2. Final absorbance was A1–A2. The DPPH radical scavenging capacity was calculated by comparison with a standard curve of trolox and the results were expressed as micromoles of trolox equivalents (TE) per g dry weight (μmol TE·g^{-1} DW).

A known method with slight modifications was used to determine ABTS$^{\bullet+}$ radical scavenging capacity of extracts (Re et al., 1999). Briefly, ABTS$^{\bullet+}$ solution was made by mixing 7 mmol·L^{-1} ABTS solution with 2.45 mmol·L^{-1} potassium persulfate in a 1:1 vol ratio and incubated in the dark for 16 h. Then, the ABTS$^{\bullet+}$ solution was diluted with sodium acetate buffer solution (pH 4.5) to an absorbance of (0.700 ± 0.020) at 734 nm and equilibrated at 30 °C. A total of 5 μL of extract was added to 2 mL ABTS$^{\bullet+}$ solution, and the absorbance of the mixture was measured at 734 nm, designated A3. After incubation in the dark for 15 min, the absorbance of the mixed solution was measured at 734 nm, designated A4, and the final absorbance was A3–A4. ABTS$^{\bullet+}$ radical scavenging capacity was calculated by comparison with a standard curve of trolox, and the results were expressed as micromoles of trolox equivalents (TE) per g dry weight (μmol TE·g^{-1} DW).

2.7. Inhibition of cancer cell proliferation

All cancer cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 μg·mL^{-1} penicillin and 50 μg·mL^{-1} streptomycin, and incubated at 37 °C with 5% CO_2 and saturated humidity.

After trypsinization, cancer cells were plated in growth medium at a density of 1×10^4 cells/well in 96-well flat-bottomed cell culture plates at 37 °C for 24 h. Then, 100 μL of different concentrations of extracts determined in preliminary test were added to each well (except for control wells). The concentrations of *M.* 'Red splendor' extracts were 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250 and 500 μg·mL^{-1}, and the concentrations of *M. micromalus* 'Haihongguo', *M. micromalus* and *M. pumila* 'Fuji' were 3.91, 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1 000, and 2 000 μg·mL^{-1}. For control cells, 100 μL of 10 μg·mL^{-1} taxol (definitely anticancer effect) were added to wells as a positive control to determine the effect of fruit extract on cancer cells; solvent controls (saline) were also utilized to eliminate the effect of the solvent on cancer cells; water was acted as blank control. Each treatment was repeated 5 times. After incubation for 72 h, 10 μL CCK-8 solutions was added in each well then incubated for 4 h. Optical density of the cells was measured at 450 nm by Thermo MK3 ELISA (Thermo Fisher Scientific Inc., USA). The inhibition rate was calculated as (1–optical density (OD) of treated sample/OD of control sample) × 100%. The anti-proliferative activity of fruit extract was represented as the IC_{50} value (half inhibitory concentration).

2.8. Assessment of cellular morphology

After 72 h of treatment, the cells were observed for changes in morphology by a XD-202 inverted phase contrast microscope (Nanjing Jiangnan Novel Optics Co., Ltd., China) and photographed.

2.9. Statistical analysis

Data were expressed as the means ± *SD* values. Statistical analyses used SPSS Statistics 22 software. One- and Two-way analysis of variance (ANOVA) was used to compare the means. The results were deemed significant at $P < 0.05$.

3. Results

3.1. Identification of the phenolic compounds in fruit extracts

Before determining the phenolic compositions, we measured the pH of fruit extracts. The results showed that the pH for *M. micromalus*, *M. pumila* 'Fuji', *M. micromalus* 'Haihongguo' and *M.* 'Red splendor' was 2.81, 2.88, 2.92, 3.01, respectively, and there was no significant difference among fruit extracts.

The phenolic compositions were identified by HPLC (Table 1). The results indicated that most compositions were the same in all fruits examined. Caffeic acid, *p*-coumaric acid, hyperin and reynoutrin were only detected in some fruits. Cyanidin-3-galactoside, the main anthocyanins in apples (Peng and Moriguchi, 2013), was not found in *M. micromalus* in this study. Procyanidin B1 and B2, catechin and epicatechin were the major constituents of *M. micromalus* 'Haihongguo' and *M.* 'Red splendor', while procyanidin B1 and hyperin were the primary phenols found in *M. micromalus*.

