

Comparative Evaluation of Five Different Cultivars of Chestnut Flower (*Castanea mollissima* Blume): Phytonutrient and Chemical Contents, Antioxidant and Anti-tumor Activities

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Abstract

Chestnut flower has been a well-known Chinese traditional medicine to be consumed for clearing heat and intestinal infectious diseases. It is necessary to characterize them in accordance to nutritional value into sources of bioactive nutraceuticals with potential health benefits. The purpose of this study is to obtain the phytonutrient facts, phytochemical antioxidant capacity and anti-tumor activities from five common Chinese chestnut (*Castanea mollissima* Blume) flower cultivars. The results obtained exhibit some significant difference among the five cultivars. Zipo genus shows highest total flavonoids contents (85.1 ± 3.6 mgRE/gdm) and protein contents (4.28%), while the one with highest total phenolic content (126.4 ± 0.8 mgGAE/gdm) and total sugar (5.08%) is Yanlong. As for mineral compositions, Zipo genus shows a higher content of Ca, Fe and Cu, while Yanlong outperforms in the content of Zn and Mn. Phytochemical profiling revealed eight compounds belonging to the phenolic acids and flavonoids. Gallic acid and quercetin are the two major compounds of phenolics in all five cultivars. The Chinese chestnut flower cultivars with high phytonutrient content has significantly higher antioxidant and anti-tumor abilities than that of other cultivars. Total flavonoids content and total phenolic content could bring large benefit to antioxidant and anti-tumor activity.

Keywords

Antioxidant activity, Anti-tumor activity, *Castanea mollissima* flowers, Mineral content, Total phenolic contents, Total flavonoids contents

Introduction

Castanea mollissima Blume (Chinese chestnut) belongs to Fagaceae family, and has been widely cultivated in Eastern Asia. Its fruits are most consumed worldwide because of superior quality and flavor compared with European and American species. It has long been considered as an important nutritional and economic resource in China [1]. There are many previous studies about chestnuts, which mainly focused on the nutritional ingredients of chestnut fruits [2-4] and its biological activity [5], and few studies on flowers. Chestnut flowers are a kind of crop waste since before pollination. Large amounts of its male flowers are picked off to increase the production. In Chinese traditional medicine, *C. mollissima* flower has been associated with health, and used to clear heat, disperse knots and treating diarrhea [6], and its water decoction is applied to treat bacterial dysentery, children vomiting and other intestinal infectious diseases [7]. The freshly mashed juice is externally applied externally to treat lacquer ulcers, and the sundried flower is used as a mosquito repellent.

It has been reported that the secondary metabolites of natural plants, such as phenols, flavonoid, tannins and saponins which can act as protective agent against free radicals and pathological attack [8]. Also, there are some previous results indicate that natural antioxidant polyphenols associate with promising tumor suppressive properties [9]. Phytochemical studies on chestnut flowers revealed that the flowers have rich content of flavonoids, phytosterols, amino acid and tannins. There are several studies investigated the physiological activities [10, 11]. Although chestnut flowers could be a promising source of bioactive nutraceuticals with potential health benefits and large amounts of flowers, in the process of castration, they remained underutilized. The volatile constituent of chestnut flowers has been described, and little comprehensive information is available for the chemical contents and the biological activities of common chestnut cultivars. Same as other plant species, the concentration of phytochemicals of chestnut flowers vary from varieties and growth conditions. In this work, the phytonutrient and chemical contents of chestnut flowers were explored, and its antioxidant capacity (DPPH, ABTS, FRAP and $\cdot\text{OH}$ scavenging) and tumor suppressive properties were evaluated from five different cultivars (Zipo (ZP), Yanshanduanzhi (YSDZ), Yanlong (YL), Yanshanzaofeng (YSZF) and Yankui (YK). Moreover, an additional goal was set to find the most promising chestnut cultivars with respect to the content of bioactive compounds and health-benefited properties, so as to develop a selection procedure suitable for a chestnut breeding program for the food industry.

