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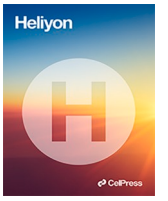

Research article

In vitro digestion and antioxidant activity of Xuan-Mugua (*Chaenomeles* fruit) peel and pulp phenolics

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ABSTRACT

Since time immortal, people have used the well-known Chinese *Chaenomeles* fruit Xuan-Mugua for both traditional medicine and nourishment. With an aim to explore the digestive and antioxidant properties of the phenolics, Xuan-Mugua peel and pulp were extracted, digested and analyzed *in vitro*. Our results indicated that the total phenolics content (TPC), total flavonoids content (TFC) and the antioxidant activity of the peel were 3.24–8.89 times higher than that of pulp. The contents and activity of the peel and pulp consistently dropped in the sequence of oral, gastric, and small intestine digestions, from 22.78 % to 52.16 %. With a level of 1.590 ± 0.060 and 0.395 ± 0.015 mg g^{-1} dried weight in the peel and pulp, respectively, chlorogenic acid was the primary phenolic ingredient in Xuan-Mugua, with a promising recovery (81.39–82.23 %) during the digestion. According to these results, Xuan-Mugua exhibited an appreciable level of phenolic content and antioxidant activity during digestion, making it a suitable ingredient for use in functional foods.

1. Introduction

Chaenomeles fruit, also known as Mugua in Chinese [1], is a traditional fruit for both medicine and food use [2]. Currently, five varieties of *Chaenomeles* fruits are found in China, whereas, the cultivar of *Chaenomeles speciosa* (Sweet) Nakai (*C. speciosa*) is the only one recorded in the Chinese pharmacopeia [3]. C. *speciose* is mainly distributed in Anhui, Hubei, Sichuan and Yunnan provinces [4], with varying chemicals and nutrition values [5]. Among them, *C. speciose*, cultivated in Xuancheng city of Anhui province is specially called Xuan-Mugua [6], which attracts growing attention for its high quality and long cultivating history. Xuan-Mugua is abundant in various phytochemicals, such as phenolic acids, flavonoids, triterpenes and polysaccharides [3,7], showing broad bioactive functions, including antioxidant, anticancer, and antibacterial activities [5]. Therefore, consumption of Xuan-Mugua is related to health benefits

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and prevention of metabolic disorders. However, the phytochemicals and bioactivity of the Xuan-Mugua are affected during processing or digestion. Previous studies have examined the change of bioactive components in Xuan-Mugua (e.g. phenolics, tannins) during the drying and fermentation [6,8], but the corresponding changes during the digestion remain elusive.

The release of bioactive ingredients from ingested foods mainly depends on the mammals' digestive system, whose enzymes and pH may lead to the degradation or transformation of bioactive components, affecting their bioactivity [9]. Therefore, exploring the digestibility of food components is crucial to evaluating their bio-function. However, detecting the digestibility of food components *in vivo* is difficult, due to either ethical issues or experimental costs [10]. The *in vitro* digestion model consisting of digestive enzymes provides a simple and easily carried-out way to explore the digestive properties of food components [11], which has been widely applied to assess the component stability in different foods, including blackberry [12], *Eugenia pyriformis* fruit [13], baobab fruit [14], jujube [15] and so on. As to *Chaenomeles* fruits, their digestibility was only reported in Mugua from Yunnan province [16], but the digestive property of Xuan-Mugua is still unknown, especially for the phenolics.

Therefore, we evaluated the digestibility of phenolics from Xuan-Mugua, using its peel and pulp extracts for simulated digestions. The variation in phenolic profile and content, and antioxidant activity were determined.

2. Material and method

2.1. Material and reagent

Xuan-Mugua was obtained from a local plantation of Xuancheng City (Anhui, China). The fruits were peeled, the seeds were removed, and the pulps were sliced into 2 mm sheets. The peels and pulp sheets were freeze-dried separately and ground into 60 mush powder.

2.2. Preparation of Xuan-Mugua extract

The dried powder of Xuan-Mugua peel or pulp (5 g) was extracted by 70 % ethanol aqueous (100 mL) under sonication for 1h. The extract was obtained by centrifugation and then concentrated under vacuum evaporation to remove ethanol. The volume of concentrated aqueous extract was made to 50 mL by water. The final solution was stored at 4 °C for further experiments.

