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Tea Polyphenols as Novel and Potent Inhibitory Substances against Renin Activity

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ABSTRACT: Renin inhibitory activities of three tea products were investigated for the first time in this work. Water extracts from fermented oolong and black tea showed strong renin inhibitory activities. By the means of ultrafiltration, gradient high performance liquid chromatography and spectroscopic analysis, four active compounds were separated from aqueous black tea extract and identified as theasinensin B, theasinensin C, strictinin, and a hexose sulfate with a galloyl moiety, which had IC_{50} values of 19.33, 40.21, 311.09, and 50.16 μM against renin activity, respectively. Further detection indicated that the potent inhibitor theasinensin B was present only in black tea, and that monomeric catechins did not contribute significantly to the renin inhibitory activities of tea products. These results revealed novel and potent tea-derived renin inhibitors and suggested another potential pathway for tea consumption to control hypertension.

KEYWORDS: *renin inhibition, black tea, polyphenol, identification, theasinensin*

INTRODUCTION

Hypertension is one of the major public health problems across the globe and is related to the development of various cardiovascular diseases. The renin-angiotensin system (RAS) plays a crucial role in modulating blood pressure in the human body, and renin (EC 3.4.23.15) and angiotensin I-converting enzyme (ACE; EC 3.4.15.1) are two key enzymes in this system. Renin can cleave the N-terminus of angiotensinogen to yield angiotensin I, which is an inactive decapeptide and may be further hydrolyzed by ACE to release the octapeptide angiotensin II. Angiotensin II is a potent vasoconstrictor that elevates blood pressure by severely constricting the arteries, causing an increase in peripheral resistance. It can also act on the kidneys to retain both salt and water, resulting in an increase in extracellular fluid volume and thus blood pressure. Finally, it causes the adrenal glands to produce aldosterone, which in turn elevates the reabsorption of water and salt in the kidney.[1] Hence, the control of the RAS has been well established as an effective therapy for the treatment of hypertension.[2]

Currently, synthetic ACE inhibitors like captopril and enalapril are widely applied clinically as antihypertensive drugs. However, some side effects, such as dry cough, allergic reaction, and taste disturbance, associated with the usage of these inhibitors, are thought to be unavoidable, as ACE is not specific for catalyzing angiotensin I but also cleaves a number of other peptides including bradykinin with vasodilator action.[1,3] In contrast, since renin is specific, with its only naturally occurring substrate being angiotensinogen, renin inhibitors are theoretically considered more selective, with attenuated side effects.[4] Furthermore, the conversion catalyzed by renin is the rate-limiting step in RAS; therefore, it is thought that direct inhibition of renin could lead to better suppression of high blood pressure than ACE inhibition.[5]

Apart from potent synthetic ACE inhibitors, many safer foodstuff-derived ACE inhibitors have been identified in the past decades.[6] Compared to these achievements, the progress on the control of renin activity seems insufficient; however, renin inhibitors have continued to draw enormous attention. Studies on the structure and function of the cellular renin inhibitor, renin-binding protein, continue to be conducted.[7] Through molecular design, the development of aliskiren, the only orally active renin inhibitor available for clinical use, has given a great boost to the research field of renin inhibition.[8] Furthermore, Ishchenko and co-workers reported the structure-based design technology Contour and its application to the design of renin inhibitors, which led to the development of a compound as a clinical candidate.[9] In recent years, several studies have reported natural renin inhibitors identified from foodstuffs, such as saponin from soybean,[10] oleic acid and linoleic acid from rice[11] and dipeptides from pea protein hydrolysate,[12] thereby presenting another significant means for screening renin inhibitors as well as developing potential antihypertensive nutraceuticals and functional foods.

It is noticeable that polyphenolic compounds were found to potentially contribute to renin inhibition, which could be supported by the renin inhibitory activities of saponins,[10,13,14] baicalin[3] and polyphenolic extracts of two green leafy vegetables *Vernonia amygdalina* and *Gongronema latifolium*.[5] In our previous work, some catechin-related compounds were also found to possess renin inhibitory activities.[15] All these studies suggested that polyphenolic ingredients are promising as candidates for renin inhibitors. Some polyphenol-rich

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materials, such as black and green tea,[16] red wine,[17] cocoa extract,[18,19] and azuki bean seed coats,[20] have been proven to reduce blood pressure in several experimental hypertensive models. The antihypertensive mechanisms of these substances were ascribed to the attenuation of vascular oxidative stress and inflammation, vasodilator action as well as the ability to reduce arginase and ACE activity.[21,22] Nonetheless, to date, little attention has been paid to their potential renin inhibitory effects. The present study aims at investigating in vitro the renin inhibitory activities of polyphenol-rich tea products, and further analyzing the responsible inhibitory substances.

