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ORIGINAL PAPER

Comprehensive Understanding of the Relationship between Bioactive Compounds of Black Tea and its Angiotensin Converting Enzyme (ACE) Inhibition and Antioxidant Activity

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Abstract

Long term regular intake of black tea (BT) can lower blood pressure, which is probably due to its antioxidant activity and angiotensin converting enzymes (ACE) inhibitory activity. This study achieves a comprehensive understanding of the relationship between bioactive compounds of BT and its ACE inhibitory activity and antioxidant activity. Phenolic compounds are closely related to antioxidant activity and ACE inhibitory activity. Catechin (C) exhibits stronger inhibitory activity on ACE enzyme than that of other compounds. Molecular docking demonstrates that C could directly bind to ACE active site pockets and Zn(II). Other bioactive compounds are involved in antioxidant and ACE inhibitory activity in varying degrees but no obvious trend is established. Our study proposes a conjecture that some bioactive compounds of BT regulate antioxidant defenses through mechanisms that involve ACE. The mixed mode of *in vitro* inhibition of ACE and oxidant of BT bioactive compounds needs to be further investigated.

Keywords Black tea · Bioactive composition · Antioxidant activity · ACE inhibition activity · Correlation

Introduction

The report of the global burden of hypertension (HTN) in 2005 has expected that over 1.6 billion individuals would suffer from HTN in 2025 [1, 2]. Unfortunately, total 1.13 billion adults had raised blood pressure in 2015 [3]. Healthy dietary habits have been proved to be involved in reducing the risk of high blood pressure (BP) [4, 5].

In the renin angiotensin aldosterone system (RAAS), angiotensin converting enzyme (ACE) converts inactive angiotensin I to angiotensin II, and the later one is a powerful vasoconstrictor and promoter of sodium retention. This transformation leads to vasodilator bradykinin deactivation and eventually raises blood pressure [6]. Therefore, blocking the transformation of angiotensin I to angiotensin II is the main target for hypertension therapy. The classical ACE inhibitor drugs, such as lisinopril, captopril, enalapril, and temocapril, are effective for hypertension treatment but associated with side effects. The exploration of ACE inhibitors from natural sources, particularly food sources, are potentially beneficial due to the safe and economical demands [7–9].

Tea is a global popular beverage and favored among people of all ages. Black tea (BT) ranks the top 1 of the world's tea production and accounts about 67% of world total consumption in 2019. It has been reported that long term (3–6 month) regular consumption of black tea could significantly lower BP in individuals [10, 11]. The *in vitro* ACE inhibitory activity of tea is probably due to phenolic compounds [12, 13]. However, the diversity of polyphenols makes it difficult to explore the mechanism of ACE inhibition. This study focuses on a comprehensive understanding of the relationship between bioactive compounds (phenolic compounds, vitamins, amino acids, and elements) of BT and its ability on ACE enzyme inhibition and antioxidant, and explain the mechanism with the aid of molecular docking.

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Materials and Methods

The Materials and Methods section is presented as Supplementary material.

Results and Discussion

Analysis of Chemical Compositions of BT

Our BT sample is the high-end Gongfu black tea, harvested in Hanzhong region in spring. The contents of (+)-catechin (C) (1.710 g/kg), (−)-epicatechin gallate (ECG) (0.228 g/kg), (−)-gallocatechin (GA) (1.958 g/kg), (−)-epigallocatechin gallate (EGCG) (25.450 g/kg), and caffeine (CAF) (0.982 g/kg) of BT are shown in Fig. 1a. EGCG was the major compound among BT catechins ($p < 0.05$), followed by C and ECG (Fig. 1b). The content of L-theanine of BT was 1.523 g/kg, which was much higher than other amino acids ($p < 0.05$). Among the essential amino acids, the content of Glu was significantly higher than other amino acids ($p < 0.05$) (Fig. 1c). These results indicated that L-theanine was the most abundant amino acid and accounted for more than 50% of the amino acid fraction of the tea samples. The elements composition of BT is showed in Fig. 1d and described in the way of atomic percentage. K was the maximum proportion of all elements ($p < 0.05$), followed by Ca, Mn, P, S, Fe, and Zn. The content of water-soluble vitamins in BT is shown in Fig. 1e. The amount of VC was much higher than that of VB_1 and VB_2 ($p < 0.05$). The high content of VC in BT might contribute to its antioxidant capacity.

