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Elsevier B.V.
2023-07-01

Wang, R, Shi, X, Li, K, Bunker, A & Li, C 2023, 'Activity and potential mechanisms of action of persimmon tannins according to their structures : A review', International Journal of Biological Macromolecules, vol. 242, no. 3. https://doi.org/10.1016/j.ijbiomac.2023.125120

http://hdl.handle.net/10138/576361
10.1016/j.ijbiomac.2023.125120

Activity and Potential Mechanisms of Action of Persimmon Tannins According to Their Structures: A Review

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Abstract:

One distinguishing feature of the persimmon, that differentiates it from other fruits, is its high proanthocyanidins content, known as persimmon tannin (PT). Despite the poor absorption of PT in the small intestine, results from animal studies demonstrate that PT has many health benefits. Our goal in this review is to summarize the literature that elucidates the relationship between PT structure and activity. In addition, we also summarize the potential mechanisms underlying the health benefits that result from PT consumption; this includes the hypolipidemic, hypoglycemic, antioxidant, anti-inflammatory, antiradiation, antibacterial and antiviral, detoxification effects on snake venom, and the absorption of heavy metals and dyes. Studies show that PT is a structurally distinct proanthocyanidins that exhibits a high degree of polymerization. It is galloylation-rich and possesses unique A-type interflavan linkages in addition to the more common B-type interflavan bonds. Thus, PT is converted into oligomeric proanthocyanidins by depolymerization strategies, including the nucleophilic substitution reaction, acid hydrolysis, and hydrogenolysis. In addition, multiple health benefits exerted by PT mainly involve the inactivation of lipogenic and intracellular inflammatory signaling pathways, activation of the fatty acid oxidation signaling pathway, regulation of gut microbiota, and highly absorptive properties.

Key words: persimmon tannin, depolymerization, health effects

1.Introduction

Persimmon (*Diospyros kaki L.*) is widely cultivated and consumed in China, Spain, and Korea [1-3]. One distinguishing feature of the persimmon, that differentiates it from other fruits, is its high proanthocyanidins content, known as persimmon tannin (PT), a molecular with a high degree of polymerization (DP). The elucidation of the detailed structure of PT is extremely challenge due to its high DP and this remains a very active field of research. For example, Matsuo et al. [4] determined that the molecular weight of PT, obtained from Japanese persimmon, was about 13.8 kDa (about 13.8 kg/mol) and found a carbon–carbon interflavan linkage (B-type). Akagi et al. [5] identified epigallocatechin-3-O-gallate (EGCG) to be the main component of PT. In 2010, our group used a combination of matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) [6], thiolysis degradation combined with high performance liquid chromatography (HPLC) [7], gel permeation chromatography (GPC) [8], and ^{13}C nuclear magnetic resonance (NMR) spectroscopy [9] to characterize the structural details of PT. Our results showed that PT from Chinese persimmon consisted of four different subunits, with epicatechin (EC), epigallocatechin (EGC), epicatechin-3-O-gallate (ECG), and EGCG as the extension units and myricetin, catechin (C), and EGCG as the terminal units (the downmost unit) [10]. Of note, the extension unit is the basic unit that is repeated in the polymerization and the terminal unit is the final unit of the PT chains, which may (not) be the same with the extension unit due to the difference in their stereochemistry and degree of hydroxylation. In this study, the novel terminal unit of myricetin was identified in PT for the first time.

Subsequently, the myricetin terminal unit was also found in Italy persimmon [11]. Recent study also identified anthocyanidins, flavanols, and phenolic acids to be the terminal unit of persimmon fruit and two specific structural features with A-type linkage and galloylation [12]. Despite the progress have been made by using suite of experimental methodologies, further work still needs to be performed to obtain a more comprehensive structural analysis on PT.

Despite its high DP, heterogeneous character, and poor absorption, numerous health benefits have been linked to PT consumption. For example, it has been found to significantly (p <0.05) ameliorate the high cholesterol (HC) or high fat (HF) diet induced hyperlipidemia and hyperglycemia through the inhibition of digestive enzymes and intestinal uptake of glucose [13]. The administration of PT was also found to relieve the pneumonia symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, additionally, even inhibit the transmission of the virus in an animal model (Syrian hamsters), showing excellent anti-viral effect [14]. In addition, other biological activities of PT, including anti-oxidant [15], anti-inflammatory [16], anti-radiation [17], and anti-bacterial [18], have also been reported in a broad range of studies.

Due to the structural complexity of PT, the current understanding regarding its mechanism of action and structure-activity relationship (SAR) of PT remains limited. Thus, a commonly used strategy is to degrade PT into small molecules, also known as depolymerization, and then infer the local structural features or overall structure of PT.

In addition, representative structural units of PT can be directly obtained through depolymerization, and then the multiple biological activities would be explained by comparing the activity similarities or differences between them and linking to its structural characteristics. Therefore, this review provides a comprehensive summary of current research on PT isolation, purification, and characterization. Secondly, we also focus on the health benefits of PT and their underlying mechanisms, aiming to elucidate the SAR between PT structure and activity.

2. Extraction, Purification, and Characterization of PT

2.1 Extraction and purification of PT

Extraction is the first step to obtain PT from persimmon fruits. However, the effective extraction of PT is difficult to achieve. Because PT molecules bind to the polar fibrous matrix of persimmon, making it difficult to be directly released from the fruit. Additionally, the high DP of persimmon tannin can enhance this binding strength. As a result, unlike the case for other fruit proanthocyanidins, the addition of acid is needed to release the PT; separation has been achieved by adding hydrochloric acid to the conventional extraction reagent methanol, resulting in a yield of 16.94 mg/g [19]. Subsequent treatment with ultrasound and heat greatly improved the extraction efficacy, increasing the yield of PT to 30.36 mg/g [19]. Recently, PT was also extracted from persimmon leaves and the yield of PT was about 34.00 mg/g (dry weight) [20]. In addition, novel extraction techniques have been developed, including supercritical and subcritical fluid extraction [21], pressurized liquid extraction [22], pulsed electric field

119 extraction [23], high-pressure processing [24], high-voltage electrical discharges [25],
120 and infrared-assisted extraction [26]. The application of novel technologies in PT
121 extraction will show promising extraction efficacy in the future. The key process
122 involved in PT isolation, purification, and structural characterization is shown in Fig.1.
123
124 Since the extracted crude PT is heterogeneous and composed of a wide variety of low
125 molecular weight substances (like sugar and organic acid), further purification is
126 necessary. Macroporous resin is a common PT purification material [19]. It is easy to
127 separate PT from other small molecules to obtain high-content polymers [27]. For
128 example, a previous study indicated that the content of PT reached 82.4% following
129 initial purification with AB-8 macroporous resin [19]. However, the obtained PT
130 remains a mixture. Further techniques, including ultrafiltration with polysulfone
131 ultrafine membrane with molecular weight cutoff of 10 KDa, were used to fractionate
132 PT into 1) low molecular weight simple phenols and 2) high molecular weight tannin
133 (11.6 KDa-15.4 KDa) [19]. Among the simple phenols, the monomeric flavanols and
134 phenolic acids are reported to be rich. However, the content of PT in the high molecular
135 weight tannin fraction reached 93.4%. In addition, the high molecular weight tannin
136 could be further finely fractionated through Toyopearl TSK HW-50F column [27, 28].
137
138 However, recent reports found a significant variance in the PT content of different
139 varieties and ripeness levels [11, 29, 30]. For example, the content of PT was found to
140 be the highest in *Hachiya* variety (321.9 mg/g), and the lowest in the *Taishu* variety

(54.8 mg/g) in a comparison of 10 different persimmon varieties [30]. Thus, the construction of a database of the PT content in different varieties is also being performed in our group; we hope that, in the future, this will be of significant utility in PT research.