MATERIALS AND METHODS

Materials. Three commercial tea products were purchased from the market in Japan. The human recombinant renin inhibitor screening assay kit, including human recombinant renin, substrate (Arg-Glu(EDANS)-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Thr-Lys(Dabcyl)-Arg) and Tris-HCl buffer (50 mM, pH 8.0, containing 100 mM NaCl), was purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.). The catechin kit, including (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECg), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCg), was bought from Mitsui Norin Co. (Fujieda, Japan). Acetonitrile, trifluoroacetic acid (TFA) and water were of HPLC grade. Other materials used were of special grade for biochemical experiments.

Preparation of Sample Extracts. All samples were powdered by a pulverizer (Wonder Blender). Two portions of each sample powder (4.0 g) were dispersed in 40 mL of either distilled water or ethanol. After mixing sufficiently, the suspension was centrifuged at 5000*g* for 10 min. The resulted supernatant was collected, followed by lyophilization with a freeze-drier for water extract or centrifugal evaporation for ethanol extract. An original concentration of 1.0 mg/mL for each dried extract powder was prepared prior to the assay for renin inhibitory activity.

Renin Inhibition Assay. Renin inhibitory activity was determined using the human recombinant renin inhibitor screening assay kit. The synthetic fluorescence resonance energy transfer peptide utilized in this assay is the normal substrate for renin and has been linked to a fluorophore at one end and to a nonfluorescent chromophore at the other end. After the peptide is cleaved by renin, the product obtained is highly fluorescent and can be easily analyzed by recording the fluorescence intensity (FI) on a fluorescence plate reader (Powerscan HT; BioTek Instruments, Inc., Winooski, VT, U.S.), with an excitation wavelength of 360 nm and an emission wavelength of 528 nm. The renin inhibitory activity was calculated as follows: Renin inhibitory activity (Inhibition%) = $[(FI_{(control)} - FI_{(sample)})/FI_{(control)}] \times 100$. The analyses were performed in triplicate. Dose-dependent suppression of renin activity was evaluated with at least four different concentrations of the inhibitor. The IC_{50} value was defined as the concentration of the inhibitor required to inhibit 50% of the renin activity, and calculated using the linear function of percentage renin inhibition versus the logarithm of the inhibitor concentration.

Ultrafiltration. Sample extract with high renin inhibitory activity was fractionated preliminarily using an ultrafiltration device (Vivaspin 20, Sartorius Stedim Biotech, Göettingen, Germany) equipped with a membrane with a nominal molecular mass cutoff of either 3000 or 10 000 Da. Three portions with nominal molecular mass of >10 000, 10 000−3000 and <3000 Da were obtained, respectively, and then subjected to lyophilization, prior to the renin inhibition assay.

High Performance Liquid Chromatography (HPLC). Renin inhibitory substances in the portion with high renin inhibitory activity were further analyzed using a reverse-phase (RP)-HPLC system (Shimadzu Co., Kyoto, Japan). The separation was carried out at 40 °C on a Capcell Pak C18 column (4.6 I.D. × 250 mm, MG II, Shiseido Co., Tokyo, Japan), employing a linear gradient of acetonitrile (5−40% in water within the elution time of 5−40 min) in the presence of 0.05% TFA. The flow rate was 1.0 mL/min; the eluate was monitored by a UV−vis detector (SPD-10AV, Shimadzu Co., Kyoto, Japan) and a photodiode array detector (PDA) (SPD-M20A, Shimadzu Co., Kyoto, Japan) scanning from 190 to 450 nm. The fractions were collected automatically every 1 min, followed by centrifugal evaporation. After the renin inhibition assay, the active peaks were recollected by recycling HPLC, using a linear gradient of acetonitrile.

Mass Spectrometry. Mass spectrometric analysis was performed using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FTICR MS) on a Brüker Daltonics APEX Ultra FTICR mass spectrometer (Billerica, MA, U.S.) equipped with a 7.0 T actively shielded superconducting magnet. Active compounds were analyzed using full scan in both positive and negative ionization modes followed by data dependent MS^2 and MS^3 scanning of the most intense ions.

Statistical Analysis. Data are the means of triplicate analyses and expressed as means ± standard errors. Data were analyzed using IBM SPSS Statistics version 19.0. Duncan's multiple range test was used to evaluate differences among samples. Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Renin Inhibitory Activities of Tea. The renin inhibitory activities of both water and ethanol extracts from three kinds of widely consumed tea products, that is, green tea, oolong tea, and black tea, are presented in Table 1. It was found that all the water extracts from green tea, oolong tea, and black tea could suppress renin activity with IC_{50} values of 477.03, 20.31, and 17.27 μg/mL, respectively. However, with respect to the ethanol extracts, much lower inhibitory effects were observed, and no activity was detected in the ethanol extract of green tea.

Table 1. Renin Inhibitory Activities of Extracts from Tea Samples[a]

samples (water extract)[b]	IC_{50} (μg/mL)	samples (ethanol extract)[c]	IC_{50} (μg/mL)
GTW	477.03 ± 6.71[b]	GTE	ND[d]
OTW	20.31 ± 0.21	OTE	357.77 ± 9.28[c]
BTW	17.27 ± 0.95	BTE	90.48 ± 1.93[a]

[a]Results are expressed as means ± standard errors from triplicate analyses. Mean values with different letters are significantly different at $p < 0.05$. [b]Samples were water extracts from green tea (GTW), oolong tea (OTW) and black tea (BTW), respectively. [c]Samples were ethanol extracts from green tea (GTE), oolong tea (OTE) and black tea (BTE), respectively. [d]ND indicates no renin inhibitory activity detected.