Antioxidant Activities of BT

Antioxidant potential of BT has received much attention because it helps to prevent *in vivo* oxidative damage, such as lipid peroxidation. The antioxidant capacity of BT was determined in DPPH, ABTS· and FRAP assay in this study (Fig. 2a). The radical scavenging rates of BT were 94.96% on DPPH and 80.95% on ABTS. In the FRAP assay, the corresponding values of BT was 0.4977. Our results on the antioxidant activity are in-line with those of Zhang et al. [14] for FRAP and DPPH, which indicated black teas presented free-radical scavenging and ferric reducing properties.

Correlations between Bioactive Components (Vitamins, Phenolic Compounds, Amino Acids, and Elements) and Antioxidant Activity

The TP ares honored as exceptional electron donors and effective scavengers of physiologically relevant reactive oxygen species *in vitro*. Antioxidant research on tea is mainly on catechins but barely on the relationship towards other active compounds. Table 1 profiled the correlations between active compounds of BT and its DPPH radical scavenging activity. VC exhibited closer relationship to DPPH than that of other vitamins. Among the polyphenolic compounds, all the catechins showed the strong association with DPPH activity at a statistical significant level (Table S1). Zhang et al. [14] has concluded that the black teas with higher phenolic contents present higher antioxidant. Our results were in agreement with the data exhibited by Zhang et al. [14], which presented a relative strong correlation between phenolic content with the chemical antioxidant activity. No trend was established between amino acids and DPPH, but L-theanine expressed a high correlation to DPPH. It has been demonstrated by the study that L-theanine is a strong antioxidant which could attenuate transport stress-induced impairment of meat quality through improving muscle antioxidant status. The macroelements of K and Ca showed high correlation to DPPH at a statistical significant level, excluding P. The trace elements of Fe and Zn exhibited high correlation to DPPH at a statistical significant level. Zn is believed as one of the important components of the antioxidant system, which can prevent cell membrane oxidation and reduce the formation of superanion and anion.

ACE Inhibitory Activity of BT and Molecular Docking

Epidemiological studies have reported that the consumption of tea flavonoids (mainly catechins) may be associated with reduced risk of coronary heart disease. We investigated the ACE inhibitory activity of BT, mainly focused on catechins. L-theanine is also evaluated because L-theanine, at high doses, significantly decreases the BP in spontaneously hypertensive rats (SHR) but does not alter BP in normal rats. As shown in Fig. 2b, BT and catechins inhibited ACE enzyme by more than 50% in comparison to Lisinopril that was a classical ACE inhibitor drug in clinical ($p < 0.05$). Among the test catechins compounds, C showed significantl activity with the maximum of 67.86%, while EGCG had the lowest activity despite its dominant proportion ($p < 0.05$). L-theanine and GA also had high activity on ACE inhibition. The alkaloid caffeine inhibited the enzyme but with the lowest activity (22.15%) ($p < 0.05$). These findings might explain that the ACE inhibitory activity of BT was attributed to its active compounds. Persson et al. [13] reported that BT and catechins (EC, EGC, ECG, and EGCG) exhibited dose-dependent inhibition of ACE activity in human umbilical veins. Ray et al. [15] expanded the ACE inhibitor family of BT with the addition of thearubigin and theaflavin [15]. Our study proved