Most phenolic compound levels showed significant differences between the four fruit extracts. The main phenolic compounds in crabapples were present at higher levels compared to apples, especially the phenolic compounds in *M.* 'Red splendor', ranging from 1.5- (gallic acid) to 242.2-fold (procyanidin B2) higher than *M. pumila* 'Fuji'. *M.* 'Red splendor' and *M. micromalus* 'Haihongguo' had more abundant phenols than the other fruits, which may be associated with their fruit color. Interestingly, the only phenolic compound whose concentration was significantly higher in *M. pumila* 'Fuji' compared to the three crabapples was chlorogenic acid ($P < 0.05$).

3.2. Total phenolics

Total phenolic levels of the four fruits were in order of *M.* 'Red splendor' > *M. micromalus* 'Haihongguo' > *M. micromalus* > *M. pumila* 'Fuji'. In detail, the total phenolic content of *M.* 'Red splendor' was the highest (46.63 mg GAE·g^{-1} DW), followed by *M. micromalus* 'Haihongguo' (20.81 mg GAE·g^{-1} DW) and *M. micromalus* (4.46 mg GAE·g^{-1} DW). *M. pumila* 'Fuji' has the lowest total pehnolic level (4.06 mg GAE·g^{-1} DW), with approximately one-twelfth of *M.* 'Red splendor'. The value differences between *M.* 'Red splendor' and *M. micromalus* 'Haihongguo' were statistically significant ($P < 0.05$), while *M. micromalus* and *M. pumila* 'Fuji' were not significant ($P > 0.05$) (Fig. 1).

Table 1 Phenolic compounds in sample extracts **(mg·kg^{-1} DW)**

	Sample	Gallic acid	Procyanidin B1	Catechin	Procyanidin B2	Epicatechin	Syringic acid
Crabapple	*M. micromalus*	16.36 ± 0.21 bc	103.57 ± 9.65 c	20.91 ± 0.88 d	20.88 ± 1.23 c	36.34 ± 1.93 d	2.28 ± 0.14 c
	M. micromalus 'Haihongguo'	19.10 ± 0.64 ab	847.88 ± 45.32 b	430.96 ± 19.29 b	203.47 ± 14.67 b	1 220.78 ± 56.83 b	27.87 ± 1.23 b
	M. 'Red splendor'	21.66 ± 0.45 a	1534.78 ± 105.6 a	1326.33 ± 98.54 a	2151.86 ± 123.43 a	2727.69 ± 112.34 a	129.99 ± 9.87 a
Apple	*M. pumila* 'Fuji'	14.06 ± 0.17 d	13.63 ± 2.12 d	38.33 ± 1.65 c	8.88 ± 0.33 d	176.42 ± 10.23 c	4.58 ± 0.43 c

	Sample	Phloridzin	Chlorogenic acid	Caffeic acid	*p*-Coumaric acid	Hyperin	Rutin
Crabapple	*M. micromalus*	24.57 ± 2.11 c	1.03 ± 0.04 d	ND	0.59 ± 0.06 a	72.09 ± 9.54 a	7.49 ± 0.56 b
	M. micromalus 'Haihongguo'	94.42 ± 10.35 b	38.15 ± 1.35 c	2.27 ± 0.44	0.32 ± 0.05 b	65.13 ± 3.53 b	5.40 ± 0.33 bc
	M. 'Red splendor'	131.02 ± 7.53 a	70.23 ± 5.66 b	ND	0.72 ± 0.35 a	13.13 ± 0.56 c	41.20 ± 4.32 a
Apple	*M. pumila* 'Fuji'	2.87 ± 0.32 d	91.62 ± 13.54 a	ND	ND	ND	2.89 ± 0.11 d