2.2 Structural characterization of PT

Due to its highly polymeric and heterogeneous character, the peak in liquid chromatography of PT is very broad and this significantly reduces the extent to which this technique can elucidate the structure of PT. Nucleophilic substitution reaction at the C-4 position of polymeric proanthocyanidins with a nucleophile has often been used to depolymerize PT, which is regarded as the most promising tool for composition analysis [31]. The most used nucleophilic reagents include benzyl mercaptan and phloroglucinol. For instance, in a previous study it was determined that the preferred reagent for composition analysis of PT is, in fact, benzyl mercaptan, through a chemical reaction known as thiolysis [31]. Thiolysis can be used to analyze the composition and DP of PT in combination with other chromatographic analysis tools, e.g., HPLC-MS, MALDI-TOF MS, and NMR [19, 32]. For example, Matsuo and Ito [4] found that PT from Japanese persimmon consisted of catechin (C), catechin-3-O-gallate (CG), gallocatechin (GC), and gallocatechin-3-O-gallate (GCG) residues in a molar ratio of 1:1:2:2 by thiolysis-HPLC. However, the terminal residues of PT remained unknown. Subsequently, our group took advantage of MALDI-TOF MS, NMR, and GPC techniques to comprehensively characterize the structural details of PT. The results

showed that the PT structure from Chinese persimmon pulps was different from that of Japanese persimmon and four different extension units of EC, EGC, EGCG, and ECG were found [10]. In addition to C and EGCG, myricetin was, for the first time, identified as a unique terminal unit of PT [10]. However, recent study also identified anthocyanidins, and phenolic acids to be the terminal unit of PT. Furthermore, PT was found to have unique A-type interflavan linkages in addition to the previously reported B-type interflavan bonds. Also, the mean DP of PT was found to be 26.0. The potential structural formula of PT is shown in Fig.1. However, the difference in mean DP was reported in recent study and their result was 10.18 from persimmon peel proanthocyanidins [12]. In addition, the molecular weight of purified PT was also measured by GPC in a previous study [19]. The results showed that PT as a single peak on chromatogram had weight average molecular weight of 1.39×10^4 Da and number average molecular weight of 1.28×10^4 Da. This result was also in agreement with that from Matsuo group [4] and suggested that PT was a macromolecular polyphenol or polymeric proanthocyanidin, with the average molecular weight of 13.9 kg/mol. However, thiolysis often produces an unpleasant smell and causes a lachrymatory effect, both of which are attributed to the presence of thiols. Briefly, thiols are known for their strong odor, which is often described as unpleasant or sulfurous. When thiols are cleaved or broken down during thiolysis, they can release volatile sulfur compounds that contribute to the unpleasant odor. Furthermore, these volatile sulfur compounds can also irritate the eyes and respiratory system when inhaled, and thus cause the lachrymatory effect. In addition, some researchers have used phloroglucinol to

depolymerize condensed tannins, but the problem of low yields of adduct in the reaction with phloroglucinol makes it unsuitable for PT degradation [5, 31]. Therefore, some environmentally friendly and more suitable nucleophilic reagents, e.g., menthol furan, captopril, L-cysteine, and L-glutathione [33], should be developed and used in the structural characterization of PT for new knowledge in the future.

3. Converting PT into oligomeric proanthocyanidins

Current depolymerization strategies mainly include the nucleophilic substitution reaction, acid hydrolysis, and hydrogenolysis.

3.1 Nucleophilic substitution reaction

In addition to structural analysis, nucleophilic substitution reactions have also been used to convert polymers into oligomers by adding some monomeric phenolic compounds as nucleophilic reagent, which include EC, EGC, ECG, and EGCG. As reported previously [34], persimmon fruits pulp (enriched in polymeric proanthocyanidins) coupled with dried green tea leaves, which contain EC, EGC, ECG, EGCG and other monomeric phenolic compounds, were heated in the presence of citric acid. Oligomeric proanthocyanidins were then obtained by Sephadex LH-20 column chromatography. Results from HPLC analysis indicated that oligomeric proanthocyanidins were mainly composed of EGC, EC, EGCG, and ECG in the unit ratios of 47, 15, 31, and 6 %. However, the structural feature and linkage type were not determined. Our group previously analyzed the structural features of PT after

depolymerization using EC and EGCG. The results showed that the obtained oligomers were mainly connected by the B-type interflavan linkage after depolymerization with EC, while the oligomers were mainly connected by the A-type interflavan linkage after depolymerization with EGCG [35]. Further, some novel procyanidin dimer analogs with a C-ring-opened structural unit by using EC and EGCG as the chain breaker were also discovered for the first time. Therefore, this method of depolymerizing PT by adding some monomeric phenolic compounds has many advantages. Firstly, it does not introduce exogenous compounds. Secondly, it does not generate unpleasant smell like adding benzyl mercaptan as a nucleophilic agent. Thirdly, it can be used to produce new types of oligomers. In short, this method has great prospects to further study the SAR of PT in the future.

3.2 Acidolysis

Acid hydrolysis is another powerful depolymerization method to cleave the structure of PT. Based on previous study [36], oligomeric proanthocyanidins, which featured both a high content of A-type linkage dimers and high galloylation, were obtained by heating PT in the presence of hydrochloric acid and methanol. Further purification could be performed by Toyopearl HW-50F column; HPLC–MS were used to identify the composition. The products of acid degradation of PT included cyanidin, delphinidin, myricetin, catechins, A-type and B-type linked dimers. Subsequently, two main galloylated dimeric proanthocyanidins, A-type ECG dimer and A-type EGCG dimer, could be obtained by high-speed counter-current chromatography and preparative high-

performance liquid chromatography, with purity of 95.6% and 96.2% respectively [37]. However, using acid hydrolysis also has some disadvantages, including low yield, low efficient, very time-consuming experiment, and environment hazard.

3.3 Hydrogenolysis

High-pressure catalytic hydrogenolysis is also an efficient strategy to convert PT into oligomeric proanthocyanidins [38]. In brief, PT was transferred to the reactor with high pressure for reacting along with the hydrogen gas, then oligomeric proanthocyanidins could quickly be obtained. Compositional analysis results from ultra-performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-QTOF-ESI-MS) indicated that catalytic hydrogenolysis products of PT (mean DP = 2.8) were composed of 16 oligomers, mainly including 3 monomers, 10 dimers, 1 trimer, and 2 new compounds. In comparison to acid hydrolysis, hydrogenolysis has the advantage of being a less time-consuming experiment and not requiring excess reagents. However, hydrogenolysis requires high-pressure equipment and it should be noted that the reaction process can possibly convert the A-type linked oligomers into B-type linked oligomers.