To date, information on natural renin inhibitors derived from food materials is poor. Recently, the IC_{50} values of the chlorophyll-enriched fractions of two green leafy vegetables *Vernonia amygdalina* and *Gongronema latifolium* were reported to be 172.0 and 513.0 μg/mL, respectively.[5] Takahashi and co-workers found renin inhibitory activity in soybean and isolated the active compound soyasaponin I with an IC_{50} value of 30.0 μg/mL.[10] Another study reported the renin inhibitory activities of minor legume (legumes except soybeans and peanuts) extracts, with IC_{50} values of 270.0−1750.0 μg/mL.[23] It was also reported that some flaxseed peptide fractions could exert moderate inhibitory activities against renin, with IC_{50} values of 1220.0−2810.0 μg/mL.[24] It was thus suggested that tea samples investigated in this work could be regarded as good candidates for renin inhibitors. Our results further indicated that the renin inhibitory substances in these samples possessed better solubility in water than in ethanol, and that water might be a more favorable solvent while sufficiently extracting renin inhibitors from tea. Moreover, some studies have proven in vivo

the antihypertensive effects of the ingestion of oolong,[25] black and green tea;[16] the mechanisms underlying this action were proposed to be changes in autonomic neurotransmission, attenuation of oxidative stress and reduction of arginase activity to regulate cellular NO production by nitric oxide synthases. To the best of our knowledge, the findings in the present work, for the first time, reported the inhibitory effect of tea extracts on renin, the crucial enzyme in the RAS, suggesting an additional potential pathway and provided further support on tea consumption to help prevent and remedy hypertension.

Tea is one of the most popular beverages worldwide and is generally divided into three basic types according to the degree of fermentation during manufacture, that is, green tea (nonfermented), oolong tea (partially fermented) and black tea (totally fermented). Nowadays, much attention is paid to the various health-promoting effects of tea consumption.[26,27] In the present work, noticeably, potent activities were detected in the water extracts of two fermented tea samples, namely, oolong (IC_{50} = 20.31 μg/mL) and black tea (IC_{50} = 17.27 μg/mL), while much weaker activity was found in the water extract of nonfermented green tea with an IC_{50} value of 477.03 μg/mL. It is known that tea fermentation leads to considerable accumulation of polymeric components from monomeric compounds in fresh tea leaves.[27] Our results indicated that the process of fermentation could be advantageous for the generation of more effective renin inhibitors in tea products.

Separation and Identification of Renin Inhibitors. As shown in Table 1, no significant difference ($p > 0.05$) was found in water extracts of oolong and black tea with potent renin inhibitory activities. These two samples were subjected to the following separation and identification of renin inhibitors. By ultrafiltration, six fractions were obtained, and their renin inhibitory activities are shown in Figure 1. It was found that the

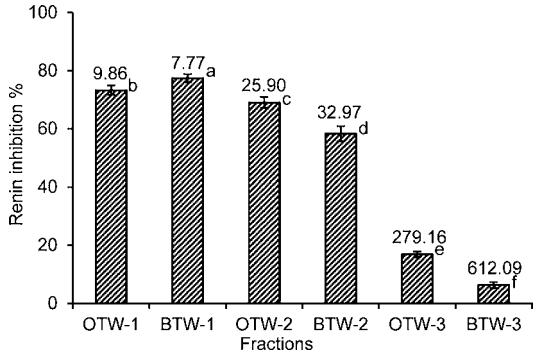

Figure 1. Renin inhibitory activities of fractions from water extracts of oolong and black tea by ultrafiltration. Results are expressed as percentage inhibition of renin activity with the inhibitor concentration of 52.63 μg/mL. Data are the means of triplicate analyses, with different letters indicating significant difference at $p < 0.05$. Values above the bars indicate the IC_{50} values (μg/mL) of the fractions. Fractions OTW-1 (BTW-1), OTW-2 (BTW-2), and OTW-3 (BTW-3) represent portions with nominal molecular masses of >10 000, 10 000−3000 and <3000 Da, respectively.

fraction with a nominal molecular mass of over 10 000 Da from the water extract of black tea (BTW-1) possessed significantly higher activity than other fractions ($p < 0.05$), with an IC_{50} value of 7.77 μg/mL, and was subsequently applied to RP-HPLC for further evaluation. Figure 2 shows the HPLC profile of fraction BTW-1 and the renin inhibitory activities of the collected fractions. On the basis of the percentage renin