	Sample	Isoquercitrin	Reynoutrin	Avicularin	Quercitrin	Cyanidin-3-galactoside
Crabapple	*M. micromalus*	25.01 ± 2.11 b	7.48 ± 0.67 c	17.17 ± 1.78 b	12.79 ± 1.89 a	ND
	M. micromalus 'Haihongguo'	49.06 ± 9.22 a	15.33 ± 0.35 a	25.07 ± 2.06 a	6.64 ± 0.54 b	29.71 ± 4.21 b
	M. 'Red splendor'	9.61 ± 1.28 c	9.27 ± 0.33 b	11.41 ± 0.65 c	6.48 ± 0.87 b	190.17 ± 3.52 a
Apple	*M. pumila* 'Fuji'	1.01 ± 0.14 d	ND	2.85 ± 0.14 d	1.16 ± 0.31 c	3.58 ± 0.55 c

Note: Values are reported as mean ± standard deviation ($n = 3$). Different small letters indicate a significant difference at $P < 0.05$. ND, not detected.

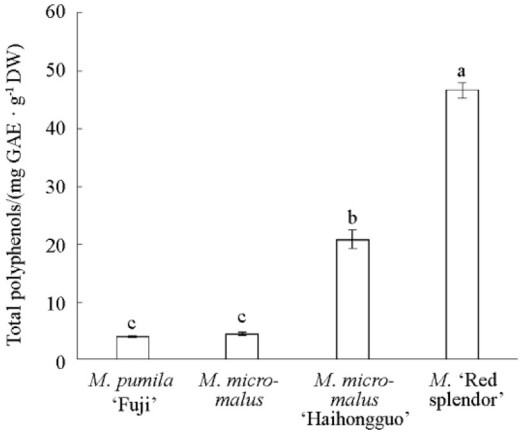

Fig. 1 Total polyphenols of fruit extracts
Different letters above the bar indicate a significant difference at $P < 0.05$.

3.3. *Antioxidant activity*

The antioxidant activity of the four fruit extracts was determined by DPPH and ABTS methods (Fig. 2). The four fruit extracts had a wide range of DPPH radical scavenging capacity, from 120.36 to 383.19 μmol TE•g^{-1} DW. *M.* 'Red splendor' had the highest DPPH radical scavenging capacity among the four fruits. The difference between *M. micromalus* 'Haihongguo' and *M. micromalus* was significant ($P < 0.05$). *M. micromalus* and *M. pumila* 'Fuji' were not statistically significant from each other (Fig. 2, A).

Similarly, the four fruit extracts exhibited the same order in ABTS$^{\bullet+}$ radical scavenging capacity. *M.* 'Red splendor' had the strongest ABTS$^{\bullet+}$ radical scavenging capacity (176.32 μmol TE·g^{-1} DW), being 2.9-fold greater than *M. pumila* 'Fuji' (60.12 μmol TE·g^{-1} DW). *M. micromalus* 'Haihongguo' had the second highest ABTS$^{\bullet+}$ radical scavenging capacity, followed by *M. micromalus*, and the difference between these two varieties was significant ($P < 0.05$) (Fig. 2, B).

3.4. *Anti-proliferative activity*

Different concentrations of fruit extracts were tested for their anti-proliferative activity in vitro against the three human cancer cells. We found that all extracts exhibited anti-proliferative effects on cancer cells, which were strongly dose-dependent (Fig. 3).

Compared with other extracts, *M.* 'Red splendor' had the strongest inhibitory effect on proliferation of all cancer cells, including those at low concentrations with maximum inhibition rate reaching 76.28% (500 μg·mL^{-1} extract on BGC-803 cell lines, Fig. 3). Besides, the inhibitory effect of *M.* 'Red splendor' increased dramatically at low concentrations. The *M. pumila* 'Fuji' curves had similar slopes among the different cell lines. The inhibition effect of *M. micromalus* was always weaker than the data of *M. micromalus* 'Haihongguo' except on CaEs-17 cell lines when their extract concentration was 2 000 μg·mL^{-1} (Fig. 3).