Materials and Methods

Plant material

Five cultivars of chestnut flowers (*Castanea mollissima* Blume) were evaluated. Flowers of 'Zipo' (ZP), 'Yanshanduanzhi' (YSDZ), 'Yanlong' (YL), 'Yanshanzaofeng' (YSZF) and 'Yankui' (YK) were collected from Qinhuangdao, China (longitude 119.6 and dimension 39.93). All of the materials were harvested in July 2017. During the sampling process, five cultivars randomly chosen flowers from ten trees. For each cultivar flowers approximately 5 kg were collected from ten individual trees. The samples were dried in a shady place and ground to a powder form and screened through 60 size mesh. Powder was stored at $-80\text{ }^{\circ}\text{C}$ until use.

Materials and reagents

DPPH, ABTS and TPTZ purchased from Sigma Chemicals Company (Saint Louis, MO, USA). Gallic acid, rutin and Folin-Ciocalteu reagents were obtained from Shanghai Yuanye Bio-Technology Co., Ltd (Shanghai, China). Formic acid and acetonitrile (mass spectrum grade) purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Sodium carbonate and ethanol were purchased from Tianjin Guangfu Chemical Research Technologies Co., LTD (Tianjin, China). All the reagents were analytical grade. HCT-116, BGC-823, NCL-H1650, HL-60, HepG2, A2780, BEL-7402, A549, EC9706 and ECA109 cells (Institute of Medicine of Chinese Academy of Medical Sciences).

Extraction process

Prepared five cultivars of *C. mollissima* flower powders were extracted with ethanol: water (3:1) for 2 h, two times. The supernatants were merged and evaporated with a vacuum rotary evaporator at $46\text{ }^{\circ}\text{C}$ before being freeze-dried. The dried samples were stored at $-20\text{ }^{\circ}\text{C}$ prior before analysis. The dried flower extract was prepared at a 0.1 g/mL in methanol and stored at $-4\text{ }^{\circ}\text{C}$ until prior to analysis of the content of total phenolics, total flavonoids and antioxidant capacity.

Physicochemical analysis

The total sugar was performed by the phenol-sulfuric acid method. The protein determination by the Micro-Kjeldahl method.

Determination of micronutrients contents: The sample power 1 g was digested with 25 mL of triacid mixture. The obtained solution was filtered with a qualitative filter paper for testing with optima 2100 DV plasma emission spectrometer (Perkin Elmer, Waltham, America). All the experimental results were means \pm SD of three measurements.

Total phenolics and total flavonoids content

The total phenolics content (TPC) was measured according to modified Folin-Ciocalteu method [12]. TPC was calculated by the standard curve of gallic acid and expressed as milligrams of gallic acid equivalents per gram of dry material ($\text{mgGAE}/\% \text{dm}$).

Total flavonoid content (TFC) was measured using a reported method previously validated [13]. TFC was calculated by the standard curve of rutin equivalents and expressed as milligrams of rutin equivalents per gram of dry material ($\text{mgRE}/\% \text{dm}$). the mixture was shaken vigorously and determined at 506 nm.

Determination of phytochemical profiles

Liquid chromatography-tandem mass spectrometry (LC-MSn) was applied in this study for phytochemical composition analysis. Thermo Scientific Hypersil GOLD C18 column ($100 \times 2.1\text{ mm}$, $1.9\text{ }\mu\text{m}$) was used for separation. The injection volume of the sample was $5\text{ }\mu\text{L}$, and the column temperature was $30\text{ }^{\circ}\text{C}$. A gradient elution mode was employed with the eluents of 0.1% formic acid deionized water mixture (A) and chromatographical methanol (B). The elution program was set as follows: 0-24 min with B of 10-95%, 24-30 with B of 95-10% and 30-35 min with B of 10% at a flow rate of $0.30\text{ mL}/\text{min}$. Wavelengths of UV absorption were set at 280 nm and 324 nm to detect phenolics and flavonoids respectively. The ions were collected in a high resolution up to 100,000 and full mass scan mass range of 100-2000 m/z. Recording and analysis is performed by Xcalibur software (Thermo Fisher Scientific, USA). The qualification of phenolic compounds was obtained by the comparison of retention time and peak area, and the calibration of external standard curves.

Anti-oxidant activities

The antioxidant activity was evaluated by DPPH \cdot radical-scavenging activity [14], ABTS $\cdot+$ radical scavenging activity

[15], ferric reducing antioxidant power (FRAP) assay [16] and •OH scavenging abilities [17]. All samples were analyzed in triplicate in at least three different experiments.