2.3. In vitro digestion

The simulated digestion was referred to previously reported method with some modifications [15]. Briefly, in oral digestion, Xuan-Mugua extract (0.5 mL) was mixed with α-amylase buffer (9.5 mL, final concentration after mixing: 150 U mL^{-1}) for 5 min under pH 6.5, and three of the nine replicate tubes were kept as oral digestion samples. In gastric digestion, samples from oral digestion were mixed with pepsin buffer (final concentration after mixing: 2000 U mL^{-1}) for 2 h under pH 2.0, and three of the six replicate tubes were kept as gastric digestion samples. In small intestine digestion, samples from the last step were mixed with pancreatin buffer (final concentration after mixing: 200 U mL^{-1}) for 2 h under pH 7.4. To detect the effect of digestive pH on the digestion, samples treated with digestion buffers that replaced the enzymes with PBS were severed as undigested groups, whereas water-diluted samples were used as controls. All the samples were made to 15 mL, and kept at 4 °C for further analysis.

2.4. Total Phenolics Content (TPC)

TPC was determined by the previous method with some modifications [17]. Briefly, the sample (400 μL) was mixed with Folin-Ciocalteu phenol reagent (2 M, 500 μL) for 5 min in the dark and followed by the addition of Na_2CO_3 (10 %, v/v, 500 μL) in the dark for another 30 min. The absorbance at 765 nm was detected and the TPC was expressed as gallic acid equivalents (GAE) per gram of dry weight (DW).

2.5. Total Flavonoids Content (TFC)

TFC was detected according to the previous method with few modifications [9]. Sample (250 μL) was added to $NaNO_2$ solution (1.6 %, m/v, 250 μL) and kept for 5 min in the dark, followed by $AlCl_3$ (3.2 %, m/v, 250 μL) addition for another 5 min. The mixture was then mixed with NaOH (8 %, m/v, 250 μL) and kept in the dark for additional 15 min. The absorbance at 510 nm was detected, and the TFC was expressed as quercetin equivalents (QE) per gram of dry weight (DW).

2.6. ABTS scavenging activity

The ABTS scavenging activity was assessed by the previously reported method [6]. The ABTS working solution was prepared by mixing ABTS stocking solution (7 mM) with $K_2S_2O_8$ (2.45 mM) at a ratio of 1:1 (v/v) for 16 h, and the solution was diluted to an absorbance of 0.700 ± 0.02 at 723 nm before use. The sample (500 μL) was mixed with ABTS working solution (500 μL) for 5 min in the dark, and the absorbance at 723 nm was detected. The ABTS scavenging activity was expressed as Trolox equivalents (TE) per gram of dry weight (DW).

2.7. Ferric Reducing Antioxidant Power (FRAP)

The FRAP was determined according to the previously reported method [15]. The FRAP working solution was prepared by mixing 10 mM TPTZ solution, 20 mM $FeCl_3{\cdot}6H_2O$ and 300 mM acetate buffer (pH 3.6) at a ratio of 1:1:10 (v/v/v). The sample (50 μL) was mixed with the FRAP working solution (950 μL) and kept in the dark for 15 min. The absorbance at 593 nm was detected and the FRAP was expressed as $FeSO_4$ equivalents (Fe^{2+}) per gram of dry weight (DW).

2.8. HPLC/MS analysis

According to previous study [6], sample was analyzed on an HPLC system (iChorm5100, Dalian Elite, China) equipped with a C_{18} reverse column (250 mm × 4.6 mm, 5 μm, Suyan Medical Technology CO., Ltd, China). The mobile phases consisted of acetonitrile (phase A) and water (both contained 0.1 % formic acid), and the sample was eluted at a rate of 0.8 mL min^{-1} under the following gradient: 0–5 min, A increased from 5 % to 15 %; 5–10 min, A increased maintained at 15 %; 10–30 min, A increased from 15 % to 25 %; 30–35 min, A increased from 25 % to 40 %; 35–40 min, A increased from 40 % to 100 %; 40–45 min, A decreased from 100 % to 5 %; 45–50 min, A maintained at 5 %. The injection volume was 100 μL and the detection wavelength was 280 nm. The condition of HPLC-MS (ACQUITY UPLC LCT Premier XE system, Waters, USA) was identical to that of HPLC, and the mass information was obtained in negative ionization mode with an m/z ratio of 100–1000. The main phenolic compounds in samples were confirmed and quantified by using corresponding standard compounds.