4. Health effects and corresponding mechanisms of PT and its degradation products

4.1 Hypolipidemic activity

Hyperlipidemia is a symptom of lipid metabolism disorder, which further increases the

risk of obesity, atherosclerosis, cardiovascular disease, insulin resistance, and diabetes. Previous study by both population surveys and animal experiments have provided evidence that PT has the capacity to act as a powerful functional ingredient to regulate lipid metabolism disorders [39-42]. For example, the male Sprague–Dawley rats fed on a high cholesterol (HC) diet showed lipid metabolism disorder, with high lipid levels in serum, excess lipid droplets in the liver, and hepatic steatosis [41]. Similar results also existed in rats feeding with high fat (HF) diet, in addition, the proinflammatory cytokines level in liver was also high. While the dietary intervention of adding 50 mg/kg - 100 mg/kg PT to HC/ HF diets significantly (p <0.05) decreased the elevated lipid profile and alleviated the above-mentioned unfavorable symptoms in a dose-dependent manner, as shown in Fig. 2. Another study also compared the hypolipidemic and hypocholesterolemic effects of highly purified PT and lyophilized whole persimmon fruit [13]. Their results showed that the administration both PT and persimmon fruit (with the same tannin content) significantly (p <0.05) reduced serum triglycerides and free fatty acids by inhibiting the activity of lipid synthesis-related proteins, and enhanced the excretion of triglycerides and cholesterol, thus improving hepatic steatosis in rats fed a HF diet. Importantly, the anti-hyperlipidemic effect by consuming PT was consistent with that of consuming lyophilized whole persimmon fruit, and confirmed that PT was responsible for the hypolipidemic and hypocholesterolemic effects of persimmon fruit. In addition, the anti-obesity ability of persimmon proanthocyanidins with different DP was also compared in C57BL/6J mice. The results showed that PT had stronger anti-obesity ability than persimmon oligomeric

proanthocyanidins (mean DP = 2.8) [43]. These studies thus showed that PT had excellent hypolipidemic activity *in vivo*.

The current understanding of the mechanism of PT in exerting hypolipidemic activity mainly includes (1) the regulation of lipid profile and enzymatic activity, (2) inactivation of the lipogenic signaling pathway and activation of the fatty acid oxidation signaling pathway, (3) regulation of gut microbiota, and (4) inhibition of the adipogenesis process. Firstly, it was shown that dietary intervention by adding PT to a HC/HF diet significantly (p <0.05) decreased the elevated lipid profile including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and free fatty acids (FFA) in a dose-dependent manner and increased the high-density lipoprotein cholesterol (HDL-C) level in the serum and liver of male Sprague–Dawley rats [41]. Further study showed that PT could increase lecithin cholesterol acyltransferase (LCAT) activity to remove the cholesterol from plasma and tissues, and further convert cholesterol to cholesteryl esters on the surface of HDL, suggesting that the elevation of the serum LCAT activity and serum HDL level was an efficient strategy to exerting hypolipidemic activity [41, 44]. Furthermore, removal of cholesterol from the plasma was also considered as a result of hepatic biosynthesis of bile acids from the cholesterol [45]. Matsumoto et al. [46, 47] studied the bile acids-binding ability of PT and found that PT could not only absorb the bile acids but also significantly (p <0.05) promote fecal bile acid excretion, in agreement with our previous study. Our results also suggested that PT supplementation significantly (p <0.05) elevated the excretion

295 of fecal total lipid, TG, and TC in comparison to the case of rats fed on an HF diet [13,
296 48]. Furthermore, PT feeding could decrease the elevated aspartate aminotransferase
297 (AST) and alanine aminotransferase (ALT) activities caused by an HF/HC diet, thereby
298 protecting the liver from damage [41]. In addition, results of a previous study also
299 suggest that PT could effectively inhibit the activity of pancreatic lipase and reduce the
300 TG absorption by the strong hydrogen bonding and π-π stacking interaction [49].
301
302 Secondly, studies found that PT could exert strong hypolipidemic activity through the
303 inactivation of the lipogenic signaling pathway and activation the fatty acid oxidation
304 signaling pathway, as shown in Fig. 2. It was found that the proinflammatory cytokine
305 level in the liver, e.g., tumor necrosis factor α (TNF-α), C-reactive protein (CRP), and
306 interleukin-6 (IL-6), were elevated in rats fed with an HF diet. While the overproduction
307 of CRP, TNF-α, and IL-6 in the liver of rats induced by an HF diet was blocked by PT
308 administration, thus alleviating liver injury caused by these proinflammatory cytokines
309 [13]. Furthermore, PT administration could inhibit the lipogenic signaling pathway and
310 activate the fatty acid oxidation signaling pathway. For example, the dietary
311 intervention by PT in an HC diet could significantly (p <0.05) inhibit lipogenic enzymes
312 or transcriptional factors, including acetyl CoA carboxylase (ACC), fatty acid synthase
313 (FAS), sterol regulatory element binding protein 1 (SREBP1), stearoyl-coenzyme A
314 desaturase 1 (SCD1), and enhance the expression of fatty acid oxidation transcriptional
315 factor, such as carnitine palmitoyltransferase-1 (CPT-1), peroxisome proliferator-
316 activated receptor γ coactivator 1α (PGC1α), and peroxisome proliferator-activated

receptor α (PPARα) [13]. Additionally, the regulation of the above signaling pathways was achieved through the activation of AMP-activated protein kinase (AMPK) or the inactivation of microRNAs (miRNAs), e.g., miR-122 and miR-33a/b, both of which can occur due to the presence of PT [13, 50]. Furthermore, miR-122 and miR-33b could also regulate hepatic fatty acid synthesis and cholesterol homeostasis, and PT treatment could significantly (p <0.05) decrease hepatic TG accumulation in L02 cells by inhibiting the expression of miR-122 and miR-33b, thus improving hepatic steatosis [50].

Thirdly, gut microbiota plays a pivotal role in the lipid metabolism [51]. Studies found that a HC diet changed the gut microbiota composition by decreasing *Bacteroidetes* phylum and increasing *Firmicutes* phylum, leading to disorders in lipid metabolism [52]. However, PT significantly (p <0.05) altered the gut microbiota in HC diet-fed rats by enhancing the growth of *Bacteroidetes* phylum and inhibiting the growth of *Firmicutes* phylum [53]. Meanwhile, the growth of beneficial bacteria (*Bifidobacterium* and *Lactobacillus*) was increased and the growth of harmful bacteria (*Escherichia coli* and *Enterococcus*) was inhibited after PT administration, resulting in the increase of short-chain fatty acids (SCFAs) including acetic, propionic and pentanoic acids in cecum [53]. These results also suggested that regulation of the gut microbiota composition could also provide a partial elucidation for the hypolipidemic mechanism of PT. Due to the structural complexity of PT, its major degradation products, including B-type EC dimer, A-type EC dimer, A-type ECG dimer and A-type EGCG dimer, were

used to elucidate structural contribution of PT for anti-hyperlipidemic activity [50]. The results showed that its distinctive A-type interflavan linkage and high content of ECG and EGCG units were responsible for the hypolipidemic effect of PT.