Inhibitory effect against tumor cell

The anti-tumor activity assay was evaluated by MTT method. Cells were prepared into suspension of 1×10^5 concentration and cultured in 96-well plate with 90 μ L per well for 24 h. 10 μ L different concentrations of flower extracts were added to in each well in test group. After 72 h incubation, cells in each well were treated with 100 μ L 0.5 mg/mL MTT for 4 h. After aspirating supernatant, 200 μ L DMSO was added in each well to dissolve formazan crystals. The absorbance values were recorded at 544 nm by microplate reader (Versa Max, Molecular Devices, California state, USA). Each concentration was analyzed in triplicate in at least three independent experiments. These outcomes from experiments were used to calculate the IC_{50} values in each cell line.

Statistical analysis

Every experiment was run in triplicates and the data are expressed as mean \pm S.D. The statistical significance was carried out using ANOVA followed by Tukey's test for post hoc analysis. A p value of less 0.05 was considered as statistically significant.

Results

Physicochemical analysis

Values obtained from the total sugar show that the most outstanding variety of chestnut flowers was YL, which has a maximum value of 5.08%, whereas ZP has the minimum value of 4.05% (Figure 1). From five cultivars of chestnut flowers, protein value ranged from 3.72% to 4.28%. The variety of ZP is the most remarkable and highest in term of concentration (4%) compared to the others. And the variety of YSDZ shows the lowest concentration (3%).

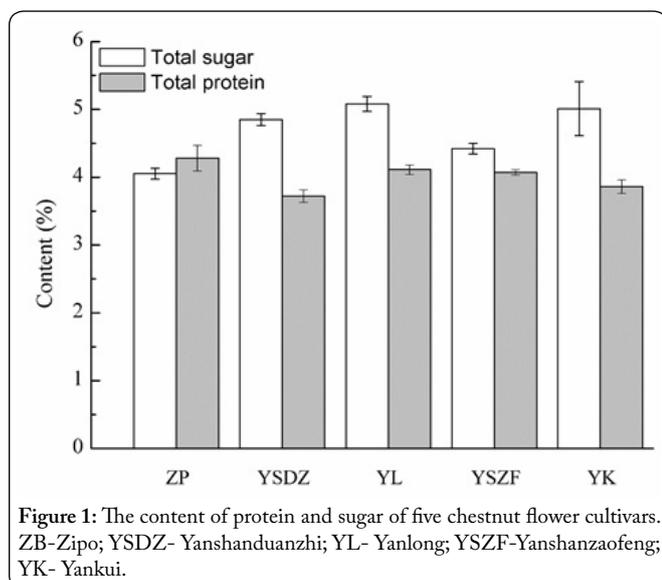


Figure 1: The content of protein and sugar of five chestnut flower cultivars. ZB-Zipo; YSDZ- Yanshanduanzhi; YL- Yanlong; YSZF-Yanshanzaofeng; YK- Yankui.

Six elements' concentrations (Ca, Mn, Zn, Fe, Cu, Cr) are presented from five kinds of chestnut flowers in figure 2. Regarding to the calcium concentration, the notable value ranges from 244.00 to 422.25 mg/kg, i.e. the variety of ZP is 422.25 mg/kg and the variety of YSDZ is with 244.00 mg/kg. Manganese value ranges between 409.10 and 766.43 mg/kg, and it is the abundant element presented in chestnut flowers. YSDZ shows the maximum level (766.43 mg/kg) and YK shows the minimum level (409.10 mg/kg). With regards to iron, the content is found ranging from 6.00 mg/kg and 21.09 mg/kg, and the variety of ZP has the higher content of 21.09 mg/kg, while YSZF has the lower content of 6.00 mg/kg. The difference of zinc concentration in five kinds of chestnut flower is not obvious, ranging from 26.90 mg/kg to 33.57 mg/kg. But the obtained copper and chromium value is in the lowest concentrations, ranging from 0.46 mg/kg to 0.87 mg/kg and 0.31 to 0.42 mg/kg, respectively.