2.9. Data analysis

The samples were prepared in triplicates and the data was analyzed by one-way variance (ANOVA) and Duncan's test at a significance level of $p \leq 0.05$.

3. Results and discussion

3.1. TPC and TFC

The TPC in Xuan-Mugua peel was approximately 3.4 times greater than in pulp, as seen in Fig. 1, suggesting that the fruit's peel contained majority of the phenolics. This was the case for most fruits, such as apples [18], jujubes [15], and *Chaenomeles* fruits [19], implying that fruit peel could be a good resource of phenolics. The TPC typically dropped in the order of the subsequent digestions following the simulated digestion. In oral digestion, the TPC respectively decreased 6.04 % and 11.52 % in peel (Fig. 1A) and pulp (Fig. 1B) extracts as compared with the control group, while the corresponding decrease was 13.75 % and 17.16 % after the gastric digestion. In small intestine digestion, the TPC continuously decreased by 22.78 % and 23.98 % in the peel (Fig. 1A) and pulp (Fig. 1B). Similar results were detected in the undigested group, and the final decrease at the small digestion step was respectively 6.87 % and 8.64 % in peel (Fig. 1A) and pulp (Fig. 1B) as compared with the control. The decrease of phenolics in the digested group were higher than that in undigested group, indicating that besides the pH drove-partial degradation, enzyme may improve the decrease of phenolics, by forming complex or transforming them to other undetectable or insoluble phenolic derivatives [20]. This trend of TPC change during the digestion was also reported in other food materials, including chrysanthemum [21] and coffee pulp [22], implying that different plant extracts may share the comparable variations during the simulated digestion.

The flavonoids changed similarly to that of phenolics during the digestion, and the TFC was shown in Fig. 2. In the oral digestion step, compared with the control, the TFC was reduced by 20.96 % and 1.71 % respectively in peel (Fig. 2A) and pulp (Fig. 2B) extracts, and the reached to 29.95 % and 15.36 % at gastric step. In the small intestine step, the decrease in TFC reached to 52.16 % and 34.89

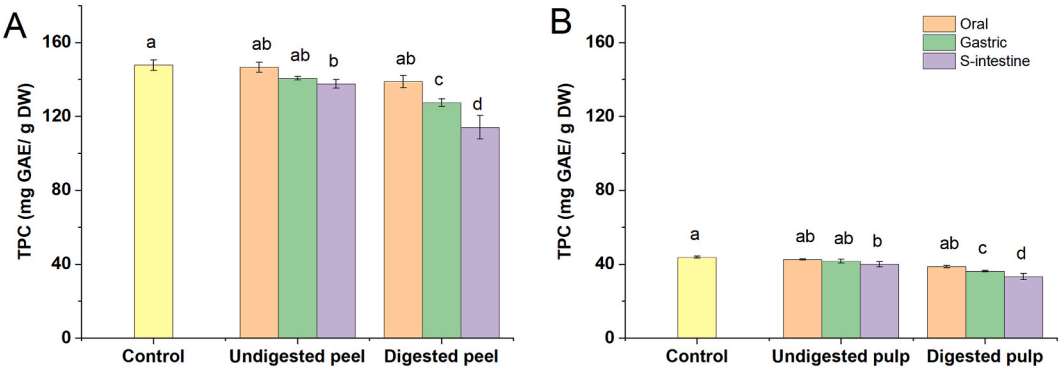

Fig. 1. TPC of Xuan-Mugua peel (A) and pulp (B) extracts as affected by the simulated digestion. Data were represented as means ± SD. Means are significantly different with different letters ($p < 0.05$).

%, displaying a significant decrease as compared with the control and the corresponding undigested group ($p < 0.05$). The sharp decline in TFC after digestion was previously reported in maqui berry [23] and blackthorn [24], indicating that part of the flavonoids were not stable during the digestion, especially against small intestinal digestion, and they may be transformed or degraded into non-flavonoid structures under the action of digestive enzymes and pH [23,25]. Besides, partial binding of the flavonoids with proteins such as enzymes and other macromolecules may also lead to the decrease of TFC, because flavonoids could easily interact with proteins, especially under alkaline conditions [26], e.g. at the pH (7.4) of small intestinal digestion.

