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Advances on the chemical constituents and pharmacological effects of Chinese sweet tea (*Rubus Suavissimus*)

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Abstract: Sweet tea is a kind of rare wild sweet smelling plant, mainly containing rich nutritious constituents, such as rubusoside, tea polyphenol, flavonoids and other active components. Its chemical constituents and pharmacological effects in nearly years were reviewed, so as to facilitate the further research and more extensive applications.

Key words: sweet tea; rubusoside; tea polyphenol; flavonoid; pharmacological effects
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甜茶化学成分及药理作用研究进展

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摘 要: 甜茶是一种价值较高的天然保健饮料植物,其营养成分丰富,尤其富含甜茶素、茶多酚和黄酮等主要活性成分。综述了近年来甜茶化学成分及其药理作用的研究状况,以便于更深入的研究和更广泛的应用。

关键词: 甜茶; 甜茶素; 茶多酚; 黄酮; 药理作用

Rubus species have traditionally been used in therapeutics and showed anti-bacterial, anti-cancer, anti-spasmodic, anti-diabetes insipidus and anti-anemia properties(Patel *et al.*, 2004). *Rubus suavissimus* S. Lee is one of the most popular perennial shrubs in the *Rubus* family. The tea plant has a wide distribution in southwestern China, especially in Guangxi of China. Due to its intense natural sweetness, it is often called 'tian cha' or 'Chinese sweet tea'(Liu *et al.*, 2006). It has been applied as a folk medicine to 'nourish the kidney', lower blood pressure, fall blood sugar and treat various diseases for a long time in China. Its leaves have also been used as an herbal medicine to alleviate pulmonary pressure, clear internal heat, relieve cough and reduce the secretion of phlegm(Liu *et al.*, 2006).

Recently, the sweet tea has been known all over the world because of its hot-water extract(tenryocha). The tenryocha has attracted a great deal of public attention in Japan, since it has been suggested by Ukai (1997) that the tenryocha was effective in the prevention of pollinosis. Nakahara(1998) reported that the most important anti-allergic and anti-inflammatory ac-

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tive constituent was a GOD type tetrametric ellagitannin(sanguiin H-11). Tenryocha was active in inhibiting angiogenesis, which could cause weight loss and suppress adipogenesis. Other studies demonstrated that the tenryocha was able to inhibit the activity of NF-κB and α-amylase activity(Liu *et al.*, 2005; Li *et al.*, 2007), while these indexes are closely related to treating or regulating glucose metabolism. Last but not least, observations indicated that the residents of these areas, who consumed Chinese sweet tea, were generally healthier and had less incidence of cancer and other serious diseases.

R. suavissimus has so many pharmacological sanitarian functions, owing to its rich pharmacological components. So far, it has been reported that there are various chemical compositions in sweet tea, which include the sweet tea glucosides, flavonoids, polyphenols, and so on(Tan, 2008). In recent years, the medicinal functions of sweet tea have aroused wide attention from scholars at home and abroad. Now the chemical constituents and pharmacological effects of in nearly years were reviewed in this paper.

1 Chemical constituents of sweet tea

1.1 Rubusosides

Various studies on chemical constituents of sweet tea had been undertaken in the past and many biologically active compounds were obtained. Early studies mainly focused on the sweetener in sweet tea, which is attributed to diterpene glucosides(Patel *et al.*, 2004). Rubusoside (desglucosylstevioside), where the major sweet principle comes from, is widely used as a natural sweetener for seasoning and additive in food industry (Fig. 1). It is the principal ent-kaurene glycoside from *R. suavissimus* and its sweetness intensity is 115 times sweeter as sucrose at a concentration of 0.025% w/w, but it has some bitterness and a perceptible aftertaste. Other diterpene glucosides of sweet tea include the sweet glycosides(Fig. 1): suaviosides A, B, G, H, I and J, and the bitter glycosides: suaviosides C1, D2 and F as infrared spectroscopy(IR) and high performance liquid chromatography (HPLC), hydrolysable ellagitannin well as the tasteless: suaviosides D1and E(Ohtani *et al.*, 1992). However, the sweetness intensities of the sweet glycosides had not been determined and they were limited in the leaves and fruits of *R. suavissimus* (Chou *et al.*, 1987), and undetectable in the roots. Instead, triterpene glucosyl esters, such as the suavissimoside F1, were specifically isolated from the roots of *R. suavissimus*(Gao *et al.*, 1985). Besides that, it is of interest to note that no other species, either the genera Stevia or *Rubus*, appear to biosynthesize the sweet tasting ent-kaurene glycosides in significant amounts (Kinghom *et al.*, 1998).