Anti-proliferative activity is represented as IC_{50} values, with lower IC_{50} values representing higher anti-proliferative activities. Table 2 shows the IC_{50} values of different extracts. Compared to other extracts, *M.* 'Red splendor' had the lowest IC_{50} value for cancer cell lines, while *M. pumila* 'Fuji' had the highest. The IC_{50} values were significantly different among *M.* 'Red splendor', *M. micromalus* 'Haihongguo', *M. micromalus* and *M. pumila* 'Fuji' ($P < 0.05$). These results indicated that the inhibitory effect of *M.* 'Red splendor' extract on cancer cell proliferation was superior to the other three fruit extracts, particularly when compared with the apple variety.

After treatment with extracts for 72 h, different cancer cell lines exhibited dramatic changes in cellular morphology (Fig. 4). Taxol was used as positive control in this assay. Extract-treated cells and the positive control exhibited the following changes: decreases in cell density, the development of irregular shapes, and cytoplasmic shrinkage, from intact spindles to scattered rounds (SW480 cells). *M.* 'Red splendor' fruit extract had the strongest effect on cellular morphological changes at 500 μg·mL^{-1}, even better than the positive controls (Fig. 4).

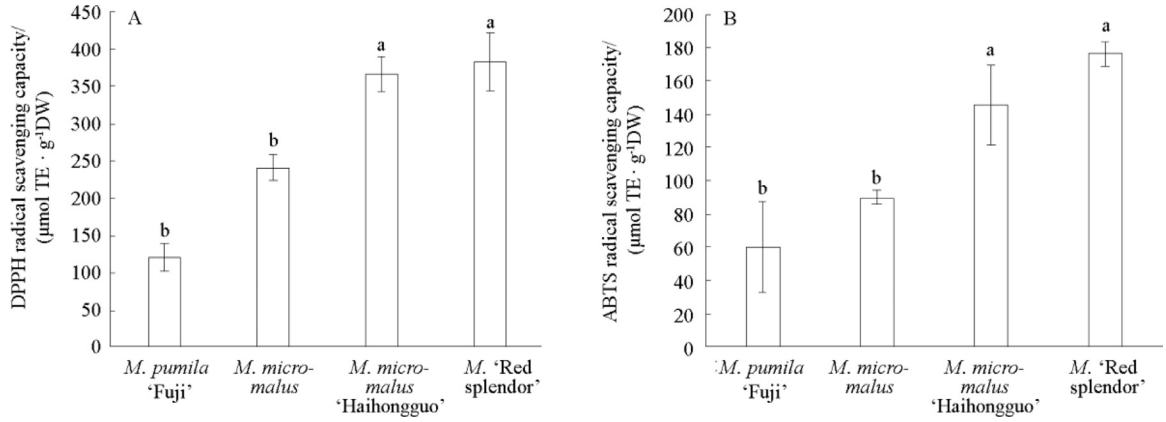

Fig. 2 Antioxidant activity of fruit extracts
Different letters above the bar indicate significant difference at $P < 0.05$.