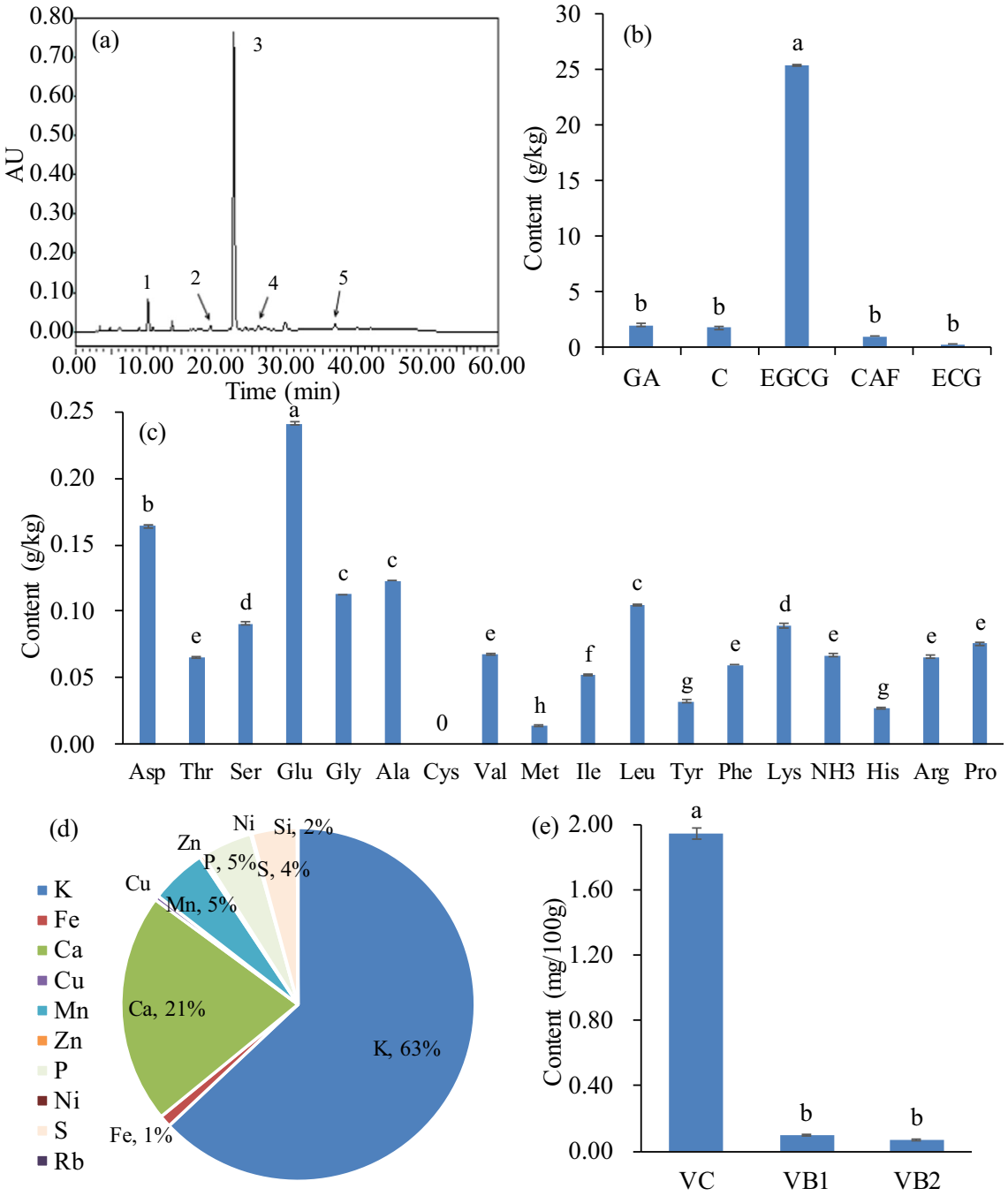

Fig. 1 Chemical composition of BT. **a** HPLC chromatogram of TP. 1, (−)-gallocatechin (GA), 10.230 min; 2, (+)-catechin (C), 19.135 min; 3, (−)-epigallocatechin gallate (EGCG), 22.422 min; 4, caffeine (CAF), 25.943 min; 5, (−)-epicatechin gallate (ECG), 36.789 min; **b** The content of TP; **c** Amino acids composition; **d** Elements composition; **e** Vitamin content. Statistical results were analyzed by Duncan's multiple-range tests, with $p < 0.05$ considered statistically significant, and multiple comparison results were marked by the letter-marking method

that C was the strongest inhibitory on ACE enzyme and explained the mechanism by molecular docking.

