Review

Tea Polysaccharides and Their Bioactivities

Ling-Ling Du [1,2], Qiu-Yue Fu [1], Li-Ping Xiang [2], Xin-Qiang Zheng [1], Jian-Liang Lu [1], Jian-Hui Ye [1], Qing-Sheng Li [1], Curt Anthony Polito [1] and Yue-Rong Liang [1,*]

[1] Tea Research Institute, Zhejiang University, # 866 Yuhangtang Road, Hangzhou 310058, China; 1052368529@qq.com (L.-L.D.); 21516103@zju.edu.cn (Q.-Y.F.); xqzheng@zju.edu.cn (X.-Q.Z.); jllu@zju.edu.cn (J.-L.L.); jianhuiye@zju.edu.cn (J.-H.Y.); qsli@zju.edu.cn (Q.-S.L.); curtpolito@outlook.com (C.A.P.)

[2] National Tea and Tea product Quality Supervision and Inspection Center (Guizhou), Zunyi 563100, China; gzzyzj_2009@vip.sina.com

* Correspondence: yrliang@zju.edu.cn

Abstract: Tea (*Camellia sinenesis*) is a health beneficial beverage and is also a source for extracting bioactive components such as theanine, tea polyphenols (TPP) and tea polysaccharides (TPS). TPS is a group of hetero-polysaccharides bounded with proteins. There were tests showing that TPS had various bioactivities, such as antioxidant, antitumors, antihyperglycemia, anti-inflammation and improving immunity. However, inconsistent results concerning chemical composition and bioactivity of TPS were published in recent years. The advances in chemical composition and bioactivities of TPS were reviewed in the present paper. The inconsistent and controversial results regarding composition and bioactivities of TPS were also discussed.

Keywords: *Camellia sinensis*; tea ploysaccharides; chemical composition; antioxidant; antitumors; antihyperglycemia; anti-inflammation

1. Introduction

Tea (*Camellia sinenesis*) is a beverage widely drunk in the word [1] and its extracts have been used as medicinal and dietary supplements in many countries such as China, Japan and USD [2]. Tea contains a variety of bioactive compounds including tea polyphenols (TPP) [2], theanine [3] and tea polysaccharides (TPS) [4], which contribute to the health benefits of tea. Polysaccharide is a high molecular weight (MW) polymer, consisting of at least ten monosaccharides mutually joined by glycosidic linkages. The glycosyl moiety of hemiacetal or hemiketal, together with the hydroxyl group of another sugar unit, formed the glycosidic linkages [5]. TPS is a group of heteropolysaccharides extracted from leaves, flowers and seed peels of tea plant [4]. Great advances have been made in chemical and bioactive studies of TPP or catechins and related tea products in last decades. However, TPS has received rare attention. There have been studies showing that TPS has many healthy benefits including antioxidant and anti-ageing, antitumor, inhibiting diabetes, improve immunity, alleviating hepatotoxicity, anti-skin ageing and antibacteria [6,7,8]. The preparation, chemical composition and physiological activities of TPS were reviewed in the present paper.

2. Polysaccharides in Tea

2. 1. Basic Composition of TPS

TPS is a nonstarch protein bounded acidic polysaccharide, which contains 44.2% neutral sugar, 43.1% uronic acid and 3.5% protein [9]. The carbohydrate composition of TPS includes

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glucose (128.4 μM), galactose (101.4 μM), arabinose (71.1 μM), rhamnose (47.1 μM), xylose (25.0 μM), galacturonic acid (24.0 μM), mannose (16.3 μM), ribose (10.3 μM) and glucuronic acid (5.6 μM) [10]. The second-derivative IR spectra of TPS had peak intensity around 1075 cm-1 and 1045 cm-1, showing TPS was characterized galactopyranose in the backbone and arabinofuranose units in side branches [11].

TPP is a group of abundant bioactive components in tea and crude TPS usually contains partial TPP. The carbohydrate, protein and polyphenols are conjugated with each other in the crude TPS. The composition of crude TPS varies with processing methods including extraction and drying [12]. Crude TPS1 and TPS2 were obtained when water extracts of green tea were precipitated using 40% and 70% ethanol, respectively. The TPS1 could be further separated on gel permeation into homogenous water soluble TPS1-2a and TPS1-2b, which were homogalacturonan (HG) pectins with MW *ca.* 20 kDa, consisting of a backbone of 1,4-linked α-D-galacturonic acid (GalA) residues with 28.4% and 26.1% of carboxyl groups as methyl ester, respectively [13]. The TPS1-2a and TPS1-2b gave a higher phagocytic effect than TPS2.

TPS can be divided into neutral polysaccharides (NTPS) and acid polysaccharides (ATPS). The crude water soluble TPS could be separated by anion-exchange chromatography into five fractions, i.e., fractions A, B, C, D and E, among which fractions A and C had significant glucokinase-stimulating activity, in which fraction C showed the highest activity and could be further separated by gel filtration chromatography into fractions C-1 and C-2. The FC-1 is an acidic polysaccharide containing 8% galacturonic acid but no protein, with MW *ca.* 60 kDa. [14].

Sugars and uronic acids are abundant in TPS. NTPS contains 82.7% total sugar among which 12.9% was uronic acids, but ATPS contains 85.5% total sugar among which 39.8% was uronic acids. Sugar composition was mainly galactose (67.6%) in NTPS, but rhamnose, arabinose, galactose and galacturonic acid in ATPS [15]. Nucleic acid was also detected in ATPS [16]. TPS from some tea sources was coordinated with rare earth elements (REE) including La, Ce, and Nd, in which La was more than 75% of total REE. Iron, magnesium, zinc and selenium were also detected in TPS [17].

2. 2. TPS Variation Between Tea Cultivars and Plant Organs

TPS in leaf cuticular membrane varies with tea cultivar and cell partitions. Tea cultivar 'Gokou' has markedly higher TPS than cultivars 'Samidori' and 'Yabukita'. Among various cell partitions, adaxial side usually has higher level of TPS than abaxial side [18]. Tea leaf TPS (TLPS) is increased with maturity of the tea leaf, with 0.23% in the first leaf and 0.58% in the sixth leaf below apex bud on the same tea shoot [19]. Tea flowers contained 5.24% TPS, which was higher than tea leaves (3.64%) [20]. Three kinds of TPS were extracted from tea seeds and the tea seed TPSs (TSPS) had MW 500 kDa, 130 kDa, and 5 kDa respectively, and they showed typical characteristics of polysaccharides and protein. TSPS is mainly consisted of rhamnose, xylose, arabinose, glucose and galactose, glucuronic acid (GulA) and GalA, with a molar ratio of 4.9 : 1.7 : 11.1 : 27.2 : 14.0 : 3.4 : 1. The sugar backbone of TSPS might be consisted of glucose, but branched chain may be consisted of rhamnose, xylose, arabinose, and galactose [21]. Tea fruit peel TPS (TFPPS) contained 4.98% of polysaccharides and it was a group of acid protein-bound hetero-polysaccharides. The major sugars in TFPPS were rhamnose, mannose, glucose, galactose, arabinose, xylose and fucose [22]. Polysaccharides extracted from a hawk mature leaf tea (a herbal tea) (HMPS) were mainly composed of arabinose, galactose, glucose and mannose and the HMPS can be classified into two fractions, i.e., HMPS-1 with MW 133 kDa and HMPS-2 with MW 100 kDa [23].

2.3. Effect of Tea Processing on TPS

Teas can be classified into green tea, black tea, oolong tea and pu-erh tea owing to

different processing methods [2]. As early as 1998, two kinds of green tea TPS (GTPS) were separated from green tea infusion, i.e., GTPS-A with MW over 100 kDa and GTPS-B with MW 10 kDa [24]. Crude GTPS was a conjugate consisted of a polysaccharide part and a protein part [25]. GTPSs from four green tea sources such as 'Xihu Longjing', 'Anxi Tieguanyin', 'Chawentianxia' and 'Huizhoulucha' contained 36.06-38.71% neutural sugar, 31.76-37.99% acid sugar, 4.60-8.51% protein and 6.53-9.65% TPP [26]. Black tea TPS (BTPS) was protein bounded polysaccharides [27]. The MW distribution of TPS varied with teas used to prepare TPS, ranging from 9.2 kDa to 251.5 kDa for GTPS, from 5.3 kDa to 100.9 kDa for oolong tea TPS (OTPS) and from 3.8 kDa to 32.7 kDa for BTPS [28]. Based on dry tea weight, OTPS content (4.6±0.2 %) was higher than GTPS (4.0±0.3 %) and BTPS (4.2±0.3 %) [28]. Content of pu-erh tea TPS (PTPS) was 1.21% [29]. Crude PTPS could be separated into PTPS-1 and PTPS-2 by DEAE-52 and Sephadex G-150 column chromatography. PTPS-1 contained lower content of uronic acid, but higher contents of neutral sugar and protein than PTPS-2. The average molecular weight of PTPS-1 and PTPS-2 was 16.8 kDa and 12.1 kDa respectively [30]. PTPS was acid hetero-polysaccharides bound with proteins and its content was increased with extension of pu-erh tea fermentation [7].