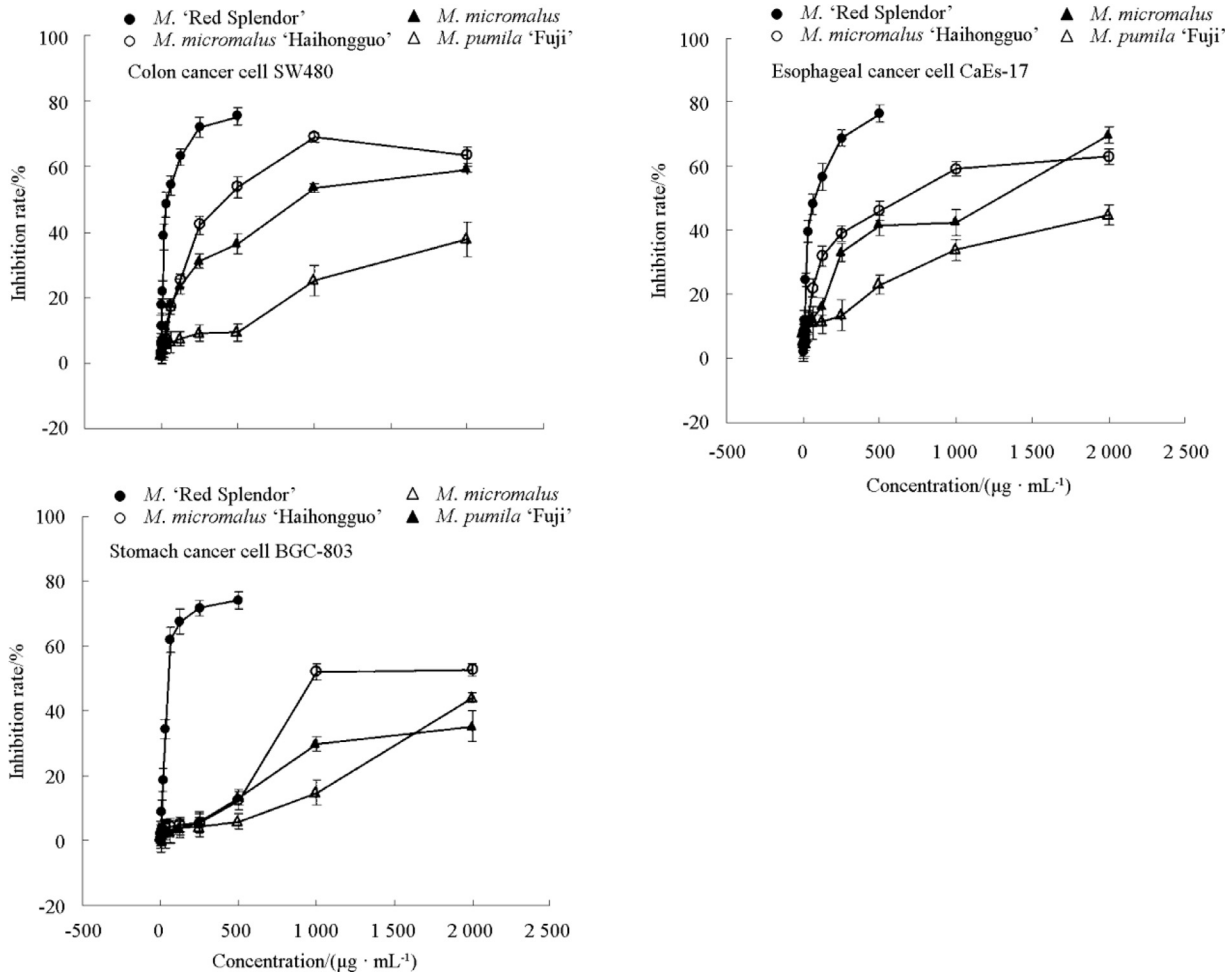

Fig. 3 Anticancer activity of fruit extracts ($n = 5$)
Concentration of *M.* 'Red splendor' extract ranged from 0.98 to 500 μg·mL^{-1}. Concentration ranges of *M. micromalus* 'Haihongguo', *M. micromalus* and *Malus pumila* 'Fuji' were from 3.91 to 2 000 μg·mL^{-1}. The inhibition rates of positive control (100 μL of 10 μg·mL^{-1} taxol) for SW480, CaEs-17 and BGC-803 were 72.69%, 76.96% and 70.11%, respectively.