Finally, the inhibition of the adipogenesis process could also provide systematic understanding for the hypolipidemic mechanism of PT. Adipogenesis often occur during the conversion of preadipocytes to adipocytes and excess adipogenesis can lead to obesity problems, seriously endangering human health [54, 55]. Numerous studies found that PT were able to suppress adipogenesis and reduce TG accumulation [15, 56]. For example, PT from astringent *Cheongdobansi* cultivar could inhibit 83.0% of adipogenesis at a concentration of 100 μg/mL; this had no effect on the viability of 3T3-L1 preadipocytes [15]. In agreement with this result, our previous study also showed that PT from astringent persimmon (*Diospyros kaki Thunb*) could inhibit 68.6% of adipogenesis of 3T3-L1 preadipocytes at a concentration of 60 μg/mL [57]. The concentration used was far lower than the cytotoxicity of PT on 3T3-L1 cells (200 μg/mL). Further study found that PT delayed the entry of 3T3-L1 cells into S and G2/M phases and blocked adipogenesis in the early stage of adipocyte differentiation. Subsequently, the effect of PT on downstream lipid-regulated transcription proteins was also explored. The result showed that PT significantly (p <0.05) inhibited the expression of peroxisome proliferator activated receptor γ (PPARγ) and CCAAT/enhancer-binding proteins α (C/EBPα), which are major regulators for modulating adipogenesis [58]. Then the expression levels of the downstream lipogenic

transcription factors, including ACC1, SCD1, and FAS, and lipolysis transcription factors, covering hormone sensitive lipase (HSL) and lipid protein lipase (LPL), were notably (p <0.05) inhibited under PT treatment. Besides that, the effect of PT on upstream gene expression of PPARγ was also studied. The results indicated that expression of miR-27, which affects adipocyte differentiation by controlling PPARγ expression [59, 60], was significantly (p <0.05) increased by treatment with PT in a dose-dependent manner. Collectively, the strong inhibitory effect of PT on adipogenesis was produced by upregulating the expression of miR-27 and downregulating the expression of PPARγ, leading to the inhibition of the downstream lipogenic signaling pathway. The signaling pathway of PT regulating adipogenesis in 3T3-L1 preadipocytes is shown in Fig. 3.

To explore the structural contribution of PT to the anti-adipogenic activity, the major degradation products of PT, including B-EC dimer, A-EC dimer, A-ECG dimer, and A-EGCG dimer, were obtained by acidolysis and their anti-adipogenic activity was compared in a 3T3-L1 preadipocytes differentiation model. Results showed that the A-ECG dimer could inhibit 65% of adipogenesis at a concentration of 20 μg/mL and the A-EGCG dimer could inhibit 58% of adipogenesis at a concentration of 60 μg/mL [61]. However, similar anti-adipogenic effects were obtained at a PT level of 60 μg/mL. Meanwhile, only 20% of the adipogenesis was inhibited by a 60 μg/mL A-EC dimer treatment, while the B-EC dimer showed no inhibitory effect for adipogenesis at the same concentration. These data suggested that two galloylated proanthocyanidins

dimers (A-ECG/EGCG dimer) significantly (p <0.05) decreased the intracellular TG content than non-galloylated proanthocyanidins dimers (A-EC/B-EC dimer) and A-type linkage enhanced the anti-adipogenic ability. Subsequently, their underlying mechanisms for the inhibition of preadipocytes differentiation were also investigated and compared. The result showed that the A-ECG/EGCG dimer blocked adipogenesis in the early stage of adipocyte differentiation by inhibiting the PPARγ-regulated adipogenesis signaling pathway, consistent with that of PT.

Lipid rafts (size 10–200 nm) are distinct functional nanodomains in the inner and outer leaflets of plasma membranes that are enriched in cholesterol, sphingolipids, and certain signaling proteins. Due to the high concentration of cholesterol in lipid rafts, they are more ordered and less fluid than the surrounding membrane, allowing them to form signaling platforms for the regulation of cellular processes and diseases [62, 63]. Studies showed that the structural integrity of lipid rafts was necessary for breast cancer cell growth [64], activation of B-cell [65], and differentiation of 3T3-L1 preadipocytes [66]. Therefore, compounds that could disturb the structure of lipid rafts would affect intracellular signaling pathways, thus exerting their activities. Further study revealed that the PT/A-ECG/EGCG dimer had an extremely strong propensity to adsorb to the membrane rafts, reducing the fluidity and order of lipid rafts and thus disrupting their structure [67, 68]. The ability to disrupt lipid rafts was highly positively correlated with the ability to inhibit 3T3-L1 preadipocyte differentiation (R^2 =0.9328) [68-70]. Both the mitotic clonal expansion process of 3T3-L1 cells and the PPARγ-regulated lipid

405 metabolism signaling pathway were inhibited. Altogether, the data suggested that the
406 structural features, including the unique A-type interflavan linkages and high ratios of
407 two galloylated extension units (ECG/EGCG), were responsible for the anti-adipogenic
408 activity of PT. The schematic diagram of PT and its degradation products inhibiting the
409 adipogenesis signaling pathway by affecting the structure of lipid rafts is shown in Fig.
410 3.
411
412 **4.2 Hypoglycemic activity**
413 Chronic postprandial hyperglycemia increases the risk of type 2 diabetes (T2D), so the
414 regulation of postprandial carbohydrate digestion and absorption is regarded as
415 effective strategy for preventing T2D [71, 72]. It has been shown that PT is a potential
416 dietary regulator in the management of hyperglycemia [73, 74]. For example, Tsujita
417 group [74] found that the oral administration of PT at 100 mg/kg body weight in rats
418 could inhibit the blood glucose levels by 20.00%. Similar result was obtained by our
419 group. The administration of PT at a 75 mg/kg dose resulted in a reduction of blood
420 glucose levels of 11.33% [73]. This hypoglycemic activity of PT was also confirmed
421 by human trial [75]. The current mechanism through which PT exerted hypoglycemic
422 activity mainly included the inhibition of enzyme activity, bound to starch granules, and
423 inhibited glucose transporters. The activity of two digestive enzymes of starch showed
424 that α-amylase and α-glucosidase were significantly (p <0.05) suppressed by PT, with
425 half inhibitory concentration values of 0.35 and 0.24 mg/mL, respectively. Further
426 study indicated that the binding of PT to the active sites of two digestive enzymes

resulted in a change in their conformations [76]. Thus, the inhibition of starch digestive enzymes could decrease the excess glucose uptake in the small intestine. In addition, PT also directly bound to starch granules, thus directly decreasing its digestibility. It was also found that its binding ability with amylose was stronger than that with amylopectin [73, 77]. Another study, using Caco-2 human intestinal cell monolayers models, suggested that the uptake of glucose in the intestine was significantly (p <0.05) inhibited (26.36%) by 20 μg/mL of PT and the inhibitory capability was shown to be dose-dependent [73]. Thus, the inhibition on the glucose uptake and transport induced by PT could be another efficient approach in regulating blood glucose level. However, the detailed inhibitory mechanism of PT on glucose uptake and transport remains unclear and need further investigation in the future.