Chemical compositions of TPS are changed with tea materials. The ratio of protein, uronic acid and neutral sugar was 32.6% : 20.8% : 27.3% for GTPS; 32.7% : 25.4% : 26.5% for OTPS; 38.0% :16.1% :18.8% for BTPS [28] and (4.2-19.7)% :(32.6-40.4)% :(15.3-20.2)% for PTPS [7]. The molar ratio of neutral monosaccharides D-rhamnose: L-arabinose: D-xylose: D-mannose: D-galactose: D-glucose in GTPS was 7.8 :41.8 :7.1 :7.3 :18.7 :17.0. OTPS and BTPS contained no D-xylose and D-mannose and the molar ratio of neutral monosaccharides D-rhamnose: L-arabinose: D-galactose: D-glucose was 16.2 :43.7 :18.0 :21.9 for OTPS and 14.4 :36.4 :19.7 :29.4 for BTPS [28]. PTPS-1 and PTPS-2 were composed of L-arabinose, D-galactose, D-glucose, D-rhamnose, D-xylose and D-mannose with molar ratios of 24.2 : 23.6 : 5.9 : 3.2 : 1.8 : 1.1 and 19.3 : 26.9 : 3.2 : 2.7 : 1.3 : 5.5, respectively [30].

2. 4. Effect of Preparation Methods on TPS

TPS is usually extracted from tea leaves using hot water, then precipitated using ethanol of various concentrations and finally purified by chromatography. The optimal conditions for extracting TPS from green tea leaf of 'Anjibaicha' were 22.53 L water per kg tea leaf, extracted at 76.79 ◦C for 2.48 h [31]. However, the optimum conditions for extracting individual components of TPS were differentiated. Microwave heating to 170 °C was beneficial to solubilization of L-arabinose and D-galactose whereas heating above 200 °C was necessary to solubilize D-xylose [32]. Enzymatic treatments will induce bioconversion of bioactive components, which can improve biological activities of TPS. Simultaneous processing with tannase and Rapidase could improve the extraction of TPS and biotransformation of catechins with enhanced radical scavenging activity from GTPS [33]. Extrusion treatment of tea can change the monosaccharide composition, MW distribution, thermal properties and the morphological properties of TPS, resulting in improvement of yield and antioxidant property of TPS. Extrusion treatment could also increase the extraction yield of TPS from 1.26% to 6.14% [34]. Supercritical CO2 extraction can improve the yield and bioactivity of TPS and the optimum conditions for supercritical CO2 extracting TPS from tea leaf were：leaf particle size 380μm, 20% ethanol, extracting pressure 35 Mpa, extracting temperature 45°C and extracting time 2 h, in which 92.5% of tea leaf TPS could be extracted [35]. Reverse micelles extraction technology has the advantages of high selectivity, fast mass transfer and relatively low cost, it can be used in extraction of bioactives from plant materials. Sodium di-2-ethylhexyl sulfosuccinate (AOT) is extensively used as surfactant to form an AOT/heptane reverse micellar system in which TPS can be extracted. About 34% of forward recovery and nearly 100% of backward recovery of TPS were achieved under optimal conditions in the AOT/heptane reverse micellar system [36].

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The crude TPS extracted from tea leaf using hot water could be isolated by absorbent chromatography and ion exchange chromatography into three fractions, which were heteropolysaccharides bounded with protein. The monosaccharides were differentiated between various fractions. Fraction-1 was composed of L-arabinose, D-ribose, D-xylose, D-glucose, D-galactose and D-mannose, with MW 268 kDa and 2.8% protein; fraction-2 was composed of L-arabinose, D-ribose, D-glucose and D-mannose, with MW 118 kDa and 3.8% protein while fraction-3 contained no D-mannose, with MW 42.0 kDa and 4.0% protein, among which fraction-1 showed the highest antioxidant activities [37].

The cruse TPS from tea seed could also be purified by chromatography on macroporous resins AB-8 column, in which water soluble impurities were washed using deionized water, pigments were removed using 0.25% NaOH solution, and tea seed saponin was eluted using 90% ethanol. 18.7 g of TPS with 89.2% purity could be isolated from 100 g tea seed [38].

Drying methods had significant influence on yield and composition of TPS. Vacuum drying gave the highest TPS yield, with 418 mg per kg green tea (418 mg/kg), and spray-drying gave the lowest yield (106 mg/kg), with freeze drying (403 mg/kg) and microwave-vacuum drying (383 mg/kg) in between. Though total sugar contents were not significantly different between products obtained by various drying methods (ranging from 41.08±0.799 % to 42.71±0.799 % by dry weight). Contents of protein, TPP and glucose were the highest in TPS obtained by vacuum drying, and contents of rhamnose, ribose, arabinose, galactose and galactose acid were the highest in TPS obtained by freeze drying, while contents of glucose, xylose, galactose, mannose, galactose acid and glucose acid were the lowest in TPS by spray drying and content of ribose was the lowest in TPS by microwave-vacuum drying [12].

Sulfation of TPS can improve hypoglycemic activity. Sulfated NTPS and ATPS could be synthesized by pyridine-sulfonic acid method [15]. Furthermore, thermal treatments, such as incubation at 98 °C for 1 h or above, will improve the stability and antioxidant activity of ATPS [16].

3. Bioactivities of TPS

3.1. Bioavailability and Toxicity of TPS

An *in vitro* test on dendritic cells (DCs) showed that the cell viability showed no significant difference between TPS-treated cells at concentrations 0.2-200 μg/ml and media-tread cells (RPMI media 1640, Gibco, BRL), and TPS did not induce any apoptosis in DCs, showing TPS can be used for a long period without cytotoxicity [39]. *In vivo* test by oral administration of TPS (5.0 g/kg BW) in mice showed that TPS had no toxicity to the liver, kidney, heart, thymus, or spleen of the mice and none of the mice died throughout the fifteen days of experiment. There was no significant difference in the thymus index, spleen index, and liver index of the mice between the test and control groups ($P > 0.05$) [9]. Based on the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) and OECD (Organization for Economic Co-operation and Development) Test Guideline 420 (fixed dose procedure), TPS was classified as GHS Category 5 [40]. Therefore, TPS can be classified as a very low toxicity substance which can be used as a candidate of dietary supplements and as additive used in food processing [9,41,42].

Test showed that TPS is orally ingested and will reach the gastrointestinal tract before performing a biological function [43]. TPS with small MW is beneficial to improvement of bioactivities [37]. TPS can form a TPS-iron complex (TPSIC) in a reaction system containing TPS and FeCl3 (1: 2.4, by weight) and reacting at 60 °C for 3 h. The obtained TPSIC contained 14.60 % iron and its bioavailability ranged from 101.85% to 116 % in human. Therefore, the TPSIC is considered to be a good iron supplement source for increasing uptake and bioavailability in the body [44].

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3.2. Alleviating Oxidative Stress

TPS is a group of hetero-polysaccharides bounded with proteins which can alleviate oxidative stress. The antioxidant activities of TPS vary with its molecular size. When ATPS was separated by chromatography into three fractions with different molecular sizes, the fraction-1 with MW 268 kDa had stronger antioxidant activity than the fraction-2 with M.W. 118 kDa and fraction-3 with MW 42 kDa. The ability to scavenge hydroxyl radicals and superoxide radicals is related to uronic acids level in TPS. The higher the uronic acid level, the stronger the ability to scavenge hydroxyl and superoxide radicals [37]. The 50% inhibitory concentration (IC50) of TPS extracted from 'Anji Baicha' tea leaf was 83.25 μg/mL on superoxide radical and 1.69 μg/mL on hydroxyl radical [31]. However, *in vivo* test in gastric cancer mice showed that TPS fraction with small MW showed stronger promoting effect on stomach antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [45]. When exhausting training mice were orally administrated by TPS (100-300 mg/kg body weight) for 30 days, SOD, CAT, GSH-Px activities in blood, liver and heart were significantly increased, whereas the MDA level in plasma, lever and heart were reduced, compared to control mice [46].