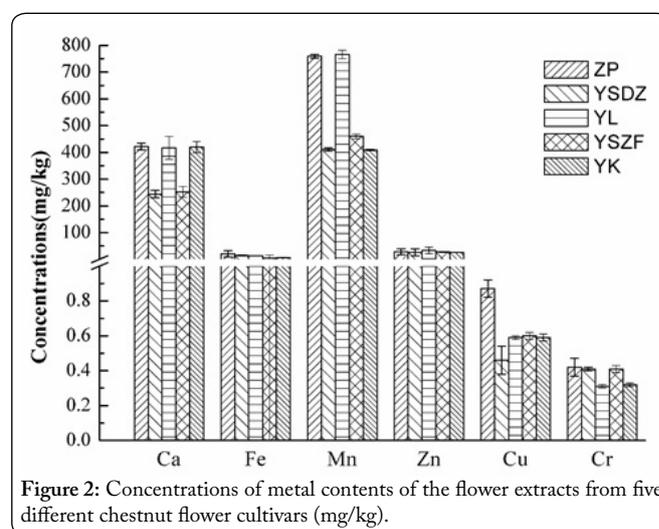


Figure 2: Concentrations of metal contents of the flower extracts from five different chestnut flower cultivars (mg/kg).

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The TPC and TFC of the extracts from various chestnut flowers are compared in table 1. It can be concluded that TPC ranges from 126.4-75.5 $mgGAE/gdm$, while TFC ranged from 32.1 to 85.1 $mgRE/gdm$. Among the five chestnut flowers studies, YL has significantly higher TPC ($126.4 \pm 2.0 mgGAE/gdm$) followed by ZP ($111.4 \pm 2.0 mgGAE/gdm$), then YSDZ ($9 \pm 1.4 mgGAE/gdm$) and YK ($75.5 \pm 4.8 mgGAE/gdm$). The TFC of ZP is significantly higher ($85.1 \pm 3.6 mgRE/gdm$) than other samples. However, the lowest one is also detected in YK with the TFC of $42.1 \pm 2.8 mgGAE/gdm$.

The Antioxidant activities of five different chestnut flowers cultivars

The antioxidative capacities of chestnut flowers extracts from different *C. mollissima* cultivars cultured *in vitro* were evaluated by four methods to fully characterize them *in vitro* antioxidant properties. The results of the antioxidant activity are shown in table 1.

It can be observed that ZP and YL present the strongest

DPPH activities ($IC_{50} = 45.2$ and $41.3 \mu\text{g/mL}$, respectively), followed by YSDZ with IC_{50} values of $48.5 \mu\text{g/mL}$. This IC_{50} is lower than the one reported for water extracts of chestnut flowers by Lee et al. [18] which ranges from 73.01 and $119.36 \mu\text{g/mL}$ [18], demonstrating that all five cultivars contribute to a high DPPH activity.

The ABTS free radical scavenging activity shows a similar order in the DPPH reaction. Statistical analysis shows that YL extract has the highest activity value ($1.3 \pm 0.14 \text{ mg/mL}$), while YK extract has the lowest value ($3.43 \pm 0.82 \text{ mg/mL}$).

In terms of reducing power, YL ranks the highest, but YK is significantly lower than that of other four pear cultivars. In this paper, the reducing power of the five chestnut flowers cultivars is: $YL > YSDZ > ZP > YSZF > YK$.

Finally, hydroxyl radicals ($\bullet\text{OH}$) scavenging was assayed by the Fenton-type reaction. The IC_{50} (Table 1) reveals that the scavenging activity of YL and ZP exhibited the highest $\bullet\text{OH}$ scavenging abilities (4.5 mg/mL and 4.8 mg/mL , respectively), while YSZF and YK have the least (5.7 mg/mL and 5.9 mg/mL , respectively).

amount (11.2%) in YSDZ. The cultivar ZP also shows the highest contents (16.8%) of kaempferol than that of others. Isorhamnetin is only detected in cultivar YSDZ and YL, but was not detected in other three cultivars, while (epi)-Catechin gallate is detected in four cultivars except cultivar YL.