4.3 Anti-oxidant activity

The imbalance of oxidative and anti-oxidative factor in the body often take place during metabolic processes or stimulus from the external environment and will produce many reactive oxygen species (ROS) [78, 79]. These ROS such as superoxide anion, hydroxyl radical, and hydrogen peroxide can, at high concentrations, cause a detrimental effect on the biomolecules, including lipid, protein, and DNA [80, 81]. As a result, multiple metabolic diseases occur due to this oxidative imbalance. Studies have shown that PT can compensate for the oxidative damage induced by these ROS. For example, a previous study showed that PT had the strongly scavenging effect on the hydroxyl radical, superoxide anion radical and an inhibitory effect on the peroxidation of linoleic

449 acid by four different *in vitro* test system [19]. PT was thus found to be a potent
450 antioxidant. However, the antioxidant capacity of PT *in vitro* model system might not
451 represent its activity in complex biological systems. To verify the capacity of PT to act
452 as an antioxidant in complex biological systems, the oxygen radical absorbance
453 capacity (ORAC) method, considered as a reliable assay to assess the antioxidant
454 activity of substances, was used [82]. The result showed that the plasma ORAC values
455 in rats after dried persimmon administration were about 1.5 times higher than that of
456 the control rats, confirming the strong antioxidant activity of PT [83]. Our previous
457 studies showed that PT is the main component that accounts for the antioxidant
458 activities of persimmon pulp [19]. Subsequently, the antioxidant capability of PT *in*
459 *vivo* was also tested by bromobenzene-induced oxidative stress in mice [84]. The result
460 indicated that mice under oxidative stress showed loss of activities on superoxide
461 dismutase (SOD) and glutathione peroxidase (GSH-Px), and an increase in the
462 malondialdehyde (MDA) content in the liver. However, an oral dose of PT at 200 mg/kg
463 prevented these adverse results and improved the oxidative stress in mice [84].
464 Furthermore, senescent mice induced by D-galactose also showed oxidative damage by
465 increasing the level of MDA and decreased the activities of antioxidant enzymes in the
466 serum, liver, and brain [85, 86]. However, supplying mice with PT via oral gavage at
467 100 mg/kg could reverse the changes in the mentioned antioxidant enzyme activities
468 and the MDA level [87]. Additionally, the memory dysfunction and neuronal
469 degeneration in mice induced by D-galactose were ameliorated in a dose-dependent
470 manner upon PT administration, suggesting that PT ameliorated age-related cognitive

471 decline by reducing oxidative damage [87]. Although preliminary research found that
472 an A-type linkage mode, high degree of galloylation, and DP were the key structural
473 features accounting for the antioxidant activity of PT [36], the current research on the
474 mechanism of PT antioxidant activity is rare, probably due to the structural complexity
475 of PT. A recent study by computational theory explored the antioxidant mechanism of
476 structural units from PT, including ECG, EGCG, A-type ECG dimer, and A-type EGCG
477 dimer [88]. Their results indicated that the positional differences of phenolic hydroxyl
478 in monomeric and dimeric phenols lead to different active site. For example, the most
479 preferential site of monomeric phenols was located on the phenolic hydroxyl group of
480 the B ring, while that for dimeric phenols was the phenolic hydroxyl group of the A ring
481 and D' ring. Thus, the difference in phenolic hydroxyl position was the main reason for
482 their different antioxidant activity, providing new knowledge for the action mechanism
483 of PT.
484
485 **4.4 Anti-inflammatory activity**
486 Inflammation is a physiological disorder due to foreign stimulus and classified into
487 acute and chronic inflammation [89]. This inflammation was related to the development
488 of some diseases, including inflammatory bowel disease (IBD), pulmonary
489 nontuberculous mycobacteria infection (PNMI), diabetes, obesity, cancer, and so forth
490 [90, 91]. Therefore, inhibition of inflammation represents a powerful strategy for
491 preventing the above-mentioned diseases. Studies found that PT is a potential candidate
492 as an agent that achieves the suppression of inflammation and its associated diseases.

493 For example, infection with *Mycobacterium avium* complex (MAC) severely damaged
494 the host immune system, causing chronic pulmonary infection disease. Ito et al. [16]
495 explored the effects of PT on pulmonary inflammation in MAC infected mice and found
496 that PT administration decreased the level of inflammatory genes and proteins in lungs,
497 including TNF-α and inducible nitric oxide synthase (iNOS). Furthermore, they also
498 found that the upregulation of inflammatory genes and proteins (TNF-α, IL-1ß, IL-6,
499 iNOS), induced by MAC stimulation, was significantly (p <0.05) inhibited after
500 treatment with PT, suggesting the potential for powerful anti-inflammatory activity of
501 PT. Also, the imbalance of gut microbiota, which showed that the high abundance of
502 proteobacteria and the low abundance of actinobacteria, is closely related to IBD [92];
503 treatment with PT could not only reverse this trend but also inhibit inflammation by
504 decreasing the expression of inflammatory cytokines. Thus, the anti-inflammatory
505 activity caused by PT may be a result of the inhibition of the nuclear factor-kappa B
506 (NFκB) signaling pathway and the regulation of the gut microbiota. Current studies
507 mainly focused on the anti-inflammatory effects of PT by detecting changes in key
508 inflammatory markers, while its SAR and anti-inflammatory mechanisms were poorly
509 understood and required further research.
510
511 **4.5 Anti-radiation activity**
512 Radiation, including ultraviolet, γ-ray, and x-ray, can produce reactive oxygen species
513 (ROS) and induce multiple damages to human skin or organs, such as epidermal
514 pigmentation, skin wrinkles, hematopoietic and cerebrovascular dysfunction [93, 94].

For example, under the irradiation of ultraviolet B (UVB), melanocytes tyrosine is converted into melanin through multiple reactions under the action of tyrosinase. ROS can further promote the process of melanin synthesis [95, 96]. Thus, inhibiting tyrosinase or the production of ROS provide potential ways to withstand damage caused by radiation. It has been shown that PT significantly (p <0.05) decreased UVB induced pigmentation by inhibiting tyrosinase and enhancing the activities of various antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) [17]. Similar results were also found in the degradation products of PT, which protected human keratinocyte cells (HaCaT) and mice from UVB induced damage by increasing the above-mentioned enzymatic activities and attenuating the activation of NF-κB pathway to inhibit intracellular ROS production [97]. Other studies suggested that PT also protected the cells from damage caused by ionizing radiation. For example, under the irradiation of 8 Gy γ-ray, the HEK 293T cell apoptosis was significantly (p <0.05) decreased by 93.30% and the production of ROS was significantly (p <0.05) inhibited by 30.00% after treatment with 200 μg/ml PT [98]. The addition of *Aloe* gel could further reduce the HaCaT cell apoptosis (95.70%) and ROS generation (64.00%) induced by 8 Gy X-ray irradiation, enhancing the anti-ionizing radiation activity of PT [99]. The mechanism of the radioprotective activity of PT is, however, still unknown.