The monosaccharide composition and molecular size range of TPS change with plant materials, resulting in difference in antioxidant activity. TPS containing mannose extracted from tea leaf and tea flower had higher antioxidant activity than that extracted from tea seed [47]. Tea flower TPS (TFPS) containing high level of sulfate and complicated monosaccharide composition had strong antioxidant activity by enhancing the activities of SOD and GSH-Px in carbon tetrachloride (CCl4)-induced liver injury mice and reducing the formation of malondialdehyde (MDA) [48].

TPS composition varies with tea materials and their places of origin, leading to difference in antioxidant activity. Test using TPS products extracted from unfermented green tea (GTPS), semi-fermented oolong tea (OTPS) and fully fermented black tea (BTPS) revealed that BTPS showed the highest antioxidant activities on hydroxyl radicals and DPPH radicals, and OTPS the least, with GTPS in between [28]. TPS extracted from green tea 'Huizhoulvcha' produced in Anhui Province exhibited significant higher superoxide anion scavenging activity than that extracted from green tea 'Xihulongjing' produced in Zhejiang Province in China [26]. Oolong tea fermentation enhanced the conjugation between TPS and protein, and so the OTPS extracted from deeply fermented oolong tea showed increased antioxidant activity [8].

Preparation methods affect the TPS composition, resulting in differentiation in antioxidant activity. When tea fruit peel was used as material to extract tea fruit peel TPS (TFPPS), the fraction extracted in hot water contained high level of uronic acid and showed stronger ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] antioxidant activity but weaker FRAP (ferric-reducing antioxidant power) antioxidant activity than that extracted in ethanol [22]. When crude TPS was separated by stepwise ethanol precipitations, the TPS-I obtained by precipitation in 30% ethanol contained high level of sulfuric radical and low level of uronic acid showed lower scavenging activities on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical, superoxide anion radical and hydroxyl radicals than the TPS-II prepared using the supernatant which had less sulfuric radical but higher level of uronic acid [49]. The free radical scavenging activity of TPS was also influenced by drying method. Freeze-dried TPS exhibited strong activity of metal chelating and superoxide radicals scavenging, while vacuum-dried TPS showed high activity of inhibiting α-glycosidase and α-amylase [12].

Free radical scavenging activity of TPS depended on its concentration. TPS showed lower DPPH scavenging activities than vitamin C at low concentrations such as 25-200 g/mL, but exhibited similar DPPH scavenging activity with vitamin C at high concentrations such as 200-400 g/mL [26].

There was synergistic interaction between TPS and other bioactive tea components.

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Epigallocatechin gallate (EGCG) caused a synergistic increase in the antioxidant activity of TPS. Low concentration of EGCG (6.15-8.0 μg/ml) significantly enhanced DPPH radical scavenging potential and reducing power of TPS [50]. There was also synergistic interaction between TPS and polysaccharides from *Pyracantha fortuneana* (PFPS). *In vivo* test on Kunming mice showed that combined oral administration of Se-enriched TPS and PFPS significantly enhanced the activities of GSH-Px and SOD, but remarkably decreased MDA level, compared to individual TPS or PFPS alone [51].

3.3. Antitumor

Many *in vitro* tests revealed that TPS showed antitumor potential. TSPS significantly inhibited the growth of human immortalised myelogenous leukemia cell K562 at concentration 50 μg/ml, with an inhibition ratio 38.44 ± 2.22% ($P< 0.01$) [21]. When the TSPS was further separated into NTPS, ATPS-1 and ATSPS-2, they showed inhibitory effects on K562 cells in dose dependant manner, with inhibition ratios of 30.13 ± 3.54% for NTPS, 36.61 ± 2.75% for ATPS-1 and 32.33 ± 2.53% for ATPS-2 at 400 μg/mL respectively [52]. TPS from Se-enriched 'Ziyang' green tea significantly inhibited the proliferation of human osteosarcoma U-2 OS cancer cells in a dose dependent manner at 25- 200 μg/ml [53]. TFPS with high level sulfate and complicated monosaccharide composition showed strong inhibitory activity on growth of human gastric cancer BGC-823 cells [48]. These experiments suggest TPS will be a potential candidate to be used as natural antitumor drugs.

The antitumor activity of TPS was also confirmed by in vivo tests. In vivo test on U-2 OS cancer xenograft model BALB/c athymic mice showed that oral administration of TPS at doses of 100- 400 mg/kg BW daily for 28 consecutive days resulted in obvious tumor regression as compared to model control [53]. The growth of transplanted sarcoma 180 tumor (S180) on S80-bearing mice was inhibited by oral administration of TFPS (75-300mg/kg BW) for 10 day [54]. TPS could inhibit the growth of H22 transplantable hepatocarcinoma (HCC) tumor in mice [55]. Test on Wistar rats with H22 HCC cells confirmed that oral administration of TPS (100, 200 and 300 mg/kg BW, once a day for 40 consecutive days) inhibited tumour growth and decrease microvessel density in tumor tissue. The altered amount of serum white blood cells (WBC), interferon-gamma (IFN-γ) and tumor necrosis factor-α (TNF-α) in HCC animals were dose-dependently increased, whereas activities of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were dose-dependently decreased in the drug treated animals. The suppressive effect of TPS on tumor growth is considered to be related to its inhibiting the expression of vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA) in H22 tumor tissue [56].

3.4. Anti-hyperglycemia

In vivo test showed that TPS had inhibitory effect on blood glucose (BG) increase and diabetes mellitus (DM). When 7-week old C57BL/8 mice were injected with TPS with MW 107 kDa -110 kDa, the BG levels in normal mice and model mice with high BG were significantly decreased by 13.54% and 22.18%, respectively [19]. Four-week oral administration of PTPS (40 mg/kg BW daily) could significantly lower the blood glucose levels in alloxan-induced diabetic mice, accompanying with improvement of activities of SOD and GSH-Px as well MDA level both in serum and liver [57]. Oral administration of GTPS (200 and 400 mg/kg BW daily) for 6 consecutive days could also suppress BG increase in alloxan-induced mice [25].

DM is an endocrine disorder caused by inherited and/or acquired deficiency in the amount of insulin from the pancreas, or by the defects in insulin action. Glucokinase is the first enzyme in glycolysis and glycogenesis, also a key enzyme in diabetes management and so it serves as a signal to both the b-cells and the liver that glucose levels in the blood are high. Glucokinase plays a role in promoting insulin secretion and reducing glucose production by

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the liver. Glucokinase facilitates phosphorylation of glucose to glucose-6-phosphate, which is regulated by insulin. Glucokinase influences glucose uptake by liver. Increase in glucokinase activity is beneficial to alleviating the symptoms of diabetes. TPS had elements related to reducing blood sugar (ERBS) whose percentage ranged from 0.03% to 9.57% [58]. The bioactivities of OTPS were proportional to its contents of protein and uronic acid [8]. The protein and uronic acid in TPS had inhibitory effect on α-glucosidase activities and had potential for prevention of type 2 diabetes (T2D) [10]. Pu-erh tea extracts containing TPS had beneficial effects on glucose homeostasis in T2D and in amendment of insulin resistance [29]. Purified ATPS significantly stimulated glucokinase activity, resulting in reduction of blood glucose level and suppression of MD [14].

Dysfunction of the vascular endothelium contributes to the etiology of diabetic micro- and macro-angiopathy [59]. Excessive increase in intra cellular glucose induces serious loss of vascular endothelial cells [60] and accelerates the occurrence of atherosclerosis in DM patients [61]. Exposure of human umbilical vein endothelial (HUVE) cells to HG (33 mM) for 12 h significantly decreased cell viability, compared to normal glucose control. As compared with cell injury group, GTPS exhibited remarkably protective effects on HUVE cells against impairments induced by HG in a dose-dependent manner [62].

α-Amylase and α-glucosidase are key enzymes to digest starch in mammals [63]. Inhibition of starch digestive enzymes or glucose transporters can suppress postprandial hyperglycemia by reducing the rate of glucose release and absorption in the small intestine [64]. TPS improved the impaired glucose tolerance (IGT) from developing into DM through its inhibiting digestive enzymes [65]. BTPS at 25-200 μg/ml suppressed α-glucosidase activity in concentration dependent manner [28]. TFPS could also inhibit the activity of α-amylase and α-glucosidase *in vitro*. The possible mechanism for TFPS protecting against rapid blood glucose rise in alloxan-induced Sprague-Dawley (SD) rats was that TFPS donated hydrogen to protect SD rats from oxidative damage and inhibited digestive enzymes activities [66]. PTPS decreased blood sugar by inhibiting α-glucosidase activity *in vitro*, with IC50= 0.438 - 2.192 μg/ml [67].

