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SHORT COMMUNICATION

Phenolic profiles and antioxidant capacities of crude extracts and subsequent fractions from *Potentilla fruticosa* L. leaves

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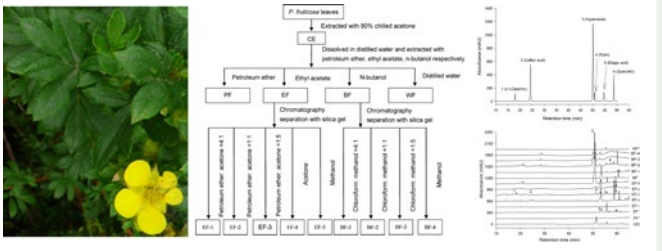
ABSTRACT

This work aimed to further investigate the phenolic profiles and antioxidant capacities of the crude extracts and the subsequent fractions of *Potentilla fruticosa* leaves. Result showed that *P. fruticosa* leaves contained high amounts for hyperoside, ellagic acid and (+)-catechin contents, and the highest amount being registered for hyperoside (17.67 mg g^{-1}). Nine sub-fractions were obtained after column chromatographic separation. EF-3, EF-4, EF-5 and BF-2 presented higher values for their total phenolic or flavonoid, (+)-catechin, ellagic acid and hyperoside content. Besides, EF-3, EF-4, BF-2 and BF-3 showed significant *in vitro* antioxidant capacities and protective effects on *Escherichia coli* under peroxide stress. The correlation between chromatograms and antioxidant activity showed that (+)-catechin, ellagic acid and hyperoside may play crucial roles in the antioxidant capacities of *P. fruticosa* and could be used as chemical markers for its quality assessment. Moreover, this is the first time *P. fruticosa* leaves have been systematically studied.



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1. Introduction

Potentilla fruticosa L., a perennial deciduous shrub of the *Rosaceae* family, is widely distributed in cool temperate, high altitude regions of the northern hemisphere, especially in the alpine areas of China (Tomczyk et al. 2008). In Tibet, it is known as 'Gesanghua' and is particularly widespread and used for fine pastures. In other areas, it is called 'Jinlaomei' and 'Yaowangcha'

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and is primarily used as a functional tea. In recent years, the genus *Potentilla* has withdrawn the attention of some researchers (Miliauskas, van Beek et al. 2004; Zhao et al. 2008; Tomczyk & Latté 2009; Jia, et al. 2013; Rauf et al. 2014, 2015). A few reports indicated that the extracts of *P. fruticosa* possess varying degrees of antioxidant, antibacterial, hypoglycaemic, anti-inflammatory, antitumour and antiulcerogenic properties (Mitich 1995; Gürbüz et al. 2005; Tomczyk et al. 2013). Additionally, research has shown that *P. fruticosa* contains abundant tannins (hydrolysable and condensed tannins), triterpenoids, coumarins and organic acids (Miliauskas, van Beek et al. 2004; Tomczyk 2011). However, in a preliminary study from our group, we detected far more chemical compounds and showed that *P. fruticosa* possessed the highest contents of total phenolic and flavonoids among the three *Potentilla* species (*P. fruticosa, Potentilla glabra* Lodd and *Potentilla parvifolia* (Fisch ex Lehm) Sojak) (Wang et al. 2013). Besides, its extracts have been shown to be safe and free of toxic effects in humans (Shushunov et al. 2009; Tomczyk et al. 2010). *P. fruticosa* leaves have a large number of applications in the food, cosmetic and medical industries. Therefore, comprehensive studies on its bioactivities and phytochemical constituents are of significant relevance (Mocan, Crișan et al. 2014; Mocan, Vlase et al. 2014). However, most *P. fruticosa* studies have focused only on the epigeal organs of the plant and not on the roots, leaves or stems. A few reports have studied the leaves as food additives and as ingredients in cosmetic products (Elkington 1969; Miliauskas et al. 2007). Though our previous work analysed the antioxidant activities and phenolics of the leaves (Wang et al. 2013), the research had an exploratory nature. In order to rationalise its use in pharmaceutical products, functional food ingredients and tea products, we conducted a systematic and comprehensive study on *P. fruticosa* leaves, specifically in the separation, purification, measurement and correlation between its antioxidant activities and phenolic profiles.