Antitumor activity

To the best of our knowledge, this is the first study that evaluates the antitumor activity of *C. mollissima* flowers extract on HCT-116, BGC-823, NCL-H1650, HL-60, ECA109, HepG2, A2780, BEL-7402, A549 and EC9706 cells. As reported in table 3, all samples have an obvious inhibition function to the ten cancer cells with an impressive antitumor activity of ZP and YL against HCT-116 cells (IC_{50} values of $62.0 \mu\text{g/mL}$ and $95.0 \mu\text{g/mL}$, respectively). A lower activity is recorded for YSDZ against BEL-7402 ($IC_{50} = 99.5 \mu\text{g/mL}$) followed by A2780 cancer cells, whose IC_{50} value was found to be $100.0 \mu\text{g/mL}$. In summary, among the five studied chestnut flowers, ZPs which pose a significant antitumor activity, followed by YL with respect to the same cell (Table 3). Interestingly, the variety of ZP extract has the highest TFC, but YL has the highest TPC. Thus, the antitumor activity

Table 1: Contents of TPC, TFC, DPPH, ABTS and FRAP scavenging assay of five different chestnut flower cultivars.

Cultivars	Bioactive concentration		Antioxidant activity			
	TPC	TFC	DPPH IC_{50} [$\mu\text{g/mL}$]	ABTS IC_{50} [mg/mL]	FRAP	$\bullet\text{OH}$
	[$\text{mg}_{\text{GAE}}/\text{g}_{\text{dm}}$]	[$\text{mg}_{\text{RE}}/\text{g}_{\text{dm}}$]			[$\mu\text{molFe(II)}/\text{g}$]	IC_{50} [mg/mL]
ZP	$111.4 \pm 2.0\text{ab}$	$85.1 \pm 3.6\text{a}$	$45.2 \pm 0.7\text{d}$	$1.44 \pm 0.99\text{e}$	$851.4 \pm 2.43\text{d}$	$4.8 \pm 1.3\text{d}$
YSDZ	$97.1 \pm 1.4\text{bc}$	$76.2 \pm 5.9\text{a}$	$48.5 \pm 0.93\text{c}$	$1.61 \pm 1.00\text{f}$	$916.9 \pm 3.57\text{c}$	$5.2 \pm 0.8\text{c}$
YL	$126.4 \pm 0.8\text{a}$	$54.4 \pm 2.1\text{b}$	$41.3 \pm 0.20\text{a}$	$1.30 \pm 0.14\text{a}$	$972.1 \pm 5.88\text{g}$	$4.5 \pm 0.3\text{a}$
YSZF	$89.3 \pm 5.3\text{bc}$	$43.3 \pm 0.32\text{b}$	$68.1 \pm 0.66\text{a}$	$2.37 \pm 0.73\text{b}$	$791.6 \pm 12.16\text{e}$	$5.7 \pm 0.4\text{b}$
YK	$75.5 \pm 4.8\text{c}$	$42.1 \pm 2.8\text{b}$	$72.6 \pm 0.15\text{f}$	$3.43 \pm 0.82\text{g}$	$623.2 \pm 3.58\text{a}$	$5.9 \pm 1.1\text{e}$

Phenolic profiles of five different chestnut flowers cultivars

A total of seven phenolic compounds are unambiguously revealed by comparing significant molecular ion peak, major fragment ions and the retention time with spectral data published previously. All the compound excimers have a mass deviation less than 8 ppm. The identified phenolic compounds are classified in two major phenolic groups: the phenolic acids and flavonoids. As shown in table 2, there are six main phenolic compounds detected in all five cultivars, including quinic acid, gallic acid, quercetin derivative, ellagic acid, kaempferol and acacetin derivative. Although the five cultivars extracts show almost the same compounds in their phenolic profile, the total yields are significantly ($p < 0.05$) different. Gallic acid and quercetin are the two major compounds of phenolics in chestnut flowers, and quercetin is mainly in the form of bound (derivatives of flavan-3-ol and flavonols), with 36.2% in YL and 29.6% in YK around of the total phenolic content respectively. The content of gallic acid is the highest amount (28.3%) in YSDZ than that of other cultivars. Ellagic acid and acacetin derivative mainly exists in ZP with the amount 16.3% and 12.2%, while the quinic acid is just the opposite to the lowest amount of 4.7% in ZP and the highest

must be undoubtedly ascribed to the different compositions. These results could stimulate the reuse of *C. mollissima* flowers as nutraceuticals for instance.