Type 1 diabetes (T1D) is an autoimmune disorder induced by dysregulation of the immune system. During development of functional regulatory T cell (Treg), interleukin 2 (IL-2) is a necessary second signal after T cell antigen receptor (TCR) signaling that upregulates Tregs CD25 and Foxp3. IL-2 not only may cause proliferation of Tregs, but also compensate for a genetic defect associated with T1D [68]. IL-1 has a major role in inflammation. The blockade of IL-1 activity (especially IL-1β) is a standard therapy for patients with autoimmune diseases [69]. TPS treatment promoted production of IL-2 in spleen cells but suppressed production of IL-1 in adjuvant arthritis rats *in vivo* [19]. The hypoglycemic mechanism of TPS is also considered to be involved in its regulation of the PI3K/Akt signal pathway because TPS was found to upregulate the expressions of PI3Kp85/p-Akt/GLUT4 in T2D mice [70].

Anti-glutamic acid decarboxylase (anti-GAD) antibody is considered to be an important marker for T1D [71]. Daily oral administration of 150 mg/kg green tea water-soluble TPS and alkali-soluble TPS suppressed spontaneous DM in non-obese diabetic (NOD) mice by decreasing the levels of anti-GAD antibody and blood glucose [72]. The hypoglycemic activity of TPS can be further improved by molecular modification such as sulfation [15].

3.6. Improving Immunity

TPS can improve immunity by enhancing the activity of immunocytes such as splenocytes. Splenocytes consist of a variety of cell populations such as lymphocytes, DCs and macrophages, which have different immune functions. TPS significantly improved the splenocyte proliferation induced by concanavalin A (ConA) or lipopolysaccharide (LPS), and notably enhanced the macrophage phagocytosis towards neutral red [55]. TPS promoted both

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phenotypic and functional maturation of murine bone marrow-derived DCs, achieving potentiation of immune responses to alleviate the diseases [39]. TPS promoted the phagocytic activity of monocyte-macrophage system, resulting in enhancement of self-protection activity and it increased phagocytosis through toll-like receptor 7 [73].

Cytokine is a group of proteins with small MW released by cells and they have specific effects on the interactions and communications between cells, or on the behavior of cells. The cytokines includes the interleukins (IL), lymphokines and cell signal molecules, such as tumor necrosis factor (TNF) and the interferons, which trigger inflammation and respond to infections. *In vivo* test on Kunming mice showed that oral administration of TPS could significantly decrease the level of pro-inflammatory cytokines such as TNF-α, but could increase the level of anti-inflammatory cytokines such as serum immunoglobulin A (IgA), IgG, IgM, IL-2, IL-4, IL-10 [45] as well as IL-6 which plays an important role in T cell activation [33]. Oral administration of TFPS could also improve the percentages of T-lymphocyte subsets CD4+and CD4+/ CD8+[54]. The effect of TPS on immune stimulation was superior to that of TPP to some extent [55]. Therefore, TPS can be used as an immunopotentiator.

However, the immunological activities of TPS were differentiated between various sources. It was showed that strictinin and catechin were important for the immunomodulating activity of TPS. The TPS from immature leaves had higher immunostimulating activity than that from mature leaves [74]. ATPS showed stronger immunological activity than NTPS at concentrations 0.5- 400 g/mL. The detail mechanisms of immunological activity of TPS have not been clear [52].

3.7. Anti-hepatotoxicity

TPS plays a role in anti-hepatotoxicity through ameliorating hepatic oxidative injury [6] and improving metabolic syndrome [27]. Oral administration of TFPS for 28 consecutive days protected liver from lipid peroxidation induced by bromobenzene in mice through increasing SOD activity, resulting in reduction of MDA in dose-dependent manner [75]. *In vivo* test on exhausting training mice showed that oral administration of TPS (100, 200 and 300 mg/kg BW) for 30 days increased the activities of SOD, catalase (CAT), GHS-Px and reduced MDA level in plasma, liver and heart [46].

Carbon tetrachloride (CCl4) induced hepatotoxicity accompanying with increase in serum alanine transaminase (ALT), aspartate transaminase (AST), triglycerides (TG), cholesterol (TC), hepatic MDA and 8-iso-PGF2α (8-iso-prostaglandin F2 alpha). Administration of GTPS or BTPS (200, 400 and 800 mg/kg BW) in mice ahead of CCl4 injection could antagonize the CCl4-induced increases in levels of ALT, AST, TG, TC, hepatic MDA and 8-iso-PGF2α. The TPS-treated mice displayed a better profile of hepatosomatic index and improved GSH-Px and SOD activities. These protective effects can be attributed to that TPS enhanced the effects on enzymatic and non-enzymatic antioxidants and restrained lipid peroxidation in liver tissue [27,48,76].

Nitric oxide (NO) is a free radical which can be produced by nitric oxide synthase (NOS) in the body. There are three NOS isoforms identified in the body, i.e., endothelial nitric oxide synthase (eNOS), neural nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS). The iNOS is inducible in response to various stimuli, such as LPS which can activate Toll-like receptor 4 (TLR4) signal pathway [77]. Test showed that PTPS suppressed the increase in level of LPS-induced NO in SD rats by inhibiting iNOS expression through reducing TLR4 signaling [78].

3.8. Anti-skin Aging

In vitro test on senescent human diploid fibroblast (HDF) showed that PTPS promoted proliferation of HDF significantly and the anti-aging effect of TPS on HDF was stronger than vitamin C and TPP [79]. The abilities of TPS and TPP to protect the skin were assessed from

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four aspects, i.e., moisture absorption and retention, sunscreen, promoting the proliferation of fibroblast cells, and tyrosinase inhibitory ability. Purified TPS had better moisture absorption and retention abilities than TPP. TPP protected skin against the sun's ultraviolet (UV) radiation, enhanced proliferation of fibroblast cells and had inhibitory effect on tyrosinase, whereas purified TPS hardly protected the skin from UV rays and showed weak ability to inhibit tyrosinase. TPS and TPP had complementary advantages and they should be appropriately combined to achieve higher performance when applied as active components in cosmetics [80]. A 6-month double blind, placebo controlled, randomized study on healthy post-menopausal females showed that a dietary supplement containing white tea extract and fish protein polysaccharides provided improved condition, structure and firmness of the skin in the post-menopausal women, showing improvement of forehead, periocular and perioral wrinkles, mottled pigmentation, laxity, sagging, under eye dark circles and overall appearance [81].

3.9. Anti-infection of Pathogenic Bacteria

The adhesion of the pathogen to host cells is one of the first steps during bacterial infection. Anti-adhesion therapy is an efficient way to prevent or treat bacterial infections. TPS showed selectively strong inhibition on bacteria-host adhesion. ATPS with a MW 80 kDa showed marked anti-adhesive effects against pathogenic bacteria such as *Helicobacter pylori, Propionibacterium acnes*, and *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) between 0.01 and 0.1 mg/mL, which was lower than polysaccharides from *Panax ginseng* and *Artemisia capillaries* [82]. A TPS like green tea extract containing 40% uronic acids, but lack of catechins showed strong inhibitory effects on the adhesion of some pathogens to host cells, with IC50 (50% inhibition of adhesion) values being 0.14-2.3 mg/mL for pathogens *H. pylori, P. acnes* and *S. aureus*. The TPS exhibited the highest activity against *P. acnes*, but no inhibition against *Lactobacillus acidophilus, Bifidobacterium bifidum, Escherichia coli,* or *Staphylococcus epidermidis*, suggesting TPS exerted a selective anti-adhesive effect against certain pathogenic bacteria with no adverse effects against some commensal bacteria [83].

The detailed mechanisms for TPS interfering bacteria-host adhesion remain to be investigated. The negatively charged groups may perform a crucial role in the process of bacteria-host adhesion. TPS and pectin have a similar carbohydrate composition, in which uronic acids are abundant. However, pectin alone did not show any significant activity on the bacteria-host adhesion in a concentration-dependent manner. Some carbohydrate components of TPS other than uronic acid might play a role in the observed inhibition of host-bacterial adhesion [84].

4. Inconsistent Results

Although *in vitro* and *in vivo* tests showed that TPS exhibited many bioactivities, there were inconsistent or controversial results published in this research area.

First, unstable chemical composition of TPS. Crude TPS could be separated into 2 fractions [13], 3 fractions [37] or 5 fractions [14] according to isolation method and materials used, resulting in variation in chemical composition and bioactivities. Content of major component uronic acids in TPS varied from 1.95-7.90% [49] to 45.89-63.11% [8]. Content of protein changed from 1.5-2.9% [85] to 32.6-38.0% [28]. TPS MW distributed from 1-800 kDa [26] to 10-2640 kDa [8]. Because the composition varied greatly, the extraction yield of TPS changed vastly from 0.23-0.58% [19] to 4.0-4.6% by dry weight [28].