2. Results and discussion

2.1. Total phenolic and flavonoid contents

In our preliminary work, results showed that *P. fruticosa* leaves extracts had higher values for total phenolic and flavonoid contents than that of *P. glabra* and *P. parvifolia* (Wang et al. 2013). To further figure out the antioxidant active chemicals of *P. fruticosa* leaves, a bioassay-guided chromatographic fractionation was conducted to produce nine sub-fractions (Figure S1).The total phenolic and flavonoid contents of the crude extracts and the subsequent fractions of the *P. fruticosa* leaves are presented in Table 1. The results showed that CE contained the highest value for total phenolic content (349.03 ± 6.82 mM GAE 100 g^{-1}) and flavonoid content (191.71 ± 4.60 mM QE 100 g^{-1}). After the chromatographic separation, EF-3 contained the highest value for total phenolic content (2014.21 ± 9. 81 mM GAE 100 g^{-1}), followed by EF-4 (1872.11 ± 9.03 mM GAE 100 g^{-1}) and EF-5 (1483.91 ± 7.32 mM GAE 100 g^{-1}). Besides, EF-4 contained the highest value for total flavonoid content (407.00 ± 8.56 mM QE 100 g^{-1}), followed by BF-2 (330.1 ± 2.10 mM QE 100 g^{-1}) and EF-3 (327.79 ± 9.95 mM QE100 g^{-1}). In addition, we observed positive correlations between the phenolic and flavonoid contents in many fractions, such as those in EF and BF. All in all the crude extracts of the *P. fruticosa* leaves were rich in phenolics and flavonoids. Most of the phenolics and flavonoids were concentrated in EF-3, EF-4, EF-5 and BF-2 after chromatographic separation.

Table 1. Contents of total phenolic, flavonoid and six phenolic compounds and *in vitro* antioxidant capacities of crude extracts and subsequent fractions of *P. fruticosa* leaves.