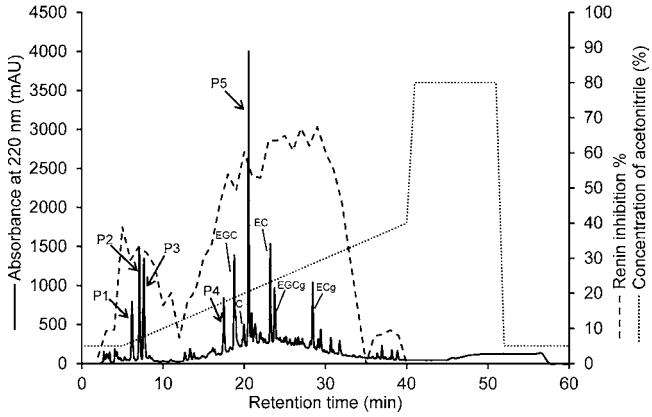

Figure 2. RP-HPLC chromatogram of fraction BTW-1 (solid line) and renin inhibitory activities of the collections (dash line). Elution was performed on a Capcell Pak C18 column at 40 °C using a linear gradient of acetonitrile (with 0.05% TFA) as shown by the round dotted line, with a flow rate of 1.0 mL/min and detection at 220 nm. The peaks for five kinds of catechins, i.e., C, EC, ECg, EGC, and EGCg, were confirmed by standards. Peaks P1, P2, P3, P4, and P5 indicate the fractions to be further analyzed.

inhibition of the collections as well as the apparently present peaks detectable under the experimental conditions, with the exclusion of those peaks for catechins representing a major class of polyphenols in tea, that is, C, EC, EGC, ECg, and EGCg, confirmed by authentic standards, five other main peaks, namely, P1, P2, P3, P4, and P5, were finally recollected using recycling HPLC. Figure 3 shows these five peaks purified and their renin inhibitory activities. Since no effect was observed in P3, the compounds P1, P2, P4, and P5 were subjected to subsequent characterization.

Table 2 as well as Figure 4 present the spectroscopic analysis of the compounds separated. P1 (t_R, 6.18 min; λ_{max}, 269 nm) generated a $[M - H]^-$ at m/z 609 which on MS^2 yielded major fragments at m/z 565 and 471. P2 (t_R, 7.09 min; λ_{max}, 270 nm) generated a $[M - H]^-$ at m/z 411 which on MS^2 yielded major fragments at m/z 331, 241, and 169. In the MS^3 spectra, the ion at m/z 331 had fragments at m/z 169 and 125, while the ion at m/z 241 had fragments at m/z 223, 181, 139, and 97. P4 (t_R, 17.48 min; λ_{max}, 273 nm) generated a $[M - H]^-$ at m/z 761, producing MS^2 fragments at m/z 609 and 591 through the cleavage of one galloyl group and one gallic acid, respectively. In the MS^3 spectra, the ion at m/z 609 had fragments at m/z 565, 525, and 471, while the ion at m/z 591 had two fragments at m/z 547 and 453. P5 (t_R, 20.54 min; λ_{max}, 273 nm) generated a $[M - H]^-$ at m/z 633 and MS^2 fragments at m/z 463 and 301, which corresponded to the loss of gallic acid and gallic acid plus a glucosyl residue, respectively.

It was reported that HPLC combined with UV and mass detection is suitable for the reliable identification of constituents in tea extract.[28−30] In this work, the identification of the four active constituents was performed on the basis of their retention time, absorbance spectra and mass fragmentation patterns, coupled with previous literature data.[28,31,32] In accordance with previous findings,[28,32] components P1 and P4 were tentatively identified as theasinensin C and theasinensin B, respectively, which were dimers of two epigallocatechin-type derivatives. Namely, theasinensin C was formed via the dimerization of two molecules of EGC, while theasinensin B consisted of one molecule of EGC and one of EGCg, with the structures depicted in Figure 4. The compound P5 was

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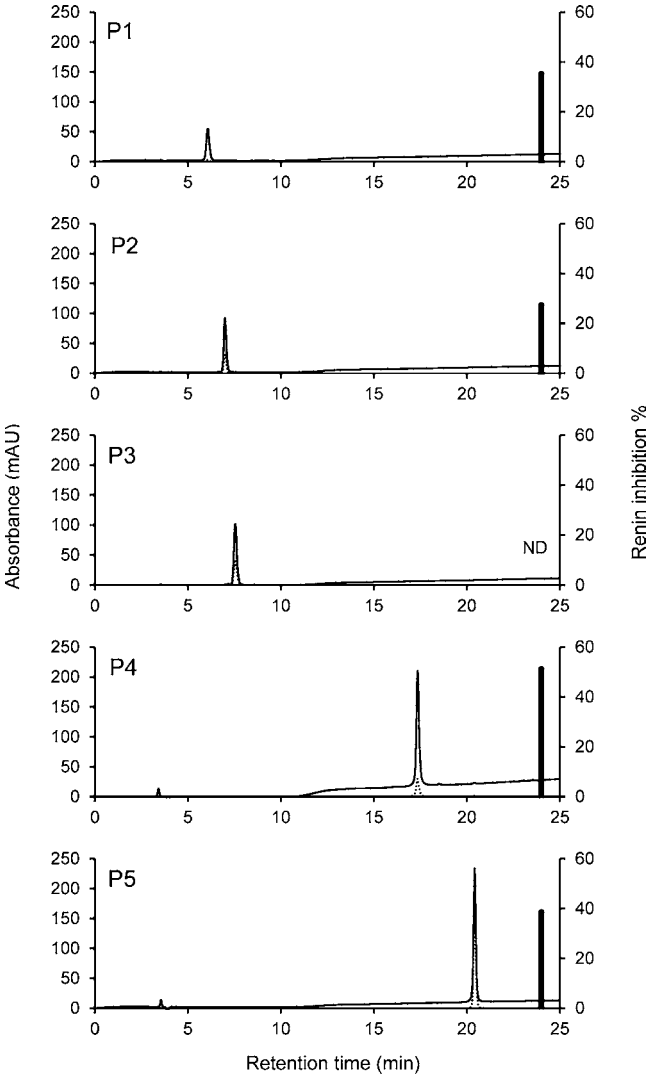