Second, controversial antioxidant activities. Crude TPS usually contained TPP and so showed good antioxidant activities [12]. However, purified TPS fractions free from TPP hardly exhibited antioxidant activities, which were similar to that of dextrans. TPS as food antioxidants was considered to be an old woman's tale [86]. Furthermore, inconsistent results of antioxidant activities of TPS came from experiments on TPSs extracted using different

kinds of teas with various degrees of fermentation. Early test showed that BTPS from fully fermented black tea had the highest antioxidant activities on both hydroxyl radicals and DPPH radicals and OTPS from semi-fermented oolong tea the least, with GTPS from unfermented green tea in between [28]. Fermentation of oolong tea increased the conjugation between TPS and protein, leading to the increased antioxidant activity [8]. However, the late experiment showed that TPS from less intensively fermented tea had higher antioxidant activity than those from more deeply fermented teas [49].

There was a causal relationship between the unstable chemical composition and the inconsistent results of antioxidant activities of TPS, in which the former might be the cause and the latter be the consequence. Purified TPS without contaminants should be obtained before it can be used in validation test or as functional food additives. Difference in preparation methods was an important factor leading to variation in chemical composition of final TPS products. It is necessary to establish a set of effectively standardized method to purify TPS for scientific research and industrial use in medicinal and functional food areas.

5. Conclusion

TPS is a group of bioactive components in tea. Crude TPS was usually prepared by extracting tea leaf (or flower, fruit peel) in hot-water and then precipitating in ethanol solution at different concentrations. The crude TPS was further purified on chromatography, such as gel filtration chromatography, ion-exchange chromatography or affinity chromatography. TPS is mostly glycoconjugates in which a protein carries one or more carbohydrate chains covalently attached to a polypeptide backbone.TPS also is typically heteropolysaccharides in which uronic acids are abundant.

TPS has many bioactive activities, including relieving oxidative stress by enhancing endogenous antioxidant enzymes or directly scavenging free radicals; antitumor activity by suppressing the expression of VEGF and TNF and inhibiting tumor cell proliferation; anti-hyperglycemic activity by increasing IL-2 production and inhibiting starch digestive enzymes, IL-2 and anti-GAD antibody; improving immune activity by enhancing immunocyte activity, increasing the level of anti-inflammatory cytokines such as IgA, IgG, IgM, IL-2, IL-4, IL-10 but decreasing pro-inflammatory cytokines such as TNF-α AND IL-6; anti-hepatotoxicity by increasing enzymatic and non-enzymatic antioxidants and inhibiting iNOS expression via reducing TLR4 signaling; anti-skin ageing by increasing moisture absorption and retention abilities; anti-infection of bacteria by interfering bacteria-host adhesion. Furthermore, TPS plays a role in weight control by downregulating the genes related to fatty metabolism, such as gene Lpin2 in the pathway of triacylglycerol biosynthesis [87] (Figure 1).

Difference in preparation methods is considered to be important factor leading to variation in chemical composition and antioxidant activities of TPS. A set of effective standardized method for purifying TPS should be established so as to obtain purified TPS products for validation test or use as medicinal and food additives.

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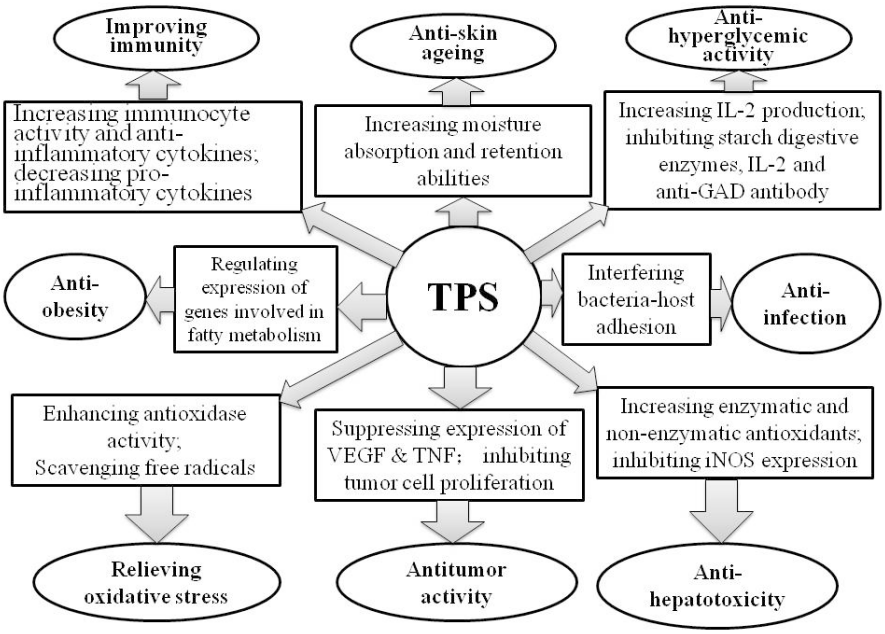

Figure 1. Bioactivities of TPS

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviation List

Abbreviation	Full name	Abbreviation	Full name
ABTS	2,2'-Azinobis(3-ethylbenzothiazoline -6-sulfonic acid) diammonium salt	IGT	Impaired glucose tolerance
ALP	Alkaline phosphatase	IL	Interleukin
ALT	Alanine transaminase	iNOS	Inducible nitric oxide synthase
AOT	Sodium di-2-ethylhexyl sulfosuccinate	LPS	Lipopolysaccharide
AST	Aspartate transaminase	MDA	Malondialdehyde
ATPS	Acid tea polysaccharides	MIC	Minimum inhibitory concentration
ATSPS	Acid tea seed polysaccharides	MW	Molecular weight
BG	Blood glucose	nNOS	Neural nitric oxide synthase
BTPS	Black tea polysaccharides	NOD	Non-obese diabetic
BW	Body weight	NOS	Nitric oxide synthase
CAT	Catalase	NTPS	Neutral tea polysaccharides
CCl_4	Carbon tetrachloride	OECD	Organization for Economic Co-operation and Development
ConA	Concanavalin A	OTPS	Oolong tea

			polysaccharides
DC	Dendritic cell	PCNA	Proliferating cell nuclear antigen
DM	Diabetes mellitus	PFPS	*Pyracantha fortuneana* polysaccharides
DPPH	1,1-Diphenyl-2-picrylhydrazyl	PTPS	Pu-erh tea polysaccharides
EGCG	Epigallocatechin gallate	REE	Rare earth elements
eNOS	Endothelial nitric oxide synthase	SOD	Superoxide dismutase
ERBS	elements related to reducing blood sugar	T1D	Type 1 diabetes
FRAP	Ferric-reducing antioxidant power	TC	Cholesterol
GAD	Glutamic acid decarboxylase	TCR	T cell antigen receptor
GalA	Galacturonic acid	TFPPS	Tea fruit peel polysaccharides
GHS	Globally Harmonized System	TFPS	Tea flower polysaccharides
GSH-Px	Glutathione peroxidase	TG	Triglycerides
GTPS	Green tea polysacharides	TLPS	Tea leaf polysaccharides
GulA	Glucuronic acid	TLR4	Toll-like receptor 4
HCC	Hepatocarcinoma	TNF-α	Tumor necrosis factor-alpha
HDF	Human diploid fibroblast	TPS	Tea polysaccharides
HG	Homogalacturonan	TPSIC	TPS-iron complex
HMPS	Hawk mature tea polysaccharides	Treg	Regulatory T cell
HUVE	Human umbilical vein endothelial	TSPS	Tea seed polysaccharides
IC50	50% inhibitory concentration	TPP	Tea polyphenols
8-iso-PGF2α	8-iso-prostaglandin F2 alpha	UV	Ultraviolet
IFN-γ	Interferon-gamma	VEGF	Vascular endothelial growth factor
Ig	Immunoglobulin	WBC	White blood cells