Samples		Content								Antioxidant capacities		
		Total phenolic content (mM GAE100 g^{-1})	Total flavonoid content (mM QE100 g^{-1})	(+)-Catechin (mg g^{-1})	Caffeic acid (mg g^{-1})	Hyperoside (mg g^{-1})	Rutin (mg g^{-1})	Ellagic acid (mg g^{-1})	Quercetin (mg g^{-1})	$DPPH_{IC50}$ (ug mL^{-1})	ABTS (uM equiv. Trolo × g^{-1})	FRAP (uM equiv. Trolo × g^{-1})
CE		349.03 ± 6.82^{e}	191.71 ± 4.60^{e}	4.52 ± 0.165^{b}	0.04 ± 0.001^{c}	17.67 ± 0.583^{g}	0.31 ± 0.008^{b}	4.77 ± 0.185^{f}	0.06 ± 0.003^{b}	16.79 ± 0.01^{g}	6261.12 ± 8.21^{f}	2458.03 ± 6.68^{e}
PE		2.45 ± 0.02^{b}	2.29 ± 0.22^{b}	ND	ND	ND	ND	0.4 ± 0.015^{b}	0.05 ± 0.002^{a}	342.42 ± 3.49^{j}	1692.35 ± 4.33^{k}	275.16 ± 2.05^{l}
EF	EF	401.79 ± 4.50^{f}	259.93 ± 1.95^{g}	3.52 ± 0.134^{a}	0.22 ± 0.004^{h}	0.3 ± 0.010^{a}	0.38 ± 0.012^{c}	11.13 ± 0.436^{i}	0.12 ± 0.005^{d}	6.76 ± 0.08^{d}	7392 ± 9.70^{d}	2830.71 ± 8.60^{d}
	EF-1	4.39 ± 0.02^{d}	1.51 ± 0.03^{a}	ND	0.08 ± 0.002^{d}	ND	0.8 ± 0.026^{e}	0.32 ± 0.011^{a}	ND	9442.41 ± 5.79^{l}	241.96 ± 7.32^{m}	112.43 ± 8.46^{n}
	EF-2	789.11 ± 8.06^{k}	258.23 ± 9.58^{gh}	17.84 ± 0.753^{i}	1.21 ± 0.041^{l}	0.33 ± 0.009^{b}	0.46 ± 0.020^{d}	21.85 ± 0.887^{k}	13.2 ± 0.521^{l}	15.76 ± 0.11^{f}	1762.78 ± 6.24^{j}	1007.78 ± 9.82^{j}
	EF-3	2014.21 ± 9.81^{n}	327.79 ± 9.95^{i}	185.11 ± 8.211^{j}	1.2 ± 0.043^{k}	2.2 ± 0.081^{c}	10.97 ± 0.038^{g}	17.75 ± 0.675^{j}	0.88 ± 0.035^{i}	5.28 ± 0.13^{b}	8314.94 ± 7.55^{b}	3667.04 ± 9.48^{a}
	EF-4	1872.11 ± 9.03^{m}	407.00 ± 8.56^{j}	10.60 ± 0.362^{g}	0.13 ± 0.005^{f}	2.09 ± 0.074^{c}	2.72 ± 0.098^{f}	23.11 ± 1.103^{l}	0.64 ± 0.198^{h}	11.65 ± 0.05^{e}	7716.28 ± 9.17^{c}	3268.89 ± 7.56^{c}
	EF-5	1483.91 ± 7.32^{l}	215.91 ± 7.45^{f}	7.19 ± 0.277^{e}	0.13 ± 0.004^{f}	100.63 ± 2.362^{h}	ND	10.3 ± 0.364^{h}	0.34 ± 0.012^{e}	25.57 ± 0.13^{h}	6635.27 ± 9.28^{e}	2005.93 ± 7.57^{g}
BF	BF	439.33 ± 7.05^{h}	268.04 ± 4.61^{h}	8.42 ± 0.224^{f}	0.03 ± 0.001^{b}	9.31 ± 0.272^{d}	ND	2.58 ± 0.102^{d}	0.51 ± 0.021^{g}	6.53 ± 0.04^{d}	8387.18 ± 9.67^{a}	3365.43 ± 8.26^{b}
	BF-1	0.28 ± 0.003^{a}	1.42 ± 0.101^{a}	ND	0.14 ± 0.005^{g}	ND	ND	ND	ND	1387.25 ± 7.21^{k}	28.97 ± 0.35^{n}	145.74 ± 1.30^{m}
	BF-2	513.11 ± 8.63^{j}	330.10 ± 2.10^{i}	14.30 ± 0.471^{h}	0.09 ± 0.004^{e}	103.07 ± 2.115^{h}	ND	4.27 ± 0.188^{e}	3.02 ± 0.124^{k}	4.81 ± 0.06^{a}	5117.02 ± 7.24^{g}	2041.11 ± 6.12^{f}
	BF-3	419.12 ± 1.90^{g}	162.40 ± 5.04^{d}	ND	0.25 ± 0.008^{i}	13.93 ± 0.493^{f}	ND	4.43 ± 0.167^{ef}	1.92 ± 0.075^{j}	6.11 ± 0.13^{c}	4895.03 ± 8.04^{h}	1932.78 ± 8.78^{h}
	BF-4	467.67 ± 7.22^{i}	218.32 ± 2.08^{f}	6.27 ± 0.156^{d}	0.44 ± 0.016^{j}	10.1 ± 0.303^{e}	ND	6.48 ± 0.285^{g}	0.45 ± 0.018^{f}	5.87 ± 0.01^{b}	3856.97 ± 3.78^{i}	1238.33 ± 9.71^{i}
WF		4.01 ± 0.06^{c}	24.90 ± 0.95^{c}	5.77 ± 0.219^{c}	0.02 ± 0.001^{a}	ND	0.09 ± 0.004^{a}	0.62 ± 0.024^{c}	0.1 ± 0.004^{c}	33.67 ± 0.51^{i}	1488.79 ± 2.97^{l}	735.8 ± 8.41^{k}