Figure 3. RP-HPLC chromatograms and renin inhibitory activities of the purified peaks P1, P2, P3, P4, and P5. Elution was performed on a Capcell Pak C18 column at 40 °C using a linear gradient of acetonitrile (with 0.05% TFA) with a flow rate of 1.0 mL/min and monitoring at 220 (solid line) and 280 nm (round dotted line). The bars indicate renin inhibitory activities determined at the concentration obtained by redissolving each collection in 150 μL of water after evaporation. ND indicates that no renin inhibitory activity was detected in P3.

Table 2. Retention Time, Spectroscopic Analysis, Purity and Identification of Active Compounds from Aqueous Extract of Black Tea

peak[a]	t_R[a]	PDA	$[M-H]^-$ (m/z)	MS^2 (m/z)	purity[b]	compound
P1	6.18	269	609	565, 471	96.72	theasinensin C
P2	7.09	270	411	331, 241, 169	100.00	unknown hexose conjugate
P4	17.48	273	761	609, 591	97.63	theasinensin B
P5	20.54	273	633	463, 301	98.79	strictinin

[a]Peak no. and retention time (t_R, min) refer to the HPLC profile shown in Figure 2. [b]Purity (%) was calculated from the peak areas recorded on the photodiode array detector.

tentatively identified as strictinin, a known constituent of tea as well.[28,31,32] As for P2, since no significant information is available from previous reports, this compound could not be fully identified based on the present analysis. However, it could be tentatively characterized as a hexose conjugate with both sulfate and galloyl moieties, a novel compound found in tea with a mass number of 412.03116 and a chemical formula of $C_{13}H_{16}O_{13}S$, labeled as M412.03.

Renin Inhibition by Identified Active Compounds. The concentration-dependent suppression of renin activity by the four active compounds identified is presented in Figure 5. It was found that theasinensin B, theasinensin C and M412.03 showed strong renin inhibitory activities, with IC_{50} values of 19.33, 40.21, and 50.16 μM (i.e., 14.73, 24.53, and 20.67 μg/mL), respectively. Strictinin possessed a comparatively moderate activity with an IC_{50} value of 311.09 μM (i.e., 197.25 μg/mL).

As for commercial or synthetic foodstuff-related compounds investigated previously as renin inhibitors, Deng et al. reported an IC_{50} value of 120.36 μM for baicalin,[3] while Takahashi et al. reported IC_{50} values of 19.4−77.4 μM for some saponins against renin.[14] Oleic acid and linoleic acid were able to inhibit renin activity, with IC_{50} values of 28.3 and 37.4 μM, respectively,[11] and three sodium houttuynin analogs were found to possess IC_{50} values of 273.3, 195.3, and 44.2 μM, respectively.[33] In line with the moderate renin inhibitory effects of peptide fractions,[24] Li and Aluko reported that the functional peptides Ile-Arg, Lys-Phe, and Glu-Phe showed renin inhibitory activities with relatively higher IC_{50} values of 9.20, 17.84, and 22.66 mM, respectively.[12] In our previous study on renin inhibitory activities of main catechin-related compounds, it was found that EGCg could exert good inhibitory effect with an IC_{50} value of 44.53 μM, ECg and EGC possessed IC_{50} values of 619.40 and 2175.30 μM, respectively, while no activity was detected in C and EC.[15] These findings supported the potent renin inhibitory activities for the novel compounds separated from black tea in this work. Noticeably, theasinensin B exerted almost the highest inhibitory effect on renin activity among the components reported, although the results might be affected slightly by the experimental conditions used individually.