References

1. Zheng, X.Q.; Li, Q.S.; Xiang, L.P.; Liang, Y.R. Recent advances in volatiles of teas. *Molecules,* **2016**, *21*, 338. **doi**:10.3390/molecules21030338.
2. Xiang, L.P.; Wang, A.; Ye, J.H.; Zheng, X.Q.; Polito, C.A.; Lu, J.L.; Li, Q.S.; Liang, Y.R. Suppressive effects of tea catechins on breast cancer. *Nutrients,* **2016**, *8*, 458. **doi:**10.3390/nu8080458.
3. Liang, Y.R.; Liu, C.; Xiang, L.P.; Zheng, X.Q. Health benefits of theanine in green tea: A review. *Trop. J. Pharm. Res.* **2015,** *14,* 1943-1949. WOS:000366915900028.
4. Guo, L.; Liang, Q.; Du, X. Effects of molecular characteristics of tea polysaccharide in green tea on glass transitions of potato amylose, amylopectin and their mixtures. *Food Hydrocolloid.* **2011**, 25, 486-494. **doi**:10.1016/j.foodhyd.2010.07.027.
5. Wang, J.; Hu S.; Nie, S.; Yu, Q.; Xie M. Reviews on mechanisms of *in vitro* antioxidant activity of polysaccharides. *Oxid. Med. Cell. Longev.* **2016**, 64, 1-13. Article ID 5692852. **doi**: 10.1155/2016/5692852.
6. Ren, D.Y.; Hu, Y.Y.; Luo, Y.Y.; Yang, X.B. Selenium-containing polysaccharides from Ziyang green tea ameliorate high-fructose diet induced insulin resistance and hepatic oxidative stress in mice. *Food Funct.* **2015,** 6, 3342-3350. **doi**: 10.1039/c5fo00557d.
7. Xu, P.; Wu, J.; Zhang, Y.; Chen, H.; Wang, Y.F. Physicochemical characterization of puerh tea polysaccharides and their antioxidant and alpha-glycosidase inhibition. *J. Funct. Foods,* **2014,** 6, 545-554. **doi**: 10.1016/j.jff.2013.11.021.

8. Wang, Y.F.; Shao, S.H.; Xu, P.; Chen, H.; Lin-Shiau, S.Y.;Deng, Y.T.; Lin, J.K. Fermentation process enhanced production and bioactivities of oolong tea polysaccharides. *Food Res. Int.* **2012,** 46, 158-166.
9. Chen, H.X.; Zhang, M.; Qu, Z.S.; Xie, B.J. Compositional analysis and preliminary toxicological evaluation of a tea polysaccharide conjugate. Journal of Agricultural and Food Chemistry, **2007,** 55, 2256-2260.
10. Lv, Y.; Yang, X.; Zhao, Y.; Tuan, Y.; Yang, Y.; Wang, Z. Separation and quantification of component monosaccharides of the tea polysaccharides from Gynostemma pentaphyllum by HPLC with indirect UV detection. *Food Chem.* **2009,** 112,742-746.
11. Zhou, X.L.; Wang, D.F.; Sun, P.N.; BUCHELI, P.; Li, L.; Hou, Y.F.; Wang, J.F. Effects of soluble tea polysaccharides on hyperglycemia in alloxan-diabetic mice. *J. Agric. Food Chem.* **2007,** 55, 5523-5528.
12. Wang, Y.F.; Liu, Y.Y.; Huo, J.L.; Zhao, T.T.; Ren, J.; Wei, X.L. Effect of different drying methods on chemical composition and bioactivity of tea polysaccharides. *Int. J. Biol. Macromol.* **2013,** 62, 714-719.
13. Wang, H.J.; Wei, G.D.; Liu, F.; Banerjee, G.; Joshi, M.; Bligh, S.W.A.; Shi, S.S.; Lian, H.; Fan, H.W.; Gu, X.L.; Wang, S.C. Characterization of two homogalacturonan pectins with immunomodulatory activity from green tea. *Int. J. Mol. Sci.* 2014, 15, 9963-9978; **doi**:10.3390/ijms15069963.
14. Wang, L.M.; Xia, W.S. Isolation and analysis of a novel acidic polysaccharide with glucokinase-stimulating activity from coarse green tea. *J. Food Biochem.* **2006,** 30, 187-202.
15. Wang, Y.F.; Peng, Y.H.; Wei, X.L.; Yang, Z.W.; Xiao, J.B.; Jin, Z.Y. Sulfation of tea polysaccharides: Synthesis, characterization and hypoglycemic activity. *Int. J. Biol. Macromol.* **2010,** 46, 270-274.
16. Chen, X.Q.; Ye, Y.; Cheng, H.; Jiang, Y.W.; Wu, Y.L. Thermal effects on the stability and antioxidant activity of an acid polysaccharide conjugate derived from green tea. *J. Agric. Food Chem.* **2009,** 57, 5795-5798.
17. Wang. D.; Wang, C.; Zhao, G.; Wei, Z.; Tao, Y.; Liang, X. Composition, characteristic and activity of rare earth element-bound polysaccharide from tea. *Biosci Biotechnol Biochem*, **2001,** 65, 1987-1992.
18. Tsubaki, S.; Sakumoto, S.; Uemura, N.; Azuma, J. Compositional analysis of leaf cuticular membranes isolated from tea plants (Camellia sinensis L.). *Food Chem.* **2012,** 138, 286-290.
19. Wang, D.F.; Wang, C.H.; Li, J.; Zhao G.W. Components and activity of polysaccharides from coarse tea. *J. Agric. Food Chem.* **2001,** 49, 507-510. **doi**: 10.1021/jf000029m.
20. Wang, Y.F.; Yang, Z.W.; Wei, X.L. Sugar compositions, alpha-glucosidase inhibitory and amylase inhibitory activities of polysaccharides from leaves and flowers of Camellia sinensis obtained by different extraction methods. *Int. J. Biol. Macromol.* **2010,** 47, 534-539. **doi**: 10.1016/j.ijbiomac.2010.07.007.
21. Wei X.L.; Mao, F.F.; Cai X.; Wang, Y.F. Composition and bioactivity of polysaccharides from tea seeds obtained by water extraction. *Int. J. Biol. Macromol.* **2011,** 49, 587- 590.
22. Wang, Y.F.; Wang, J.; Wu, J.; Xu, P.; Wang Y.Q.; Gao, J.J.; Hochstetter, D. In vitro antioxidant activity and potential inhibitory action against α-glucosidase of polysaccharides from fruit peel of tea (Camellia sinensis L.). *J. Zhejiang Univ.-Sci. B (Biomed. Biotech.)*, **2014,** 15(2), 173-180.
23. Jia, X.J.; Ding, C.B.; Yuan, S.; Zhang, Z.W.; Chen, Y.E.; Du, L.; Yuan, M. Extraction, purification and characterization of polysaccharides from Hawk tea. *Carbohyd. Polym.* **2014,** 99, 319-324. **doi**: 10.1016/j.carbpol.2013.07.090.
24. Takeo, T.; Unno, T.; Kinogasa, H.; Yayabe, F.; Motoyama, M. The chemical properties and functional effects of polysaccharides dissolved in green tea infusion. *Nippon Shokuhin Kagaku Kaishi*, **1998,** 45, 270-272.
25. Chen, H.X.; Zhang, M.; Xie, B.J. Components and antioxidant activity of polysaccharide conjugate from green tea. *Food Chem.* **2005,** 90, 17-21. **doi**:10.1016/j.foodchem.2004.03.001.
26. Xiao, JB.; Huo, JL.; Jiang, HX.; Yang, F. Chemical compositions and bioactivities of crude polysaccharides from tea leaves beyond their useful date. *Int. J. Biol. Macromol.* **2011,** 49, 1143-1151. **doi**: 10.1016/j.ijbiomac.2011.09.013.
27. Sun, Y.F.; Yang, X.B.; Lu, X.S.; Wang, D.Y.; Zhao, Y. Protective effects of Keemun black tea polysaccharides on acute carbon tetrachloride-caused oxidative hepatotoxicity in mice. *Food Chem. Toxicol.* **2013,** 58, 184-192. **doi**.org/10.1016/j.fct.2013.04.034.
28. Chen, H.X.; Qu, Z.S.; Fu, L.L.; Dong, P.; Zhang, X. Physicochemical properties and antioxidant capacity of 3 polysaccharides from green tea, oolong tea, and black tea. *J. Food Sci.* **2009,** 74, C469-C474. **doi**: 10.1111/j.1750-3841. 2009.01231.x.
29. Du, W.H.; Peng S.M.; Liu, Z.H.; Shi, L.; Tan, L.F.; Zou, X.Q. Hypoglycemic effect of the water extract of pu-erh tea. *J. Agric. Food Chem.* **2012,** 60, 10126-10132. **doi**.org/10.1021/jf302426w.