3.2. Antioxidant activity

The antioxidant activity of Xuan-Mugua peel and pulp extracts before and after simulated digestion was shown in Fig. 3. In the oral step, the ABTS scavenging activity of the peel (Fig. 3A) and pulp (Fig. 3B) extracts respectively was reduced by 20.24 % and 15.36 % as compared with the control, and reached to 28.25 % and 22.76 % at gastric digestion step, and further increased to 40.64 % and 37.05 % at small intestine digestion step. The continuous decrease of ABTS value also found in digested wild and commercial blackberries [27], which may correlate with a diminution of antioxidant agents, e.g. phenolics, vitamins. Similarly, a constant decrease of FRAP was observed after intestinal digestion, and the reduction was 39.88 % and 27.09 % in peel (Fig. 3C) and pulp (Fig. 3D), respectively. This kind of change for FRAP was also reported in bamboo leaves soup [28] and juçara fruits [29], implying that components with FRAP, such as phenolics and flavonoids, partially degraded or transformed during the simulated digestion [25]. The decrease of ABTS value and FRAP in the digested group was higher than in the undigested group, implying that digestive enzymes may play a vital role in reducing antioxidant activity. The enzymes may bind to the antioxidant compounds (e.g. phenolics), or transform them into non-antioxidant molecules [23], resulting in the decrease of antioxidant agents and antioxidant activity. Besides, the change in the antioxidant activity of Xuan-Mugua peel and pulp extracts (Fig. 3) was similar to that of TPC (Fig. 1) and TFC (Fig. 2), implying that the phenolic compounds including flavonoids may be the main antioxidant agent of Xuan-Mugua.

3.3. HPLC/MS analysis

The TPC/TFC can provide an overview of Xuan-Mugua's phenolic compounds during the simulated digestion, but the corresponding change of individual phenolics remain unexplored. To explore the variation of main phenolic compounds during the digestion, the samples were analyzed and quantified by HPLC. As shown in Fig. 4, the HPLC profiles were almost the same during the simulated digestion, and this phenomenon was previously reported in the digestion of Meghalayan cherry pomace extracts [30] and Turkish fruit wines [31], indicating that the main phenolics were stable to some degree against digestion. The main compound with the retention time of 16.36 min was tentatively identified as chlorogenic acid, one kind of caffeoylquinic acid (Fig. 5). The *m/z* of 707, 353 and 191 obtained at negative mode were respectively assigned to $[2M - H]^-$, $[M - H]^-$ and a fragment of quinic acid, which was one part released from the chlorogenic acid [32]. To differentiate chlorogenic acid from its analogs, it was further confirmed by a standard compound as reported in our previous study [6].

To elucidate the change of chlorogenic acid during digestion, the content was quantified by HPLC using the corresponding standard. As shown in Table 1, the variation of chlorogenic acid was similar to that of TPC/TFC (Figs. 1 and 2) in the undigested group. Its content constantly decreased in the order of digestion steps, and the finally decreased at small intestine digestion step with 18.61 % and 17.77 % in peel and pulp extracts, respectively. Similar decrease of chlorogenic acid was observed in the undigested group of chrysanthemum extract [21], confirming its instability toward high pH [33]. However, different from the undigested group, the corresponding decrease of chlorogenic acid in the digested group was 10.34 % and 15.21 %, which was smaller than that of undigested group, implying that protein (e.g. digestive enzyme) and other matrix (e.g. polysaccharides) in the digesta may protect chlorogenic acid by forming corresponding complexes [34], which was confirmed in the study of whey protein and phenolics complex [35]. Besides the protection, as compared with undigested group, the small increase of chlorogenic acid after intestinal digestion could also be due to