Structurally, theasinensin B, an EGC-EGCg dimer, and theasinensin C, an EGC-EGC dimer, were formed by oxidative polymerization at the fermentation stage during tea manufacturing. On the basis of our previous report that EGCg and EGC could exert renin inhibitory activities with IC_{50} values of 44.53 and 2175.30 μM, respectively,[15] despite the increase in molecular weight, it appeared that the renin inhibitory activities of epigallocatechin-type derivatives could be much improved upon dimerization. Similarly, it was reported that theasinensin A, an EGCg dimer, might function better than monomeric EGCg in some cases such as the inactivation of herpes simplex virus and the growth inhibitory effects on human cancer cells.[34,35] Moreover, from structure−function relationship analysis of catechin-related compounds, it was suggested that the galloyl moiety might be favorable for these compounds to exert renin inhibition.[15] However, strictinin, containing three galloyl moieties with two linked through a C−C bond (Figure 4) showed relatively lower renin inhibitory activity (IC_{50} = 311.09 μM) than some compounds with no or one galloyl moiety, such as theasinensin C, theasinensin B, M412.03, and EGCg, although its activity was higher than some other compounds bearing one galloyl moiety like ECg (IC_{50} = 619.40 μM) and gallic acid (IC_{50} = 890.71 μM),[15] suggesting that the manner of linkage of the galloyl moiety or the presence of the catechin skeleton might also be responsible for the potency of

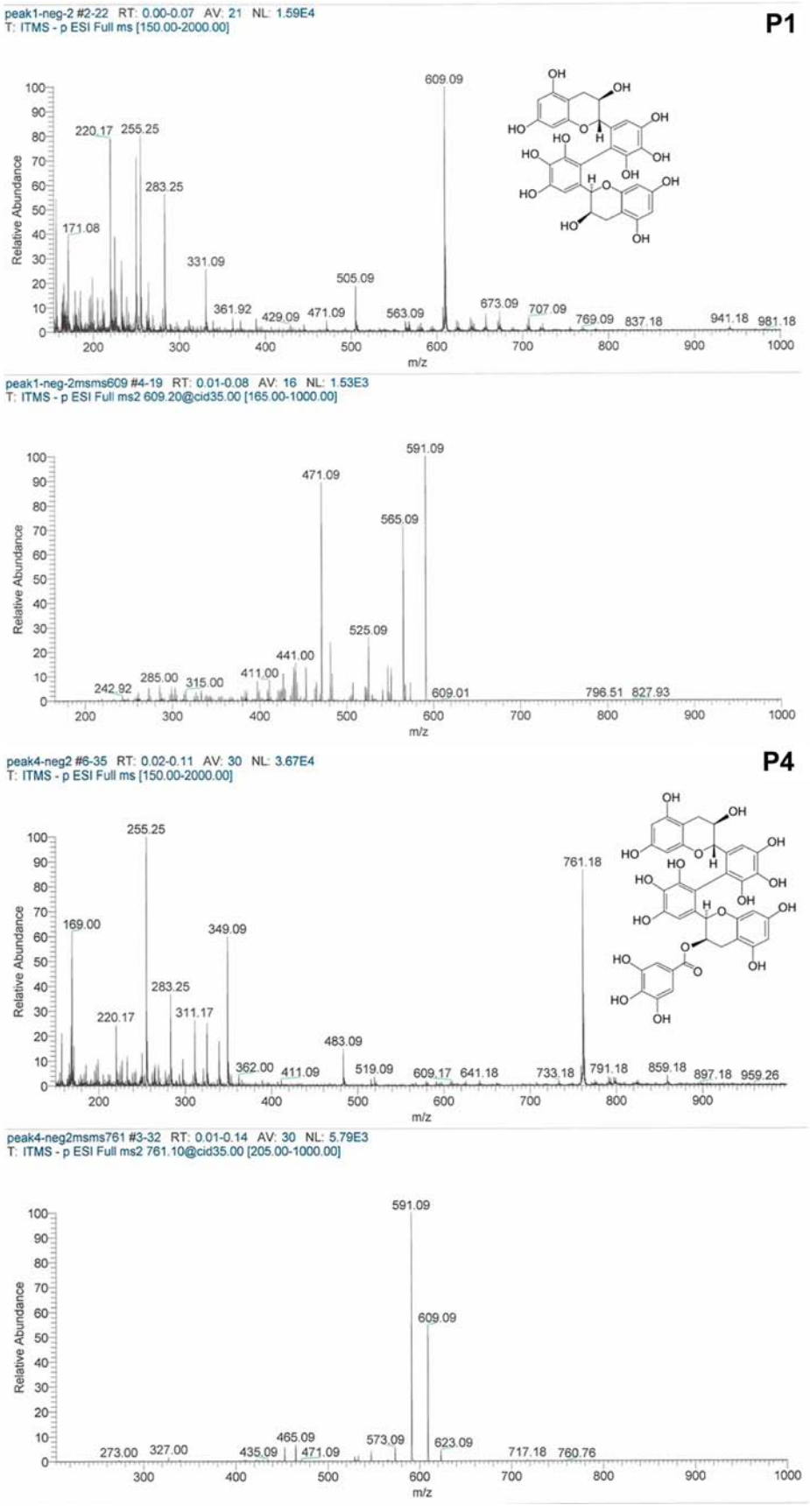

Figure 4. Full scan mass spectra of the purified compounds. Two charts for each compound represent MS and MS^2, respectively. For P1, MS^2 was the fragmentation pattern of the parent ion at m/z 609. For P4, MS^2 was that of the parent ion at m/z 761. For P5, MS^2 was that of the parent ion at m/z 633. Coupled with previous literature data, P1, P4, and P5 were putatively identified as theasinensin C, theasinensin B, and strictinin, respectively, with the chemical structures indicated.