4.6 Antibacterial and antiviral activity

Pathogenic bacteria and viruses pose a great threat to humans and animals, causing a

variety of diseases. For example, the MAC can cause seriously aspiration pneumonia. The *Staphylococcus aureus* (*S. aureus*) can form a biofilm to resist antimicrobials and secrete toxins that contaminate food products and threaten human health [100, 101]. Likewise, SARS-CoV-2 has spread rapidly around the world, causing millions of deaths [102, 103]. Evidence has been found that PT could ameliorate the harm caused by pathogenic bacteria. For example, PT showed strong bacteriostatic properties for MAC and prevented MAC-induced pneumonia in mice [16]. Furthermore, PT and their degradation products could also significantly (p <0.05) inhibit the growth of *S. aureus* with a minimum inhibitory concentration of 1.1 mg/mL and 0.7 mg/mL [38]. Due to the important role of biofilms in bacterial self-protection, Mukai et al. explored the effects of PT on intraoral biofilm formation using a polymicrobial model consisting of multiple intraoral bacteria [18]. The results from scanning electron microscopy exhibited the disruption of the polymicrobial biofilm structure and inhibition of bacterial aggregation. Our previous study also verified their results and found that PT and their degradation products could disrupt the integrity of the *S. aureus* membrane and change the conformation of the *S. aureus* membrane protein [38]. Meanwhile, the activities of the enzymes involved in energy metabolism and the genes of the glycolysis pathway were also downregulated in *S. aureus* after the treatment of PT and their degradation products. In addition, further results from transcriptomic and metabolomic analyses of *S. aureus* with the treatment of 0.5 mg/ml PT revealed that the signaling pathways related to membrane transport and energy metabolism were inhibited [104]. Through analyses, the understanding for the antibacterial mechanism of PT mainly

559 focuses on the inhibition of biofilm formation and energy metabolism signaling
560 pathways.
561
562 In addition, PT also decreased the infectivity of different viruses and blocked the
563 attachment of the influenza virus to cells within 30 seconds, thus showing strong
564 antiviral effects [105]. For example, PT could effectively inactivate the various avian
565 influenza viruses at a concentration of 1 mg/ml and inhibit the spread of several avian
566 influenza virus subtypes [106]. A recent study indicated that oral administration of 1
567 mg/ml PT in Syrian hamsters infected by SARS-CoV-2 could relieve the pneumonia
568 and inhibit the transmission of the virus [14]. Their research showed that there were
569 two ways in which PT exerted its antiviral activity. On the one hand, PT could bind
570 with viral proteins to form inactive aggregates and on the other hand, PT could decrease
571 the gene expression of inflammation-related cytokines to activate the anti-inflammatory
572 signaling pathway of the viral host. However, this aggregate-forming ability and the
573 manner, through which it occurs, should be further investigated in the future.
574
575 **4.7 Detoxification effects on snake venom**
576 Snake venoms (SV), which are composed of multiple proteins such as myotoxins,
577 acetylcholinesterase, hemorrhagic metalloproteases, proteolytic enzymes, and
578 phospholipase A2 (PLA2), can produce neurotoxicity, myotoxicity, and cardiotoxicity
579 in humans and animals [107, 108]. Studies found that PLA2 was a key component of
580 the SV of the Chinese Cobra, and thus an effective detoxification strategy were

581 demonstrated by inactivating PLA2 under physiological conditions [109]. Many *in vitro*
582 studies indicated that PT has a significant protective effect against SV from *Agkistrodon*
583 *halys Pallas* and *Naja naja atra* by forming aggregates with PLA2 [110]. Moreover, PT
584 has multiple binding sites with PLA2 and induces considerable conformational changes
585 in the secondary structure of the SV protein by reducing the a-helix. This has been
586 characterized through a combination of experimental techniques, including MALDI-
587 TOF/MS, isothermaltitration calorimetry, fluorescence quenching, circular dichroism
588 (CD) and Fourier transform infrared (FT-IR) spectra techniques [111]. Meanwhile, the
589 key active sites of PLA2, covering tyrosine, histidine, lysine, and tryptophan residues,
590 were revealed and their toxicity might be limited after the combination of PT with PLA2
591 active sites [111]. The detoxifying effects of PT *in vivo* were also evaluated in mice
592 injected with snake venom and the result showed that PT could restrain PLA2 induced
593 lethality, myotoxicity, and hemolysis in mice [112]. Therefore, the dysfunction of the
594 SV protein caused by the binding of PT with active sites of PLA2 is the dominant
595 mechanism for the detoxifying effects of PT. To explore the structural contribution of
596 PT to the detoxifying effects, the role played by seven characteristic structural elements
597 in PT, myricetin, ECG, EGCG, A-type ECG dimer, A-type EGCG dimer, A-type EC
598 dimer and B-type EC dimer, on the PLA2 were explored both *in vitro* and *in vivo*. The
599 results suggested that both the A-type ECG dimer and A-type EGCG dimer had higher
600 abilities to inactivate PLA2 than the other five components and made the survival ratio
601 of mice injected with SV more than 50%, indicating that these two dimers might be the
602 structural requirements for PT to exert its detoxifying effect [112, 113]. Therefore, PT

603 has great promising to be developed as an anti-venom agent in the future.

604

605 **5. Other benefits and application**

606 Pollution caused by heavy metal and dies is widespread and has caused serious harm to
607 organisms, humans, and the environment [114, 115]. Adsorption has been viewed as an
608 efficient strategy to remove heavy metals and dyes from an aqueous solution or
609 industrial wastewater. Thus, the adsorbents with eco-friendly, low-cost, and sustainable
610 available have aroused great attention. Due to the high reactivity and abundant phenolic
611 hydroxyl groups in PT, a series of PT-derived adsorbents has been produced. These PT-
612 derived adsorbents showed outstanding adsorption ability for heavy metals from
613 contaminated waters. For example, Li et al. [116-118] prepared environment-friendly
614 bio-adsorbents by modifying PT with graphene oxide (GO) or chitosan and evaluated
615 their adsorption capacity for Au (III), Pd (II), Ag (I), and Pb (II) from aqueous solution.
616 The results showed that PT-based bio-adsorbents had excellent adsorption capacity for
617 Au (1325.09 mg/g), Pd (797.66 mg/g), Ag (421.01 mg/g), and Pb (179.30 mg/g).
618 Moreover, this adsorption process was endothermic and spontaneous along with the
619 redox reaction, electrostatic interaction, and chelation reaction. Studies also found that
620 the anchoring of the nitrogen containing groups, like methylamine, ethylenediamine,
621 and triethylenetetramine (TETA) on the surface of PT could significantly (p <0.05)
622 enhance the adsorption capacity for metal ions. For example, ethylenediamine-
623 modified PT adsorbent significantly (p <0.05) elevated the adsorption capacity for Au
624 (1550.40 mg/g) relative to that of the previously reported PT-based bio-adsorbents

[119]. Moreover, ethylenediamine-modified PT showed excellent adsorption capacity for Mo (478.02 mg/g), higher than that of methylamine-modified PT (411.65 mg/g) [120], amine-modified PT (172.00 mg/g) [121], diethylamine modified PT (294.12 mg/g) [122], and triethylamine modified PT (287.21 mg/g) [122]. In addition, another approach to remove the precious metals from wastewater was also developed by immobilizing PT onto water-insoluble matrices, such as polyacrylonitrile fiber [123], viscose fiber [124], polyurethane foam [125], and dialdehyde waste paper [126]. These results indicated that the maximum adsorption amounts of PT-based adsorbents towards Au, Cu, Pb, U, and Cr were 801.20 mg/g, 96.06 mg/g, 112.20 mg/g, 242.30 mg/g, 178.70 mg/g, respectively. In recent study, Liu et al. [127] prepared novel bio-adsorbents by introducing PT into dialdehyde cellulose nano-fibres and evaluated their adsorption capacity for U and Th from radioactive wastewater. The results showed that PT-based bio-adsorbents possessed a high adsorption capacity for U (105.7 mg/g) and Th (95.1 mg/g). Meanwhile, the removal process for heavy metals or radionuclide from wastewater was also related to the electrostatic attraction, chelation reaction, intercalation adsorption, and surface complexation.