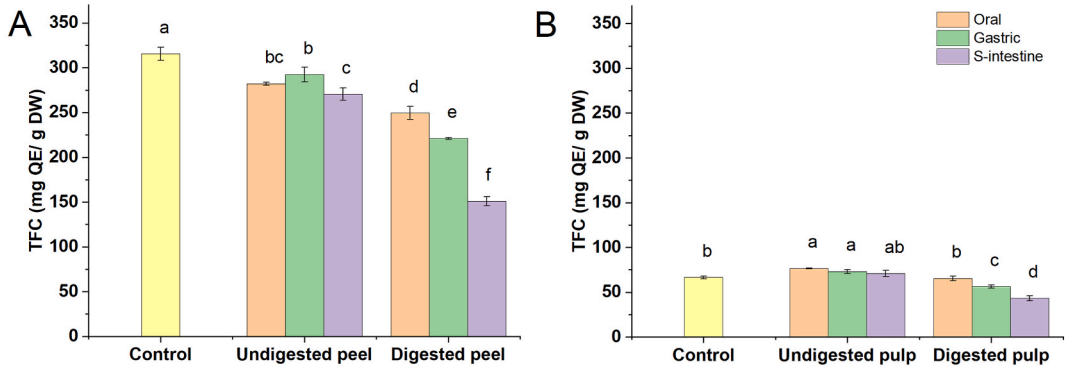

Fig. 2. TFC of Xuan-Mugua peel (A) and pulp (B) extracts as affected by the simulated digestion. Data were represented as means ± SD. Means are significantly different with different letters ($p < 0.05$).

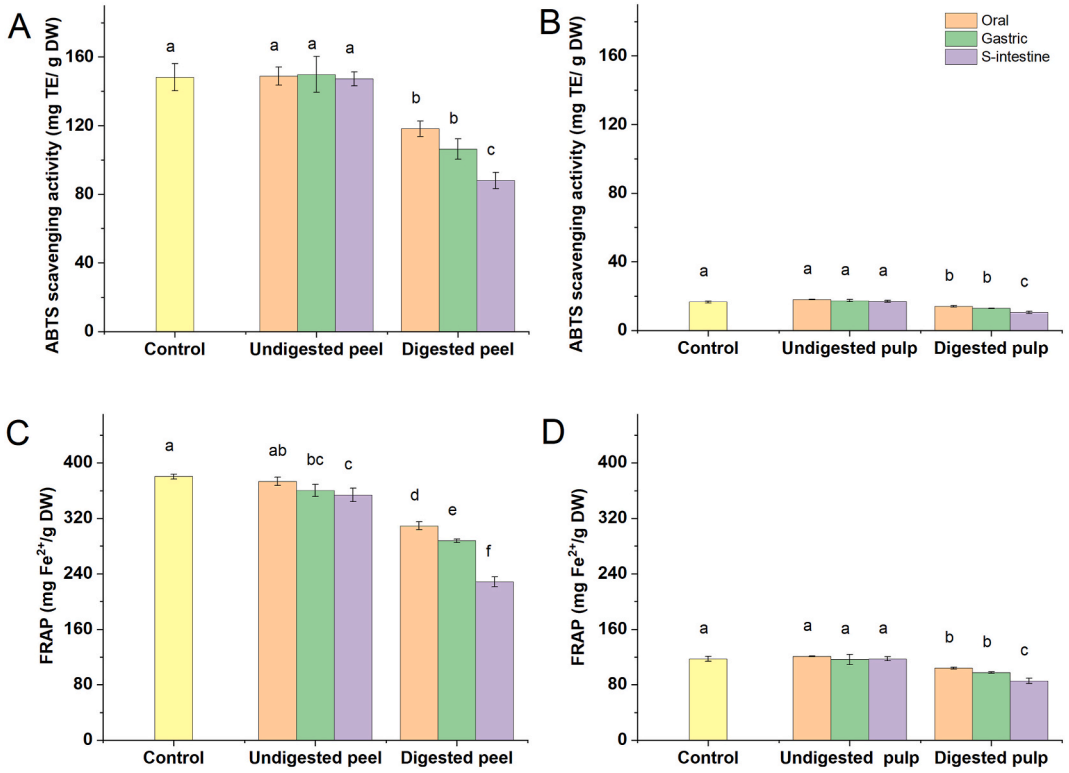

Fig. 3. Effect of simulated digestion on the ABTS scavenging activity (A, B) and FRAP (C, D) of Xuan-Mugua peel and pulp extracts. Data were represented as means ± SD. Means are significantly different with different letters ($p < 0.05$).