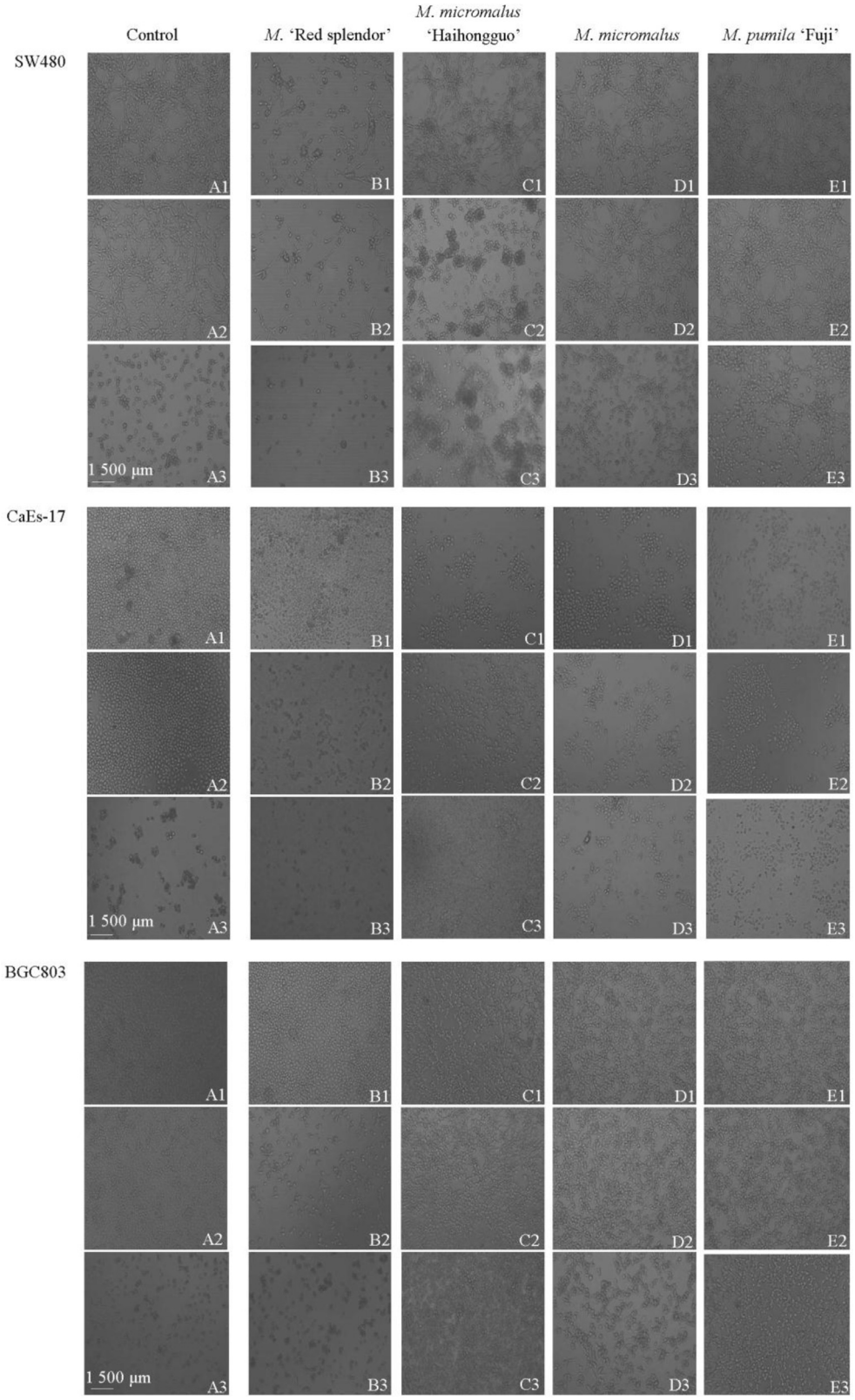

Fig. 4 Different morphological changes of cancer cells in response to different treatments

A1 to A3 are the blank control, solvent control and positive control, respectively. B1 to B3 represents extracts of *M.* 'Red splendor' of 31.25, 125 and 500 $\mu g \cdot mL^{-1}$, respectively. C, D and E represent extracts of *M. micromalus* 'Haihongguo', *M. micromalus* and *M. pumila* 'Fuji' in different concentrations (from 1 to 3 are 125, 500 and 2 000 $\mu g \cdot mL^{-1}$, respectively).