Figure 3a represents the intermolecular interactions between ACE binding site residues and predicts the pose of C. Direct interactions between C and active site pocket of ACE were observed and shown as S1 pocket (Ala354, Tyr523, Glu384), S2 (His353) and Zn(II) (Fig. 3b). This interaction with the Zn(II), stabilized by other interactions with amino acid residues of active site pockets through hydrogen bond, hydrophobic interaction, and electrostatic interaction, might empower the catechins to inhibit the ACE enzyme. It has been

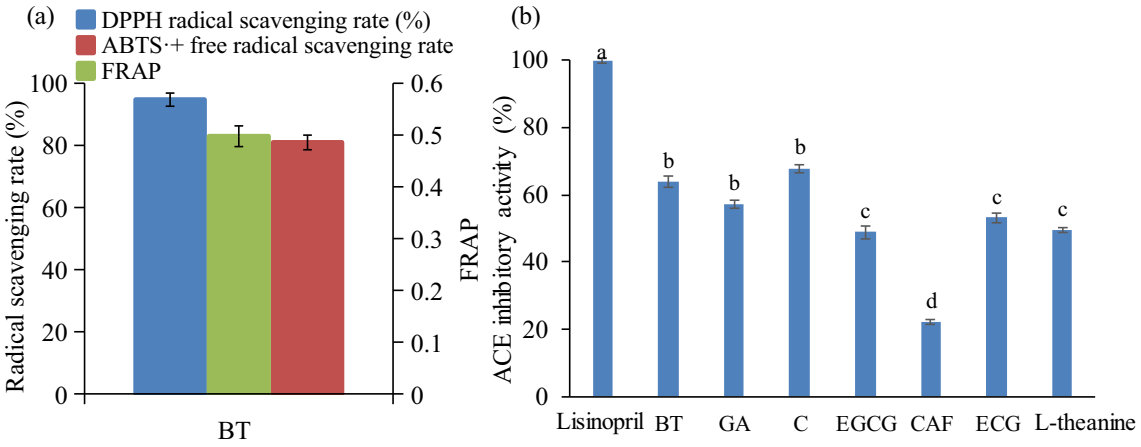

Fig. 2 BT activity profiles on (**a**) antioxidant capacities and (**b**) ACE inhibitory activity. Different letters indicate statistical differences ($p < 0.05$)

debated that the numbers of hydroxyl groups on the benzene ring encourages the ACE inhibitory activity with increasing numbers of hydroxyl groups. However, EGCG exhibited lower ACE inhibitory activity than that of ECG and C, although it maintained more hydroxyl groups on the benzene ring than that of ECG and C. We confirmed the significance of hydroxyl groups on catechins compounds for ACE inhibition, but the structure-activity relationship analysis could not be established. Al Shukor et al. [16] demonstrated that phenolic compounds inhibited ACE via interaction with the zinc ion or with amino acids at the active site, thereby blocking the catalytic activity of ACE [16]. The combination of ACE and inhibitors depends on the three active pockets in the catalytic site of ACE, namely S1, S2, and S1'. S1 includes Ala354, Glu384, and Tyr523, S2 includes Gln281, His353, Lys511, His513, and Tyr520, while S1' contains the residue Glu162. Our results were in-line with his opinion and exhibited such combinations.