Besides heavy metal, organic dyes used in the textile industry can seriously pollute the environment if they are discarded without pretreatment. PT-derived adsorbents also play important roles in the removal of dyes from wastewater. For example, polyethyleneimine-modified PT was tested for the application of the removal of methyl orange (MO) dye [128]. The results showed that it showed a strong potential to absorb

MO with a maximum adsorption capacity of 225.74 mg/g. Another effective PT-derived adsorbent was also prepared by the same group through crosslinking GO. It was found that the GO-modified PT not only showed excellent absorption for heavy metals, but also strong absorption for methylene blue dye from contaminated water with absorption capacity of 256.58 mg/g [129]. Mechanistic studies have shown that electrostatic interactions, π-π interactions, and hydrogen bonding dominated the adsorption of PT-derived adsorbents for dyes. Overall, PT-based adsorbents synthesized through anchoring a functional group onto the PT surface, crosslinking PT with other materials or immobilizing PT onto water-insoluble matrices has shown promise as an agent to remove heavy metals or dyes from contaminated waters, expanding the application scope of PT in eco-friendly materials.

Of note, there are some other reports about the activity of persimmon, including the antiaging activity [130, 131], anticancer activity [132], and antihypertensive activity [133]. For example, persimmon oligomeric proanthocyanidins showed excellent ability to slow the process of aging and extend the life span of senescence-accelerated mice in previous study, while the antiaging mechanism was lacking [130]. Then the protective mechanism of persimmon oligomeric proanthocyanidins against aging was investigated from insight of the protein kinase and the results indicated that the phosphorylation of vascular endothelial growth factor receptor was increased after the administration of persimmon oligomeric proanthocyanidins [131]. These data showed the promising role of oligomeric proanthocyanidins as a candidate for expanding the life span of

669 senescence-accelerated mice. In this regard, the antiaging effect of PT should be better
670 than persimmon oligomeric proanthocyanidins. Cancer is also severe health issue and
671 has not been completely solved until now. Previous study found that the extract of
672 persimmon leaves could induce human colorectal cancer cell death and restrain cell
673 proliferation, suggesting outstanding anticancer activity [134, 135]. Another research
674 confirmed that the promising role of flavonoids from persimmon leaves as bioactive
675 substances to induce cancer cell death [136]. Despite this, the content of PT in the
676 extract of persimmon leaves and anticancer mechanism remain unknown. Hypertension,
677 mainly caused by unhealthy diet, is a risk factor of cardiovascular diseases and damages
678 the blood vessels [137]. Regarding this point, regulating blood pressure by dietary
679 proanthocyanidins have attracted much attention. Numerous studies about the
680 antihypertensive effects of dietary proanthocyanidins from *in vivo* and clinical trials
681 have been summarized [138]. However, the action mechanism in molecular level is still
682 lacking. Therefore, further works should be performed in the future.
683
684 In addition, PT has some novel applications in recent research. For example, PT was
685 used as a biopolymer for the synthesis of bifunctional heterogeneous catalyst due to its
686 environmentally friendly and structurally specific nature, which also expands the
687 application of PT as a biodegradable material [139]. Another study in our group
688 prepared a novel emulsion with strong emulsifying properties by mixing PT and
689 persimmon pectin, and then explored the manner of action of PT with persimmon pectin
690 [140]. The results showed that PT under high concentration (>1 mg/mL) improved the

691 emulsifying capacity of persimmon pectin by forming a honeycomb network structure
692 and enhancing the viscoelasticity of the interfacial film. To increase the health benefits
693 of wheat flour-based foods, polyphenol compounds, especially PT, are introduced into
694 wheat-based foods. Thus, a recent study explored the interaction between PT and gluten
695 and the results showed that glutenin was more responsive to PT than gliadin, as
696 evidenced by more significant alterations in thermal stability, network structure, and
697 aggregation behavior [141]. This reason was attributed to the unique structural
698 characteristics of PT, including its high DP and strong interaction with glutenin. Overall,
699 these results not only offer guidance on how to incorporate phenolic compounds into
700 wheat flour-based products, but also broaden the application of PT in the food industry.
701

702 6. **Conclusion and outlook**

703 Multiple health benefits of PT, that have been found through a combination of *in vivo*
704 and *in vitro* studies, and the potential underlying mechanisms have been summarized
705 in the present study. The beneficial effects exerted by PT mainly involve the
706 inactivation of lipogenic and intracellular inflammatory signaling pathways, activation
707 of the fatty acid oxidation signaling pathway, regulation of gut microbiota, and highly
708 absorptive properties. However, due to the characteristics of heterogeneous, highly
709 polymeric, and highly galloylated, the complete structural elucidation of PT is still very
710 challenging, hindering the understanding of the PT structure-activity relationship;
711 further study of this is needed. Firstly, sophisticated techniques should be developed to
712 overcome the structural characterization challenges of large proanthocyanidins (DP >

20), in particular PT (mean DP = 26). Secondly, high-resolution detection techniques should be used to identify the terminal units of PT. Thirdly, the digestion and absorption mechanism of PT following oral administration should be explored in the future. This can fill the gap between the low level of PT in the intestine and the significant health benefits found through animal testing. Fourthly, extensive fundamental studies, aimed at identifying pharmacological targets as well as relevant mechanisms of PT, are anticipated. In addition, eco-friendly adsorbents based on PT have promising application in the field of heavy metals and dye removal from industrial wastewater in the future.

Declarations of interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by National Natural Science Foundation of China (32172201).

Author Contributions

Ruifeng Wang, Xin Shi, Kaikai Li, and Chunmei Li performed the literature research, analyzed the data, conceptualized information, and wrote the manuscript. Alex Bunker conducted grammar editing and language refinements for the manuscript.

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Figures

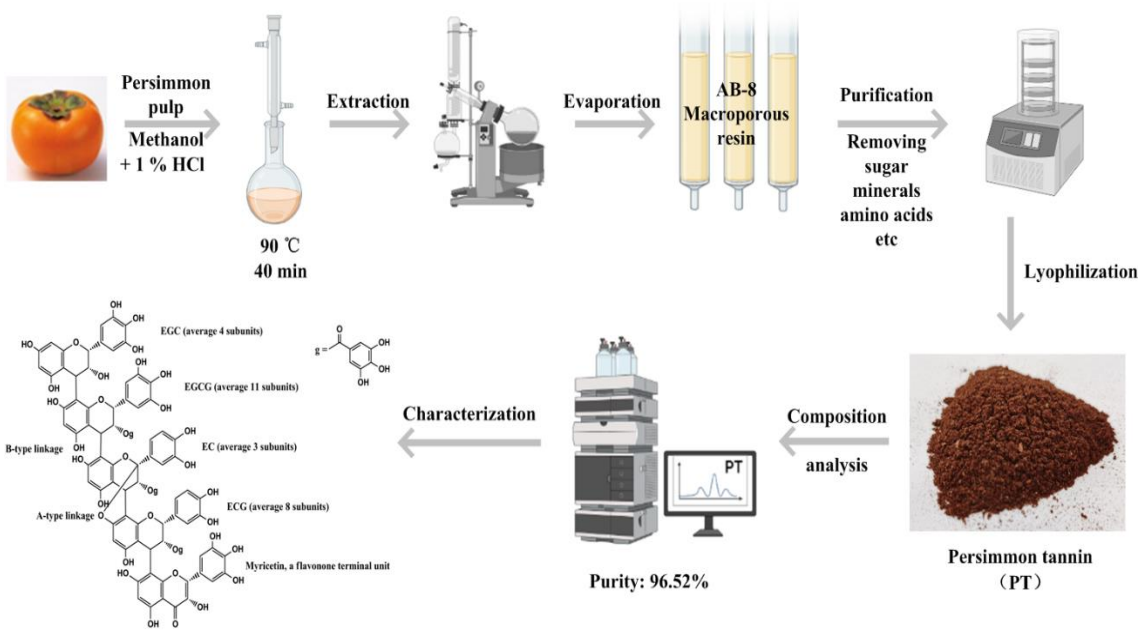

Fig. 1.