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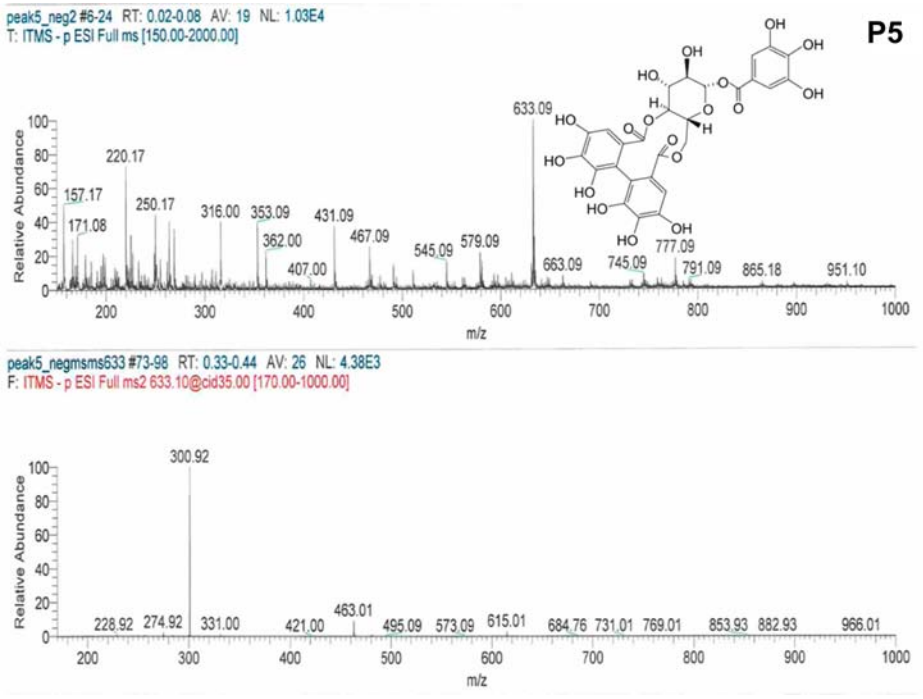

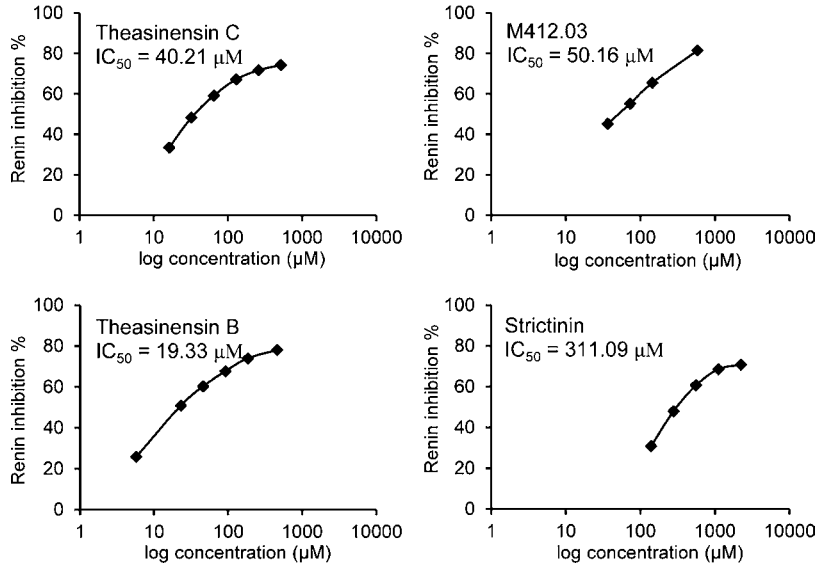

Figure 5. Concentration-dependent profiles and IC_{50} values of the active compounds identified. Data are the means of triplicate analyses.

renin inhibition by these inhibitors, which needs to be further elucidated.

Identified Active Compounds in Sample Aqueous Extracts. The HPLC profiles of water extracts from tea are depicted in Figure 6. The same amount was applied, so, the presence of the active compounds identified in this work as well as the main catechins confirmed by standards could be manifested qualitatively and quantitatively in each sample extract. It was shown that EGC and EGCg in particular were much more abundant in green tea (Figure 6A) than in fermented oolong tea (Figure 6B) and black tea (Figure 6C), and their contents decreased with increasing degree of fermentation during tea processing as well. Among the four compounds identified, theasinensin B and theasinensin C were not detected in green tea extract, while a minor amount of theasinensin C was observed in oolong tea extract, though both were relatively abundant in the black tea extract tested in the present study. Strictinin was observed with a similar amount in all of the samples tested. As for the compound M412.03, it was present in the two fermented tea extracts, with little detected in green tea extract.