Correlations between Bioactive Components (Vitamins, Phenolic Compounds, Amino Acids and Elements) and ACE Inhibition Activity

The relationship between bioactive compounds of BT and ACE inhibitory activity was analyzed in Table 2 and Table S2. Among the phenolic compounds of BT,

Table 1 Correlation between bioactive compounds and DPPH radical scavenging activity

Vitamins	VC	VB_1	VB_2			
r	0.9265	0.8848	0.9874			
Amino acids	Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Alanine
r	0.8484	0.9830	0.8463	0.9900	0.9226	0.9613
Amino acids	Cysteine	Valine	Proline	Methionine	Isoleucine	Leucine
r	0	0.9918	0.8831	0.8853	0.9160	0.9583
Amino acids	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine	L-theanine
r	0.9815	0.7706	0.7844	0.9307	0.7347	0.8824
Elements	Fe	Zn	Cu	Mn	Ni	S
r	0.9667	0.9850	0.8548	0.8988	0.9629	0.8716
Elements	K	P	Ca	Si	Rb	
r	0.9916	0.8468	0.9020	0.9733	0.7353	
Phenolic compounds	GA	C	EGCG	ECG		
r	0.9248	0.9567	0.8909	0.9613		
Alkaloid	CAF					
r	0.9637					

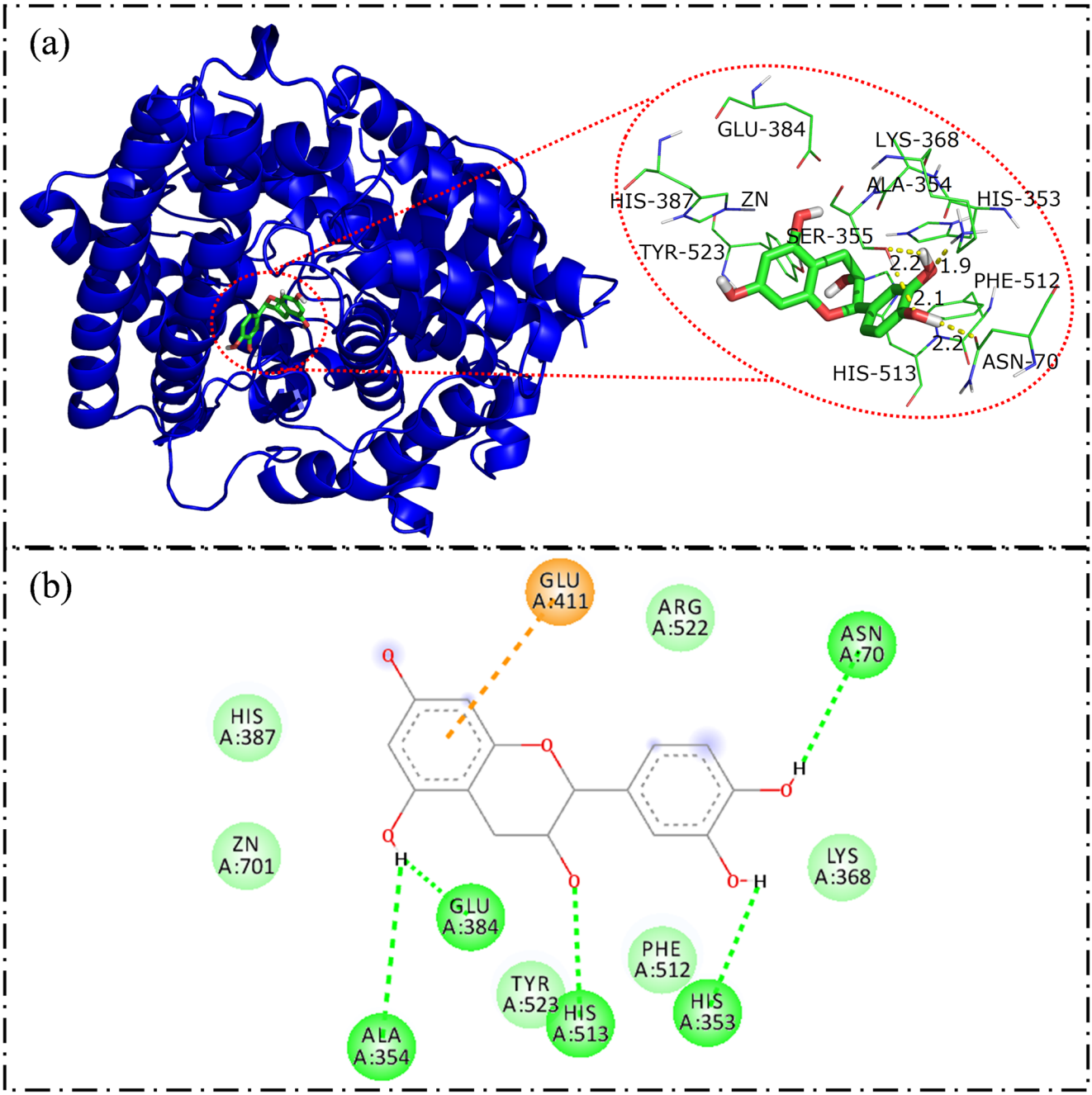

Fig. 3 Molecular docking mode for Catechin - ACE on (**a**) three-dimensional (3D) structure and (**b**) 2D diagram with active site residues. Dark green region represents hydrophobic interactions, and light green region is Van der Waals

the relationship between C and ACE inhibition was closer, followed EGCG and ECG. These results were consistent with our above mentioned findings that C exhibited the strongest inhibition on ACE enzyme. The elements of Fe and Zn failed to show a close relationship towards ACE inhibition activity at a statistical significant level. The correlation of among amino acids varied in a wide range. A list of Asp, Thr, His, Glu, Lys, and Phe showed close relationship with ACE inhibitory activity. Interestingly, all these amino acids of BT matched the active sites pocket of ACE. VC and VB1 had close correlation with ACE inhibitory activity. We profiled the correlation towards active compounds of BT and ACE inhibitory activity generally.