Discussion

In present study, the antioxidant activity of *C. mollissima* flowers extract was evaluated *in vitro* through four different methods (DPPH, ABTS, FRAP and $\bullet\text{OH}$ scavenging). Nevertheless, these assays were performed by the extracts instead of pure molecules. There are some previous reports showed that potent bioactive properties of fruits and vegetables are responsible for synergistic effects of phytochemicals. This is why no single antioxidants could replace a combination of natural phytochemicals to achieve the same healthcare effect [19].

This study is the first investigation of the antioxidant activities of chestnut flowers extracts of different cultivars through comprehensive *in vitro* methods. The results show that the extracts of all five chestnut flowers cultivars have antioxidant activity, and chestnut flowers contain a series of antioxidants in different types. To investigate relationships

Table 2: Phytochemical profiles of five different chestnut flowers cultivars.

Identification	t_r	[M-H] ⁻ (m/z)	MS/MS fragment	%					References
	(min)			ZP	YSDZ	YL	YSZF	YK	
Quinic acid	1.35	191	127	4.7 ± 0.1a	11.2 ± 0.2ab	13.6 ± 0.8a	10.2 ± 0.2a	11.1 ± 0.2a	[29]
Gallic acid	3.78	169	125	17.4 ± 0.7a	28.3 ± 1.3a	4.8 ± 0.1a	20.5 ± 0.6a	8.5 ± 0.2a	[29]
Ellagic acid	13.7	300	217	16.3 ± 0.6	8.6 ± 0.1a	15.8 ± 0.5a	9.1 ± 0.1a	16.1 ± 0.6a	[30]
Quercetin derivative	14.78	501	380,301, 229	24.5 ± 1.8a	21.8 ± 0.9a	36.2 ± 2.1a	24.3 ± 1.1a	29.6 ± 1.5a	[31]
Kaempferol	18.03	285	2,55,151	16.8 ± 1.1a	6.2 ± 0.2a	7.1 ± 0.1b	7.4 ± 0.1c	6.3 ± 0.2a	[30]
Acacetin derivative	21.16	739	673, 453, 285	12.2 ± 0.9a	5.9 ± 0.1b	9.4 ± 0.2a	7.7 ± 0.1a	8.9 ± 0.4a	[32]
Isorhamnetin	12.12	315	300	ND	7.8 ± 0.3a	3.2 ± 0.1b	ND	ND	[32]
(epi)-Catechin gallate	20.19	441	169, 289	8.1 ± 0.4a	8.9 ± 0.3a	ND	8.2 ± 0.3a	7.9 ± 0.2b	[33]

Table 3: The antitumor activity of five chestnut flowers cultivars (µg/mL).

Cells	ZP	YSDZ	YL	YSZF	YK
HCT-116	62.0 ± 1.5c	214.5 ± 1.2a	95.0 ± 1.3c	242.0 ± 3.4b	367.2 ± 1.7a
HepG2	133.3 ± 0.7c	239.5 ± 1.2a	231 ± 1.0b	296.3 ± 2.2c	347.1 ± 1.25b
BGC-823	152.9 ± 1.7b	164.1 ± 0.4a	140.1 ± 0.2c	381.4 ± 0.7b	457.7 ± 0.4a
NCI-H1650	108.1 ± 1.0c	113.1 ± 0.4b	212.0 ± 0.8a	293.1 ± 0.6b	343.2 ± 1.5a
A2780	146.0 ± 1.0b	100.0 ± 0.5c	268.0 ± 0.9a	157.1 ± 0.3c	320.1 ± 1.2b
HL-60	195.1 ± 0.9b	132.8 ± 0.3c	231.0 ± 0.3a	351.5 ± 1.7b	492.1 ± 0.5a
BEL-7402	154.0 ± 0.7a	99.5 ± 1.5b	135.0 ± 0.6b	139.1 ± 0.6c	196.7 ± 0.6b
A-549	215.5 ± 0.7a	197.0 ± 1.0b	166.0 ± 1.2c	251.8 ± 1.0c	371.4 ± 0.5b
EC9706	123.5 ± 0.5c	149.4 ± 0.4b	169.0 ± 0.3a	221.4 ± 0.6c	236.5 ± 0.3b
ECA109	265.0 ± 0.9a	108.0 ± 0.7c	118.0 ± 0.5b	180.2 ± 0.7b	154.6 ± 0.3c

between phytochemicals and antioxidant assays, there used a regression analysis. The correlation study is described as the Pearson correlation coefficients in table 4.