Note: Each values represented in tables are means ± SD ($N = 3$). Values with different letters (a, b, c, d …) within same column are significantly different ($p < 0.05$).

2.2. Content of six phenolic compounds

As shown in Table 1 and Figure S2, we found that crude extracts of the *P. fruticosa* leaves contained high values in terms of hyperoside (17.67 ± 0.583 mg g^{-1}), (+)-catechin (4.52 ± 0.165 mg g^{-1}) and ellagic acid (4.77 ± 0.185 mg g^{-1}) contents, while the other three compounds (caffeic acid, rutin and quercetin) were measured at less than 1.0 mg g^{-1}. This result was just corresponding to our preliminary work that hyperoside, (+)-catechin and ellagic acid were the predominant phenolic compounds in the three *Potentilla* species (Wang et al. 2013). Among extracts of *P. fruticosa* blossoms, catechin and ellagic acid were also proven to be the most active radical scavengers (Miliauskas, Venskutonis et al. 2004). These results confirmed its great application values in developing powerful antioxidants. After chromatographic separation, (+)-catechin was primarily concentrated in EF-3 (185.11 ± 8.211 mg g^{-1}); Hyperoside was especially high in EF-5 (100.63 ± 2.362 mg g^{-1}) and BF-2 (103.07 ± 2.115 mg g^{-1}); ellagic acid was concentrated in EF, especially in EF-2 (21.85 ± 0.887 mg g^{-1}) and EF-4 (23.11 ± 1.103 mg g^{-1}). In other words, EF-3 contained relatively high contents of (+)-catechin, caffeic acid, ellagic acid and rutin; EF-2 contained high values for(+)-catechin, caffeic acid, ellagic acid and quercetin contents; EF-4 contained high values for(+)-catechin, rutin and ellagic acid contents; EF-5 contained high values for (+)-catechin, ellagic acid and hyperoside contents; BF-2 contained high values for (+)-catechin, quercetin and hyperoside contents; and BF-3 contained high values for quercetin content. Among them, the enrichment effect of (+)-catechin, hyperoside, rutin and quercetin was quite obvious. So we concluded that these fractions have the most valuable components and should be considered in further investigations.

2.3. DPPH, ABTS and FRAP assays

All results were summarised in Table 1. We found that CE presented good antioxidant capacities, no matter in DPPH, ABTS or FRAP assays. After chromatographic separation, most of the sub-fractions of EF and BF showed very good antioxidant activities except for EF-1 and BF-1. Among them, BF-2 showed the best DPPH radical-scavenging activity with the lowest $DPPH_{IC50}$ value of 4.81 ± 0.06 μg mL^{-1}, followed by those of EF-3 (5.28 ± 0.13 μg mL^{-1}) and BF-4 (5.87 ± 0.01 μg mL^{-1}); EF-2, EF-3 and EF-4 had the best $ABTS^{\cdot+}$ radical-scavenging activities with values ranging from 6635.27 ± 28.28 to 8314.94 ± 7.55 μM equiv. Trolox g^{-1}; EF-3 and EF-4 fractions had the best ferric-reducing power with FRAP values of 3667.04 ± 9.48 and 3268.89 ± 10.56 μM equiv. Trolox g^{-1}, respectively. Besides, though EF and BF almost had no observable differences in ABTS activity, the activities of EF were significantly better than those of BF. And we presumed this more likely because of the enrichment of two different active substances. In short, EF-3, EF-4 and BF-2 presented very good DPPH, $ABTS^{\cdot+}$ radical-scavenging activities and ferric-reducing power. So we could conclude that EF-3, EF-4 and BF-2 contained the most abundant oxidation-resistant components and had the best antioxidant activities *in vitro*. Thus, further *in vivo* antioxidant activity was focused on CE and the other nine sub-fractions (EF, BF, EF-2, EF-3, EF-4, EF-5, BF-2, BF-3 and BF-4).