Table 2 Anti-proliferative activity (IC_{50}) of extracts against colon cancer cells SW480, esophageal cancer cells CaEs-17 and stomach cancer cells BGC-803 ($\mu g \cdot mL^{-1}$)

Extract tested		SW480	CaEs-17	BGC-803
Crabapple	*M.* 'Red splendor'	48.34 ± 3.66 d	78.88 ± 2.45 c	64.47 ± 4.33 d
	M. micromalus 'Haihongguo'	482.53 ± 20.77 c	610.52 ± 43.87 b	1 466.49 ± 110.79 c
	M. micromalus	974.81 ± 54.28 b	910.62 ± 64.88 b	2 291.38 ± 161.76 b
Apple	*M. pumila* 'Fuji'	4 212.54 ± 194.77 a	4 348.64 ± 278.68 a	3 476.54 ± 180.99 a

Note: Values are reported as mean ± standard deviation ($n = 3$). Different small letters indicate a significant difference at $P < 0.05$.

Table 3 Correlation coefficients of polyphenolic compounds, antioxidant activity (DPPH and ABTS assay) and anticancer activity (IC_{50} of fruit extracts)

	Total polyphenols	DPPH	ABTS	IC_{50}
DPPH	0.820	1		
ABTS	0.890**	0.729**	1	
IC_{50}	–0.657*	–0.603*	–0.823**	1
Gallic acid	0.938	0.962*	0.995**	–0.871
Procyanidin B1	0.988*	0.892*	0.972*	–0.724
Procyanidin B2	0.951*	0.641	0.792	–0.533
Phloridzin	0.946	0.956*	0.996**	–0.806

Note: ** Means significance at $P < 0.01$. * Means significance at $P < 0.05$.

Compared with apple extract, treatment with crabapple extracts always resulted in greater effects at the same concentration. As shown in Fig. 4, *M. pumila* 'Fuji' had obvious effects on morphological changes in cells but had little influence on SW480 and BGC-803 cell numbers.

Remarkably, when the concentration of *M. micromalus* 'Haihongguo' extract was increased from 1 000 to 2 000 $\mu g \cdot mL^{-1}$, the inhibition rate for SW480 cells was slightly decreased (from 69.00% to 63.49%) rather than rising continually (Fig. 3). Inhibitory effect for other two cancer cells also maintained a stable level after the extract concentration reached 1 000 $\mu g \cdot mL^{-1}$. With increasing concentrations of *M. micromalus* 'Haihongguo' extract, we observed decreased SW480 cell counts, with dead cells clumping together. This indicated that *M. micromalus* 'Haihongguo' extract may induce cell growth inhibition and cell death via a different mechanism than the other extracts, but further research is required to explain this phenomenon.

3.5. *Correlations between phenolic compounds, antioxidant activity and anti-proliferative activity*

Correlations between phenolic compounds, total antioxidant activity, and anti-proliferative activity (IC_{50} values) of the crabapple and apple extracts were analyzed (Table 3). There were extremely significant positive correlations among total polyphenolic content and $ABTS^{\bullet+}$ radical scavenging capacity ($P < 0.01$), while correlation between DPPH radical scavenging capacity and total polyphenolic content was not significant ($P > 0.05$). Total polyphenolic levels were significantly negatively correlated with IC_{50} values of the extracts ($P < 0.05$). Negative correlations were also observed between antioxidant activity and IC_{50} values of the extracts, with –0.603 for DPPH ($P < 0.05$) and -0.823 for ABTS ($P < 0.01$), respectively. In addition, gallic acid and procyanidin B1 were significantly correlated with DPPH radical scavenging capacity ($P < 0.05$). The correlations between $ABTS^{\bullet+}$ radical scavenging capacity with gallic acid and phloridzin were very significant ($P < 0.01$), with procyanidin B1 also being significant ($P < 0.05$). There were negative correlations between gallic acid, phloridzin, procyanidins and IC_{50}, but these values were not statistically significant ($P > 0.05$).

4. Discussion

Different phenolic compositions between crabapple varieties and apples may be explained by genetic variation or differences in environmental conditions and geographic locations (McRae et al., 1990; Awad et al., 2000). Previous reports (Lata et al., 2009; Kim et al., 2017) have demonstrated that chlorogenic acid, rutin, catechin and epicatechin are major phenolic compounds in apple, and our results are in agreement with these reports. Phloridzin is a typical phenol in apple and has been found in fruits and leaves of different apple varieties (Dragovic-Uzelac et al., 2005). Phloridzin was also found in crabapples and had much higher concentrations than apple.