Table 2 Correlation between bioactive components and ACE inhibitory activity

Vitamins	VC	VB_1	VB_2			
r	0.9372	0.9555	0.8582			
Amino acids	Aspartic acid	Threonine	Serine	Glutamic acid	Glycine	Alanine
r	0.9896	0.8806	0.8499	0.8993	0.9396	0.8530
Amino acids	Cysteine	Valine	Proline	Methionine	Isoleucine	Leucine
r	0	0.8261	0.8953	0.8811	0.8728	0.8735
Amino acids	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine	L-theanine
r	0.7531	0.9971	0.9697	0.8952	0.9920	0.9758
Elements	Fe	Zn	Cu	Mn	Ni	S
r	0.7647	0.8694	0.9261	0.9423	0.8954	0.9847
Elements	K	P	Ca	Si	Rb	
r	0.8427	0.9336	0.8978	0.8598	0.9759	
Phenolic compounds	GA	C	EGCG	ECG		
r	0.9100	0.9971	0.9729	0.9200		
Alkaloid	CAF					
r	0.9249					

Conclusions

Profile of bioactive compounds of BT and its relationships with antioxidant activity and ACE inhibitory activity was depicted in this study. Phenolic compounds, especially catechins, had close relationships with antioxidant activity and ACE inhibitory activity. C, on behalf of the catechins, inhibited the ACE enzyme though binding to its active site pockets and Zn(II). Other bioactive compounds were involved in antioxidant activity and ACE inhibitory activity in varying degrees but no obvious trend was established. We speculated that some bioactive compounds of BT could regulate antioxidant defenses through mechanisms that involved ACE.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11130-021-00896-6.

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Author Contributions Linlin Wang and Lin Ma contributed equally to this study. Linlin Wang, Lin Ma, and Yike Zhao performed most of the experiments and data analyses. Guowei Shu, Jianke Li, and Li Chen helped supervised and discuss the work. All authors have given approval to the final version of the manuscript. Li Chen wrote the manuscript with critical input from all the authors.

Declarations

Conflict of Interest The authors declare no conflict of interest. This article does not contain any studies with human or animals' subjects.

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