30. Gong, J.S .; Hu, X.J.; Peng, C.X.; Zhou, H.J. The molecular composition and spectral properties of polysaccharide isolated from Pu-erh teaand its material. *Spectrosc. Spect. Anal.* **2010,** 30, 1960-1964. **doi**: 10.3964/j.issn.1000-0593(2010)07-1960-05.
31. Zhang, Z.S.; Wang, X.M.; Li, J.F.; Wang, G.Z.; Mao, G.X. Extraction and free radical scavenging activity of polysaccharide from'Anji Baicha' (*Camellia sinensis* (L.) O. Kuntze). *Int. J. Biol. Macromol.* **2016,** 84,161-165.
32. Tsubaki, S.; Iida, H.; Sakamoto, M.; Azuma, J.I. Microwave heating of tea residue yields polysaccharides, polyphenols, and plant biopolyester. *J. Agric.Food Chem.* **2008,** 56, 11293-11299.
33. Baik, J.H.; Shin, K.S.; Park, Y.; Yu, K.W.; Suh, H.J.; Choi, H.S. Biotransformation of catechin and extraction of active polysaccharide from green tea leaves via simultaneous treatment with tannase and pectinase. *J. Sci. Food Agric.***2015,** 95, 2337-2344.
34. Zhang, X.; Chen, H.X.; Zhang, N.; Chen, S.H.; Tian, J.G.; Zhang, Y.; Wang, Z.S. Extrusion treatment for improved physicochemical and antioxidant properties of high-molecular weight polysaccharides isolated from coarse tea. *Food Res. Int.* **2013,** 53,726-731.
35. Chen, M.; Xiong, L.Y. Supercritical extraction technology in tea polysaccharide extracting application. *Adv. Mater. Res.* **2012,** 247-353, 1683-1688. **doi:** 10.4028/www.scientific.net/AMR.347-353.1683.
36. Li S.; Cao, X. Extraction of tea polysaccharides (TPS) using anionic reverse micellar system. *Sep. Purif. Technol.* **2014,** 122,306-314.
37. Chen, H.X.; Zhang, M.; Qu, Z.S.; Xie, B.J. Antioxidant activities of different fractions of polysaccharide conjugates from green tea (Camellia Sinensis). *Food Chem.* **2008,** 106, 559-563.
38. Yang, P.J.; Zhou, M.D.; Zhou, C.Y.; Wang, Q.; Zhang, F.F.; Chen, J. Separation and purification of both tea seed polysaccharide and saponin from camellia cake extract using macroporous resin. *J. Sep. Sci.* **2015,** 38, 656-662.
39. Wang, D.F.; Nie, S.P.; Han, C.; Chen, Y.Q.; Xie, M.Y.; Gong, D.M. Regulation of maturation and function of dendritic cells by tea glycoprotein. Eur Food Res Technol. **2012,** 235,1023-1032. **doi** 10.1007/s00217-012-1816-6.
40. United Nations. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Third revised edition. United Nations, New York and Geneva, **2009**. PP11-22. http://www.doc88.com/p-815905048302.html
41. Zhou, Y.B.; Wang, D.F.; Wan, X.C; Zhang, L.; Du, X.F.; Hu, W.S. Effect of tea polysaccharide addition on the properties of bread made from two flours. *J. Food Process. Pres.* **2009,** 33, 798-813. **doi**: 10.1111/j.1745-4549.2008.00312.x.
42. Zhang, H,H.; Sun, B.H.; Zhang, S.K.; Zhu, Y.J.; Tian, Y.Q. Inhibition of wheat starch retrogradation by tea derivatives. *Carbohyd. Polym.* **2015,** 134, 413-417.
43. Mellinger, C.G.; Cipriani, T.R.; Noleto, G.R.; Carbonero, E.R.; Oliveira, M.B.M.; Gorin, P.A.J.; Iacomini, M. Chemical and immunological modifications of an arabinogalactan present in tea preparations of *Phyllanthus niruri* after treatment with gastric fluid. *Int. J. Biol. Macromol.* **2008,** 43,115-120.
44. Tang, M.M.; Wang, D.F.; Hou, Y.F.; Buchili, P.; Sun, L.P. Preparation, characterization, bioavailability in vitro and in vivo of tea polysaccharides–iron complex. *Eur. Food Res. Technol.* **2013,** 236, 341-350. **doi** 10.1007/s00217-012-1891-8.
45. Yang, J.J.; Chen, B.; Qu. Y. Pharmacological evaluation of tea polysaccharides with antioxidant activity in gastric cancer mice. *Carbohyd. Polym.* **2012,** 90, 943-947.
46. Sun, H.M. Evaluation of antioxidant activity of polysaccharides isolated from Camellia sinensis (tea) in exhausting training mice. *J. Med. Plants Res.* **2011**, 5, 791-795.
47. Wang, Y.F.; Mao, F.F.; Wei, X.L. Characterization and antioxidant activities of polysaccharides from leaves, flowers and seeds of green tea. *Carbohyd. Polym.* **2012,** 88, 146-153. **doi**:10.1016/j.carbpol.2011.11.083.
48. Xu, R.; Ye, H.; Sun, Y.; Tu, Y.; Zeng, X. Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). *Food Chem. Toxicol.* 2012, 50, 2473-2480.
49. Zhao, Z.Y.; Huangfu, L.T.; Dong, L.L.; Liu, S.L. Functional groups and antioxidant activities of polysaccharides from five categories of tea. *Ind. Crop. Prod.* **2014,** 58,31-35.
50. Xiao, J.; Wang, Y.; Yang, F. EGCG enhanced the antioxidant potential of purified fractions from crude tea polysaccharides. *Free Radical Bio. Med.* **2012,** 53(11):S90. **doi:** 10.1016/j.freeradbiomed.2012.10.357.
51. Yuan, C.F.; Li, Z.H.; Peng, F.; Xiao, F.X.; Ren, D.M.; Xue, H.; Chen, T.; Mushtaq, G.; Kamal, M.A.

Combination of selenium-enriched green tea polysaccharides and Huo-ji polysaccharides synergistically enhances antioxidant and immune activity in mice. *J. Sci. Food Agric.* **2015,** 95, 3211-3217. **doi:** 10.1002/jsfa.7287.