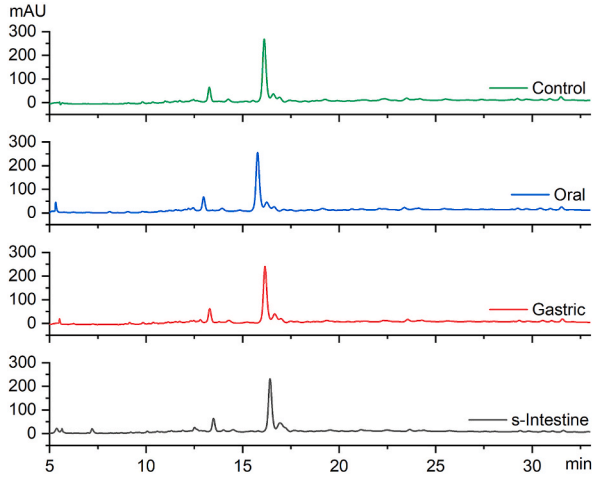

Fig. 4. HPLC profile of Xuan-Mugua peel extract during the digestion.

the release of this compound from its esterified derivatives, e.g. methyl ester [36], after the hydrolysis through corresponding digestive enzymes.

4. Conclusion

Our study revealed that the phenolic content of Xuan-Mugua was higher in peel than in pulp. After simulated digestion, the TPC/TFC and antioxidant activity of the peel and pulp extracts showed significant decrease, implying that the phenolic compounds including flavonoids, as well as other antioxidant agents may be degraded, transformed or bound during the digestion. However, due

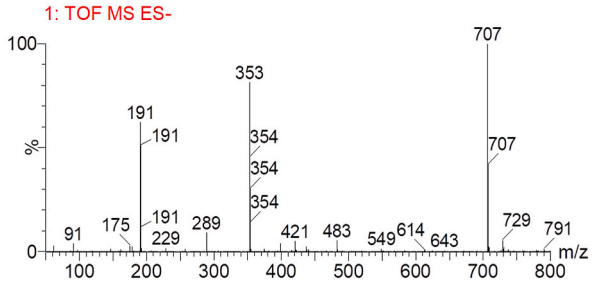

Fig. 5. MS spectrum of chlorogenic acid (the main compound in Fig. 4) from Xuan-Mugua peel extract.

Table 1
Change of chlorogenic acid in Xuan-Mugua peel and pulp extracts during the *in vitro* digestion. Data were represented as means ± SD (mg g^{-1} DW). Means are significantly different with different letters ($p < 0.05$).

	Control	Undigested	Digested
peel	1.590 ± 0.060^{a}		
oral		1.616 ± 0.034^{a}	1.450 ± 0.131^{ab}
gastric		1.441 ± 0.069^{ab}	1.414 ± 0.093^{ab}
small intestinal		1.294 ± 0.052^{b}	1.425 ± 0.061^{ab}
pulp	0.395 ± 0.015^{a}		
oral		0.393 ± 0.007^{ab}	0.358 ± 0.013^{cd}
gastric		0.359 ± 0.009^{cd}	0.365 ± 0.002^{bc}
small intestinal		0.325 ± 0.007^{e}	0.335 ± 0.008^{de}

to the protection of some residues (e.g. protein and polysaccharide) in the digestion system, chlorogenic acid, as the main phenolic compound, was found to be relatively stable against the simulated digestion, which may owe to the retained antioxidant activity of Xuan-Mugua after digestion. These findings indicate that the Xuan-Mugua peel or pulp extracts sustained proper antioxidant agents and activity after the *in vitro* digestion, which may support its further application in the functional food development. Besides phenolics, other macromolecules including proteins and polysaccharides may be degraded or transformed during the digestion, leading to the change in composition and activity of Xuan-Mugua extract, which warrants further exploration.

Data availability statement

The data that support the findings of this study are included in the article.

CRediT authorship contribution statement

Zhi Li: Writing – original draft, Methodology, Investigation, Formal analysis. **Xu-Yang Zhang:** Validation, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Yi-Long Ma:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Qian-Lan Wu:** Investigation, Data curation. **Xin Guo:** Investigation, Data curation, Kiran Thakur, Writing – review & editing. **Zheng-Fang Wu:** Methodology, Investigation. **Ya-Fang Shang:** Resources, Methodology. **Shao-Hua Yang:** Writing – review & editing, Software. **Xiang-Li Niu:** Writing – review & editing, Resources. **Zhao-Jun Wei:** Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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