2.4. Protective effect on H_2O_2-induced E. coli

To circumvent the limitations of individual assays for antioxidant activity, we adopted a microbiological method to measure the antioxidant activity *in vivo*. Experiments were conducted

to show that the treatment of aerobic *E. coli* cultures with 6.0 mM H_2O_2 could led to an inhibition in growth (Figure S3). We determined the proliferation rate of *E. coli* treated with different samples in 6.0 mM H_2O_2 to measure the protective effect of the samples. The highest protective effect was exerted in EF-3-treated cultures (4.66 ± 0.5-fold), followed by those of EF-4 (3.02 ± 0.3-fold), EF (2.60 ± 0.4-fold) and EF-2 (2.14 ± 0.2-fold). The protective effect of these extracts is not very different from that of quercetin (3.57 ± 0.3-fold), which had been proven to significantly enhance resistance of *E. coli* to peroxide stress (Smirnova et al. 2009).The other fractions also showed varying degrees of protective effect with observed 1.53 ± 0.2-fold to 2.01 ± 0.3-fold increases in growth rates (Figure S4). These results verified again that EF-3, EF-4, EF and EF-2 had very strong antioxidant capacities. This also confirmed the feasibility of this method for evaluation of antioxidant activities.

2.5. Correlation analysis

We evaluated the correlation between values for the antioxidant activities and the total phenolic content through linear regression analyses (Figure S5). We found a positive correlation between the protective effect of the treatments with the crude extracts fractions on H_2O_2-induced *E. coli* and the ferric-reducing power assay ($r = 0.64$). A similar correlation was observed between the (+)-catechin content and the total phenolic content of the extracts ($r = 0.63$), which was consistent with the high content of (+)-catechin observed in the *P. fruticosa* leaves. A significant relationship was observed between the protective effect of the treatments with the crude extracts fractions on H_2O_2-induced *E. coli* and the total phenolic content ($r = 0.74$). Additionally, the positive correlation between the protective effect of (+)-catechins on H_2O_2-induced *E. coli* and the (+)-catechin content ($r = 0.88$) was significant. This indicated that the protective effect of the extracts of the *P. fruticosa* leaves on H_2O_2-induced *E. coli* may be due to its high content of (+)-catechin, as evidenced by the role of (+)-catechin as one of the most active radical scavengers of *P. fruticosa* (Mitich 1995). However, this conclusion requires further study and verification.

3. Conclusion

The present report is the first time *P. fruticosa* leaves were systematically studied on phenolic, flavonoid and antioxidant capacities, and its *in vivo* antioxidant activity determined using a microbial system. Results showed that its crude extracts contained high values in terms of hyperoside, (+)-catechin and ellagic acid, and also a small amount of caffeic acid, rutin and quercetin (less than 1.0 mg g^{-1}). After chromatographic separations, EF-3, EF-4 and EF-5 presented higher values for their total phenolic or flavonoid content, and contained higher values for (+)-catechin, ellagic acid and hyperoside. Moreover, a positive correlation between the protective effect of sub-fractions and (+)-catechin content ($r = 0.88$, $p < 0.05$) was observed. In conclusion, these results suggested that (+)-catechin, ellagic acid and hyperoside may play crucial roles in the antioxidant capacities of *P. fruticosa* and could be used as chemical markers for its quality assessment.

Supplementary material

Experimental details relating to this paper are available online, alongside Table S1 and Figures S1–S5.

Disclosure statement

The authors declare that they have no competing interests.

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