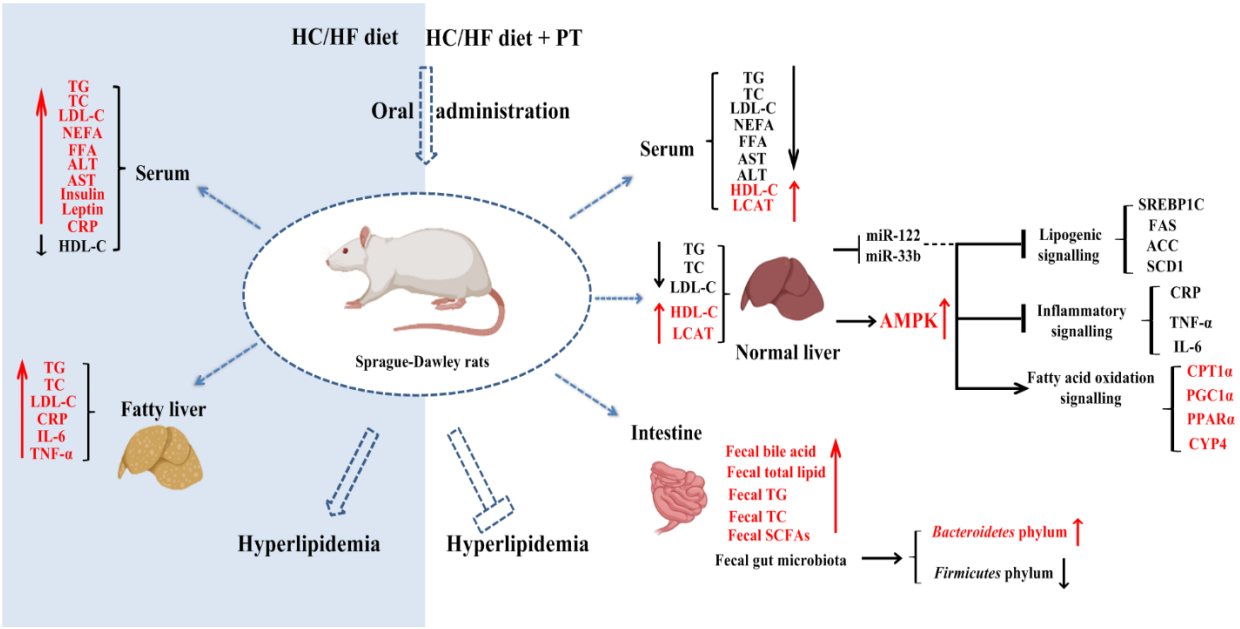

Fig. 2.

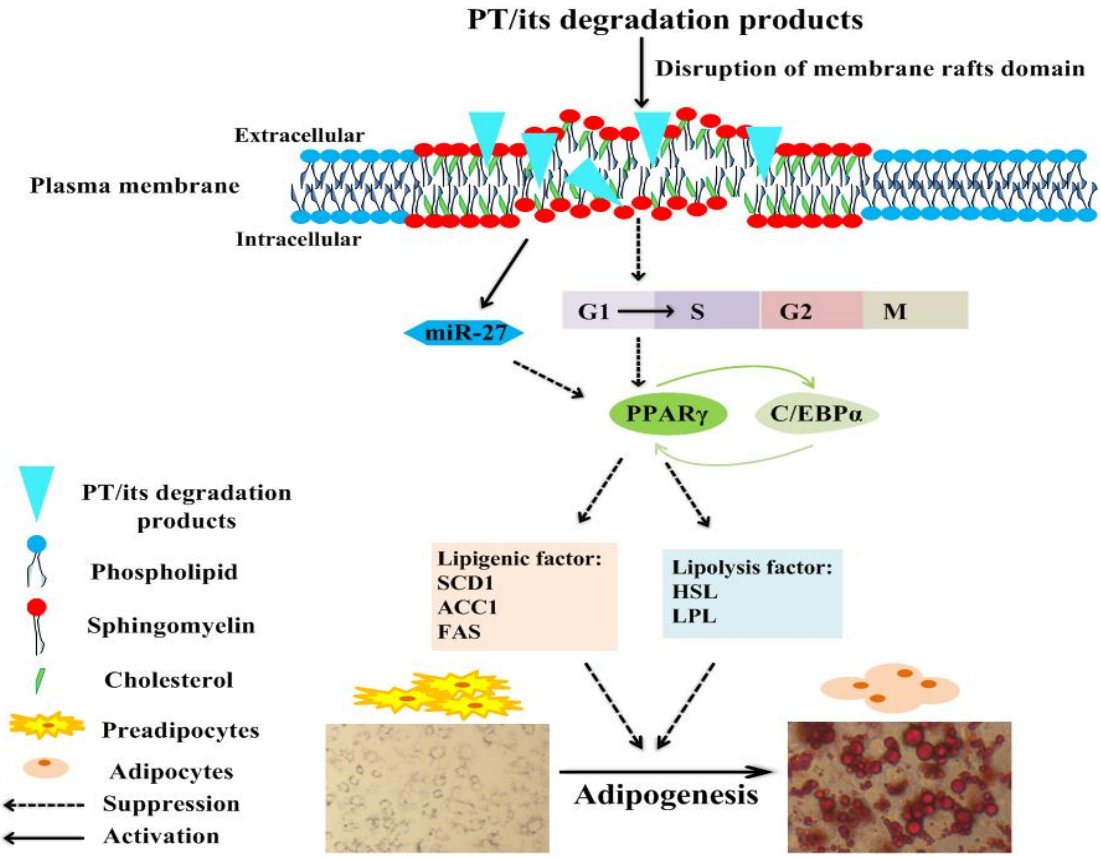

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Figure Captions

Fig. 1. Flowchart of key technologies in the isolation, extraction, purification, and characterization of PT. Potential structural formula of the PT is also shown.

Fig. 2. Underlying mechanisms of hyperlipidemia and hypolipidemic effects of PT in rats. HC, high cholesterol; HF, high fat; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; FFA, free fatty acids; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; LCAT, lecithin cholesterol acyltransferase; AMPK, AMP-activated protein kinase; SREBP1C, sterol regulatory element binding protein 1C; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; SCD1, stearoyl-coenzyme A desaturase 1; CPT1α, carnitine palmitoyltransferase-1α; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; PPARα, peroxisome proliferator-activated receptor α; CYP4, cytochrome P450 4; SCFAs, short-chain fatty acids.

Fig. 3. The signaling pathway of PT and its degradation products regulating adipogenesis in 3T3-L1 preadipocytes. PPARγ, peroxisome proliferator-activated receptor γ; C/EBPα, CCAAT/enhancer-binding proteins α; HSL, hormone sensitive lipase; LPL, lipid protein lipase.