Table 4: Correlation coefficient (R) between antioxidant activity and bioactive components content of chestnut flowers.

Parameter	DPPH	ABTS	FRAP	•OH	TPC	TFC
DPPH	1					
ABTS	0.93**	1				
FRAP	0.76	0.79*	1			
OH	0.84*	0.64	0.56	1		
TPC	0.97**	0.92*	0.87*	0.89**	1	
TFC	0.83*	0.86	0.77*	0.78	0.75	1

** p < 0.01 highly significant; * 0.01 < p < 0.05 significant.

Significant correlations are observed between various assays used to determine the antioxidant potential, especially between DPPH and ABTS assays (r = 0.93) and DPPH and •OH (r = 0.84) (Table 4). The lowest correlations were observed between the FRAP and •OH (r = 0.56) assay, and ABTS and

OH activity (r = 0.64). The similar result is reported that DPPH assay has a significant positive correlation with ABTS assay [20] and FRAP assay. The similar kind of correlation is also observed between DPPH and FRAP.

All the extracts show a significantly high DPPH activity with a great correlation with phenolics (r = 0.97*). Results obtained with ABTS, FRAP and •OH assays also show a significant correlation with TPC, with r = 0.92, r = 0.87 and r = 0.8, respectively. This agrees with earlier findings that chestnut spiny burs, catkin and leaves have a linear correlation between total phenol content and their antioxidant capacity [21, 22].

It is well known that the phenolic composition directly contributes to scavenging free radicals, chelate metal ions and inhibit oxidation chain reactions due to their hydrogen-atom donors [23]. These results are in accordance with that of other authors that there are high correlations between TPC and antioxidant activity. Similarly, flavonoids, the largest group of plant polyphenols, have also shown obvious antioxidant activities [24]. In addition, our study shows that there is a significant correlation between DPPH scavenging activity and TFC.

The cultivars of chestnut flowers with high TPC and TFC have significant antioxidant activities, which indicate that TPC and TFC of the extracts could be largely responsible for their antioxidant activity. Our results also suggest that different cultivars of chestnut flowers have varied antioxidant activities. Nevertheless, the antioxidant activities of a plant could be attributed to the combined influences, genetic factors, geographic origin and environmental conditions [25]. In addition, the extraction solvent used in this study is one of the important factors for the differentiated antioxidant activities of plant extracts [18].

According to the previous research, there is a relationship between antioxidant activity of plants and the mineral elements. The results obtained for trace elements in chestnut flowers cultivars show that chestnut flowers are a good source of copper, iron, manganese and zinc (Figure 2). The previously reported metal ions such as Zn, Fe, and Mn are important ingredients as antioxidant enzymes [26]. This is due to

the fact that the transition metals are easy to gain and lose electrons, which can affect the occurrence, transfer and loss of reactive oxygen species as well as the mutual influence and transformation [27]. This may be one of the reasons for the cultivars with high content of trace elements with obvious antioxidant activity.

Besides, cultivars flowers extracts show a higher antiproliferative activity on HCT-116, BGC-823, NCL-H1650, HL-60, ECA109, HepG2, A2780, BEL-7402, A549 and EC9706 cells. A similar study performed by Chen et al. [28] evaluating the flavonoids from chestnut flower indicated that it has a stronger antioxidant activity and can inhibit the growth of Hela cells.

Sugar, protein content and mineral compositions are characteristic indexes which are important for evaluating the quality of the plant. Additionally, active ingredients and biological activities are also important factors for appraising the characterization of cultivars flowers cultivars pertaining to their potential usage for product development. The contents and activities of total phenolics and flavonoids in cultivars flowers extracts are diverse. The results show that the highest value of TPC, total sugar as well as zinc and manganese can be obtained from YL, and so same as the highest value of TFC, protein content, as well as calcium and copper concentration, which can improve antioxidant capacity and can be extracted from ZP. In addition, YL and ZP are remarkable in antitumor assays. It is not difficult to observe the differences in components among the five cultivars flower cultivars by accurate analysis. We cannot make an arbitrary conclusion about which kind of cultivars flower is the best, but the present work can provide chemical composition characteristics of the five cultivars flower cultivars for further research on the processed products.

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Conflicts of Interest

The authors declare no conflict of interest.

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