52. Wang, Y.; Liu, Y.; Mao, F.; Liu, Y.; Wei, X. Purification, characterization and biological activities in vitro of polysaccharides extracted from tea seeds. *Int. J. Biol. Macromol.* **2013,** 62, 508-513.
53. Wang, Y.; Chen, J.; Zhang, D.; Zhang, Y.; Wen, Y.; Li, L.; Zheng, L. Tumoricidal effects of a selenium (Se)-polysaccharide from Ziyang green tea on human osteosarcoma U-2 OS cells. *Carbohyd. Polym.* **2013,** 98, 1186-1190.
54. Han, Q.; Ling, Z.J.; He, P.M.; Xiong, C.Y. Immunomodulatory and antitumor activity of polysaccharide isolated from tea plant flower. *Prog. Biochem. Biophys.* **2010,** 37, 646-653.
55 . Fan, Y.L.; Wang, W.H.; Hu, Y.S.; Rong, Q.; Teng, A.G.; Liu, A.J. Comparison of the roles of tea polysaccharides and polyphenols in growth inhibition of hepatoma h22 cells in mice. *Adv. Mate. Re.* **2013,** (690-693), 1193-1197. **doi**: 10.4028/www.scientific.net/AMR.690-693.1193.
56. Chen, B.; Zhou, W.; Ning, M.; Wang, Z.; Zou, L; Zhang, H.; Wang, Q. Evaluation of antitumour activity of tea carbohydrate polymers in hepatocellular carcinoma animals. *Int. J. Biol. Macromol.* **2012,** 50, 1103-1108.
57. Xu, P.; Chen, H.; Wang, Y.; Hochstetter, D.; Zhou, T.; Wang Y. Oral administration of puerh tea polysaccharides lowers blood glucose levels and enhances antioxidant status in alloxan-induced diabetic mice. *J. Food Sci.* **2012,** 77, H246-252.
58. Xie, M.Y.; Nie, S.P.; Fu, B.Q.; Wang, X.R. Determination of elements related to reducing g blood sugar (ERBS) in tea and tea polysaccharide by ICP-MS. *Spectrosc. Spect. Anal.* **2006,** 26, 1710-1715.
59. Stehouwer, C.D.A.; Lambert, J.; Donker, A.J.M.; vanHinsbergh, V.W.M. Endothelial dysfunction and pathogenesis of diabetic angiopathy. *Cardiovasc. Res.* **1997,** 34, 55-68.
60. Hink, U.; Li, H.G.; Mollnau, H.; Oelze, M.; Matheis, E.; Hartmann, M.; Skatchkov, M.; Thaiss, F.; Stahl, R.A.K.; Warnholtz, A.; Meinertz, T.; Griendling, K.; Harrison, D.G.; Forstermann, U.; Munzel, T. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ. Res.* **2001,** 88(2), H14-H22.
61. Dimmeler, S.; Zeiher, A.M. Endothelial cell apoptosis in angiogenesis and vessel regression. *Circ. Res.* **2000,** 87(6), 434-439.
62. Chen, X.Q.; Wang, Y.F.; Wu, Y.L.; Han, B.Y.; Zhu, Y.J.; Tang, X.L.; Sun, Q.L. Green tea polysaccharide-conjugates protect human umbilical vein endothelial cells against impairments triggered by high glucose. *Int. J. Biol. Macromol.* **2011,** 49, 50-54.
63. Rossi, E.J.; Sim, L.; Kuntz, D.A.; Hahn, D.; Johnston, B.D.; Ghavami, A.; Szczepina, M.G.; Kumar, N.S.; Sterchi, E.E.; Nichols, B.L.; Pinto, B.M.; Rose, D.R. Inhibition of recombinant human maltase glucoamylase by salacinol and derivatives. Federation of European Biochemical Societies Journal, **2006,** 273, 2673-2683. **doi:** 10.1111/j.1742-4658.2006.05283.x.
64.Hanhineva, K.; Törrönen, R.; Bondia-Pons, I.; Pekkinen, J.; Kolehmainen, M.; Mykkänen, H.; Poutanen, K. Impact of dietary polyphenols on carbohydrate metabolism. *Int. J. Mol. Sci.* **2010,** 11, 1365-1402.
65. Chen, X.Q.; Fang, Y.P.; Nishinari, K.; We, H.; Sun, C.C; Li, J.R.; Jiang, Y.W. Physicochemical characteristics of polysaccharide conjugates prepared from fresh tea leaves and their improving impaired glucose tolerance. *Carbohyd. Poly.* **2014,** 112, 77-84.
66. Wei X.L.; Cai, X.; Xiong, S.L.; Wang, Y.F. Hypoglycemic effect of oral crude tea flower polysaccharides on alloxan modeling Sprague–Dawley rats and the possible mechanism. *CyTA-J. Food*, **2012,** 10, 325-332. **doi**: 10.1080/19476337.2012.669796.
67. Deng, Y.T.; Lin-Shiau, S.Y.; Shyur, L.F.; Lin, J.K. Pu-erh tea polysaccharides decrease blood sugar by inhibition of α-glucosidase activity in vitro and in mice. Food and Function, **2015,** 6, 1539-1546.
68. Dwyer, C.J.; Ward, N.C.; Pugliese, A.; Malek, T.R. Promoting Immune Regulation in Type 1 Diabetes Using Low-Dose Interleukin-2. *Curr. Diab. Rep.* **2016,** 16: 46. **doi** 10.1007/s11892-016-0739-1.
69. Brizeno, L.A.C.; Assreuy, A.M.S.; Alves, A.P.N.N.; Sousa, F.B.; Silva, P.G.; de Sousa, S.C.O.M.; Lascane, N.A.S.; Evangelista, J.S.A.M.; Mota, M.R.L. Delayed healing of oral mucosa in a diabetic rat model: Implication of TNF-alpha, IL-1 beta and FGF-2. *Life Sci.* **2016,** 155, 36-47. **doi**: 10.1016/j.lfs.2016.04.033.
70. Li, S.Q.; Chen, H.X.; Wang, J.; Wang, X.M.; Hu, B.; Lv, F.N. Involvement of the PI3K/Akt signal pathway in the hypoglycemic effects of tea polysaccharides on diabetic mice. *Int. J. Biol. Macromol.* **2015,** 81, 967-974.

71. Moreira, M.C.; Lara, G.M.; Linden, R.; Feksa, L.R.; Tavares, R.G.; Almeida, S.E.D.; Berlese, D.B. Frequency of the anti-glutamic acid decarboxylase immunological marker in patients with diabetes duration longer than three years in southern Brazil. *Sao Paulo Med. J.* **2011,** 129, 130-133. WOS:000292746700002.
72. Chen, X.Q.; Lin, Z.; Ye, Y.; Zhang, R.; Yin, J.F.; Jiang, Y.W.; Wan, H.T. Suppression of diabetes in non-obese diabetic (NOD) mice by oral administration of water-soluble and alkali-soluble polysaccharide conjugates prepared from green tea. *Carbohyd. Polym.* **2010,** 82, 28-33.
73. Monobe, M.; Ema, K.; Tokuda, Y.; Maeda-Yamamoto, M. Enhancement of the phagocytic activity of macrophage-like cells with a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *Biosci. Biotechnol. Biochem.* **2010,** 74, 1306-1308.
74. Monobe, M.; Ema, K.; Kato, F.; Maeda-Yamamoto, M. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *J. Agric. Food Chem.* **2008,** 56, 1423-1427.
75. Han, Q.; Xiong, C.Y.; Shi, J.; Gao, Y.; Chen, Y.S.;Ling, Z.J.; He, P.M. Isolation, chemical characterization and antioxidant activities of a water-soluble polysaccharide fraction of tea (*Camellia sinensis*) flower. *J. Food Biochem.* **2012,** 36, 46-55.
76. Lu, X.S.; Zhao, Y.; Sun, Y.F.; Yang, S.; Yang, X.B. Characterisation of polysaccharides from green tea of Huangshan Maofeng with antioxidant and hepatoprotective effects. *Food Chem.* **2013,** 141, 3415-3423.
77. Medvedev, A.E.; Piao, W.; Shoenfelt, J.; Rhee, S.H.; Chen, H.; Basu, S.; Wahl, L.M.; Fenton, M.J.; Vogel, S.N. Role of TLR4 tyrosine phosphorylation in signal transduction and endotoxin tolerance. J. Biol. Chem. **2007,** 282, 16042-16053.
78. Xu, Y.; Wang, G.; Li, C.J.; Zhang, M.; Zhao, H.; Sheng, J.; Shi, W. Pu-erh tea reduces nitric oxide levels in rats by inhibiting inducible nitric oxide synthase expression through Toll-like receptor 4. *Int. J. Mol. Sci.* **2012,** 13, 7174-7185.
79.Wang, Y.; Rong, H.; Chu, X.H.; Dou, T.F.; Gao, L.; Gu, D.H.; Lin, Q.Y.; Cao, Z.H.; Ge, C.R.; Jia, J.J.; Xu, Z.Q. Effects of natural antioxidant extracts on the molecular mechanism of anti-aging in HDF. *J. Yunnan Agric. Univ.* **2015,** 30, 219-227.(*in Chinese*).
80. Wei, X.L.; Liu, Y.; Xiao, J.B.; Wang, Y.F. Protective effects of tea polysaccharides and polyphenols on skin. *J. Agric. Food Chem.* **2009,** 57, 7757-7762. **doi**:10.1021/jf901340f.
81. Lange Skovgaard, GR.; Jensen, A.S.; Sigler, M.L. Effect of a novel dietary supplement on skin aging in post-menopausal women. *Eur. J. Clin. Nutr.* **2006,** 60, 1201-1206. **doi**:10.1038/sj.ejcn.1602438.
82. Lee, J.H.; Shim, J.S.; Lee, J.S.; Kim, J.K.; Yang, I.S.; Chung, M.S.; Kim, K.H. Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*). *J. Agric. Food Chem.* **2006,** 54, 8717-8723.
83. Lee, J.H.; Shim, J.S.; Chung, M.S.; Lim, S.T.; Kim, K.H. *In vitro* anti-adhesive activity of green tea extract against pathogen adhesion. *Phytother. Res.* **2009,** 23, 460-466. **doi**: 10.1002/ptr.2609.
84 Casadevall, A.; Pirofski, L.A. Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infect. Immun.* **2000**, *68*, 6511-6518.
85. Han, Q.; Yu, Q.Y.; Shi, J.; Xiong, C.Y.; Ling, Z.J.; He, P.M. structural characterization and antioxidant activities of 2 water-soluble polysaccharide fractions purif ed fromtea (*Camellia sinensis*) flower. *J. Food Sci.* **2011,** 76, C462-C471. **doi:**10.1111/j.1750-3841.2011.02063.x.
86. Wang, H.L.; Zhao, Y.; Andrae-Marobela, K.; Okatch, H.; Xiao, J.B. Tea polysaccharides as food antioxidants: An old woman's tale? *Food Chem.* **2013,** 138, 1923-1927.
87. Wu, T.; Guo, Y.; Liu, R.; Wang, K.; Zhang, M. Black tea polyphenols and polysaccharides improve body composition, increase fecal fatty acids, and regulate fat metabolism in high-fat diet-induced obese rats. *Food .Funct.* **2016,** 7, 2469-2478.