As is known, for tea products, catechins constitute the main bioactive compounds in nonfermented green tea.[26,27] In our previous work, it was reported that, among the main catechins, EGCg showed quite much higher renin inhibitory activity than others;[15] however, green tea extract rich in EGCg possessed a much higher IC_{50} value than that of oolong and black tea (Table 1), suggesting that EGCg did not contribute significantly to the renin inhibitory activity of green tea as well as other tea products. With respect to processing techniques, green tea is produced by steaming and drying the fresh tea leaves to inactivate the polyphenol-oxidase enzyme

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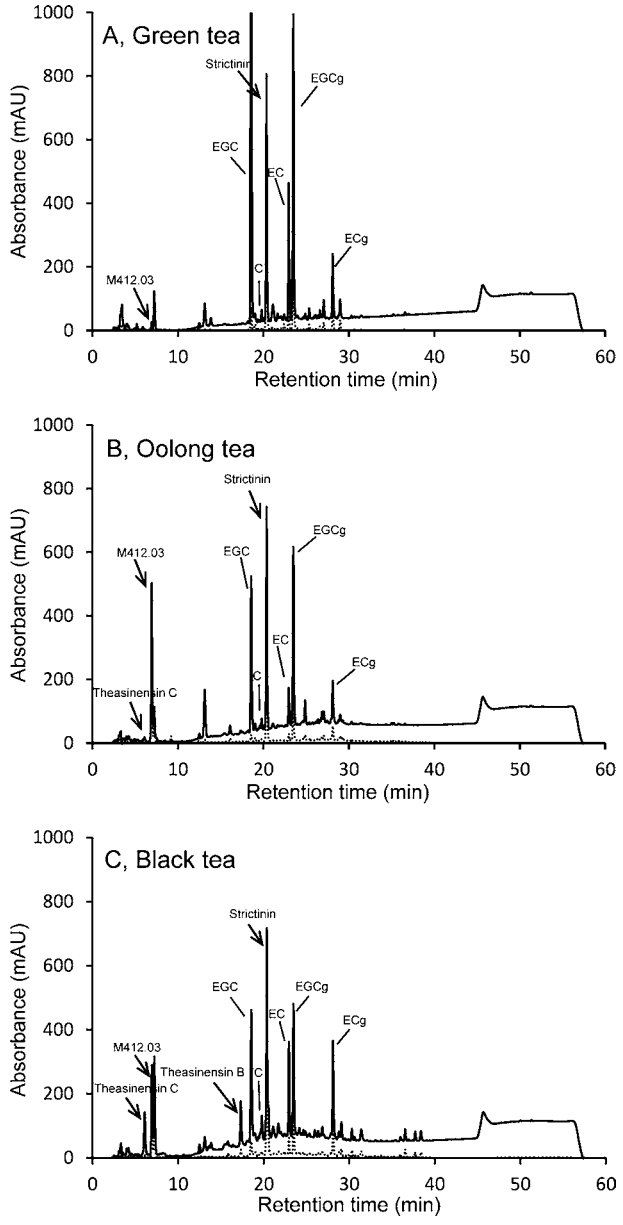

Figure 6. Active compounds identified as well as the main catechins in the aqueous extracts of tea. Each sample extract was applied at the same volume of 10 μL at a concentration of 5.0 mg/mL. Elution was performed on a Capcell Pak C18 column at 40 °C using a linear gradient of acetonitrile with a flow rate of 1.0 mL/min and detection at 220 (solid line) and 280 nm (round dotted line).

resulting in the presence of considerable amounts of polyphenols in monomeric forms. In contrast, during the production of black tea and oolong tea with extended fermentation, a large number of monomeric compounds undergo enzymatic transformation including oxidation and partial polymerization, which leads to the generation of the compounds theasinensin B and theasinensin C identified as potent renin inhibitors in this work, which also makes it difficult to structurally define the composition of these products, combined with variations in processing dependent on the producers. It was further suggested that the unknown renin inhibitor M412.03, which was mainly observed in the two fermented tea extracts as well, was a tea-derived compound and probably generated during the process of tea fermentation. The similar content of strictinin observed in all samples possessing significantly diverse renin inhibitory activities indicated that strictinin contributed to but did not play an essential role in the renin inhibitory activity of tea. Moreover, it was notable that, apart from the components separated and identified, there were other fractions with strong renin inhibitory activities as shown in Figure 2, which need to be especially analyzed in the future work.

In summary, the renin inhibitory activities of three typical tea products were confirmed for the first time in this study. In particular, fermented oolong and black tea could be regarded as excellent sources of renin inhibitors, due to the complicated biochemical transformation during tea fermentation that resulted in a variety of bioactive substances. Novel and potent renin inhibitors theasinensin B, theasinensin C and a hexose conjugate were identified from black tea extract. It would be of great value to further evaluate renin inhibitors in fermented tea, including those with trace amounts and those that are unavailable with standard compounds, so as to explore antihypertensive functional foods or ingredients with effective in vivo renin inhibition.

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Notes

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