Previous research has demonstrated that the total phenolic and flavonoid contents in wild crabapples were higher than domestic apples (Valavanidis et al., 2009; Chen et al., 2014), possibly due to genetic and environmental reasons (McRae et al., 1990; Awad et al., 2000). Moreover, the original growth conditions of crabapples were complex; their high phenolic composition helps them resist disease (Valavanidis et al., 2009). Our results indicate that red crabapples have more total polyphenolic content than yellow fruits.

Antioxidant activity was assessed using methods that yielded similar results: crabapple extract activity was higher than apple extract, and red crabapple varieties had higher activity than yellow fruits, consistent with total polyphenolic levels. Similarly, a previous study showed that red currant had lower total polyphenolic levels and antioxidant capacity than black currant (Aneta et al., 2013), and Shahidi et al. (2006) found similar result in black sesame seeds compared to white seeds. These results indicate that darker tissue colors may be relevant to the levels of total polyphenol and antioxidant activity.

Many research studies have shown that apple extract has anti-proliferative activity against various cancer cells, including colon, breast, liver and gastric cancer (Waldecker et al., 2008; Sudan and Rupasinghe, 2014; Yang et al., 2015). Moreover, different varieties of apples seem to affect cancer cell proliferation at different intensities (Liu et al., 2001; Serra et al., 2010). Our results indicate that crabapples have much higher anti-proliferation properties than apples for different cancer cells. The anticancer capacity also varies between varieties of crabapples, being especially higher in red color crabapples.

Lower concentrations of extracts resulted in little changes in cell morphology, consistent with the cell viability experiments. Both assays demonstrated that extract effects were dose-dependent manner. There are many in vitro studies that have reported similar dose-dependent effects by other plant extracts in anticancer experiments (Parry et al., 2006; Yuan et al., 2009; Bhavana et al., 2016).

The highest total polyphenolic content, antioxidant activity, and anti-proliferative activity were all detected in *M.* 'Red splendor', suggesting associations between phenolic content, antioxidant capacity and anticancer capacity. Cancer cells increase oxidation and free radical production, and phenolic contents in *M.* 'Red splendor' exhibit good antioxidant activity, which can inhibit cell proliferation (Hileman et al., 2004).

A study by Serra et al. (2010) found procyanidin B1, catechin and epicatechin were the main factors contributing to the antioxidant activity of apples, and procyanidins (B1 and B2), phloridzin and epicatechin were found to have important roles in the inhibition of human digestive cancer cell proliferation. In this study, statistical analysis indicated that phloridzin, gallic acid and procyanidins played important roles in antioxidant and anticancer activities. Some reports have found that anthocyanins had anticancer capacity through the induction of apoptotic factors (Mazewski et al., 2018). Awad and de Jager (2000) demonstrated that the presence of cyanidin-3-galactoside contributed to the red color of apples. In this study, anthocyanin was detected in *M.* 'Red splendor', *M. micromalus* 'Haihongguo' and *M.* pumila 'Fuji'. Most phenolic components, including cyanidin-3-galactoside, were the highest in *M.* 'Red splendor', whose anticancer activity was also strongest. However, *M. micromalus*, in which no anthocyanin was detected, also had higher anticancer activity than *M. pumila* 'Fuji'. These results may indicate that antioxidant and anticancer activity are not the simple sum of single phenolic compounds but a result of synergy among different bioactive compounds (Serra et al., 2010).

5. Conclusion

Based on the results of this study, it can be concluded that crabapples have more abundant phenolic components, higher antioxidant activity and stronger inhibition on cancer cell proliferation than apples. The red crabapple variety *Malus* 'Red splendor' had more potent affects than the two other crabapple varieties. Therefore, crabapple fruits, especially red varieties, have more dynamic uses aside from ornamental function, particularly for use as a functional anticancer food and source of natural antioxidants.

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