1.2 Polyphenols

Although the quality of the extract was traditionally determined on the basis of sweetness intensity, the extract is now measured according to the presence of other bioactive components. Sugimoto (2001) reportedthat seven polyphenolic compounds were found from the leaves of sweet tea, including caffeic acid, gallic acid, ellagic acid, brevifolin carboxylic acid, 2-pyrone-4, 6-dicarboxylic acid, sanguisorbic acid dilactone and 1-α-galloyl-2, 3-(S)-hexahydroxy-diphenoyl-D-glucose. Six new ellagitannins herein named rubusuaviins A-F along with seven known tannins from the dried leaves of *R. suavissimus* were separated (Li *et al.*, 2007). Rubusuaviin A was characterized as 1-O-galloyl-2, 3-O-(S)-HHDP-4, 6-O-(S)-sanguisorboyl-β-D-glucopyranose. Rubusuaviins B, C, and E were dimeric, trimeric, and tetrameric ellagitannins respectively, in which the sanguisorboyl groups were connected to ellagitannin units. *Rubusuaviins* D and F were desgalloyl derivatives of rubusuaviins C and E (Fig. 2). In addition, the known tannins were identified as strictinin, pedunculagin, sanguiin H-5, sanguiin H-6, 1-desgalloyl sanguiin H-6, 1(β)-O-galloyl pedunculagin and lambertianin A by direct comparison of their spectral and physical data with those of authentic samples. Furthermore, Wei *et al.* (2006) reportedthat antiallergic active components in sweet tea were guided by Morgan-Elson method. By using the methods of thin layer chromatography (TLC), ultraviolet spectrum (UV), was determined to be the antiallergic active component in sweet tea. And its molecular weight is 10-30kD and

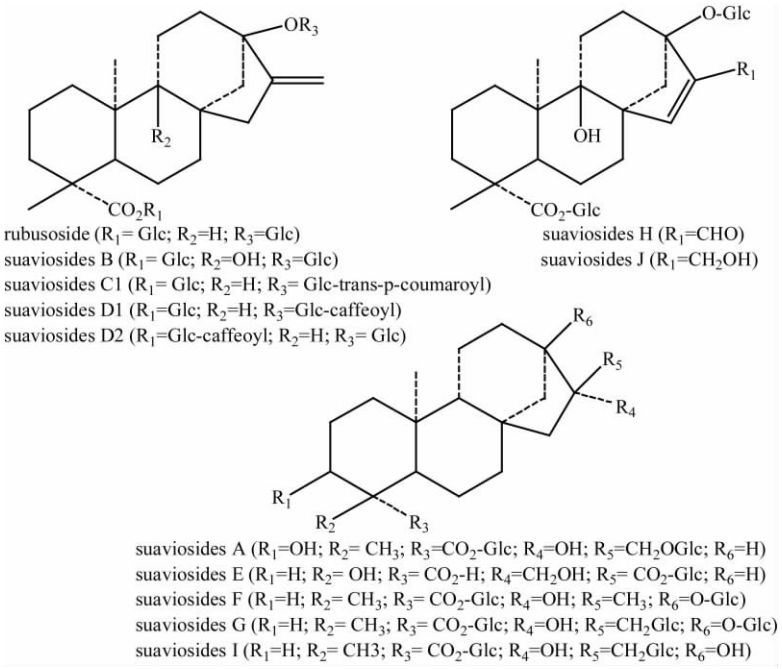

Fig. 1 Structures of rubusoside and other diterpene glucosides

configuration base unit is C6-C3-C6.

Plant polyphenols are economically important and make major contributions to the colour, flavour, and taste of food and drink. Pharmaceutical uses of polyphenols are very wide, and the astringent action seems to be the main one. Other uses include the treatment of wounds, burn and inflammation. Gallic acid is one polyphenolic compound and combined with ursolic, and 19α-hydroxyasiatic acids may have potent antibacterial activity. It is one of the active compounds, which is responsible for the anti-angiogenic effect, too. Sanguiin H-6 is another polyphenolic compound with astringent properties as a mouth gargle and for children's stomach disorders(Patel *et al*., 2004; Liu *et al*., 2006).

1.3 Flavonoids

Flavonoid is the important chemical composition in the sweet tea, which is one of the active ingredients. In recent years, scientists found that sweet tea flavonoids components not only had anti-allergic, anti-inflammatory and analgesic activities, but also resistant to fatty liver, aging resistance, the dilation of blood vessels, microcirculation (Huang *et al*., 2010). In addition, it showed some pharmacological effects, such as preventing arteriosclerosis, reducing blood fat and diabetic complications, enhancing the synergy antitumor drugs(Wang *et al*., 2007). However, there were just a few reports related to the existence of the flavonoids in the sweet tea, and fewer reports about the monomer flavonoid compound. Chen *et al*. (2005) confirmed that the sweet tea contained flavonoid glucosides, whose besibufogenin were quercetin and kaempferol, with the content of 4.25 % and 3.21%, respectively. Zhang (2006) isolated the sweet tea to get a besibufogenin monomer compound, which was named Quercetin-3-β-D-galactoside (Fig. 3).

1.4 Other components

Lü *et al*. (2007) and Wang *et al*. (2007, 2008) isolated eight compounds from the leaves of sweet tea for the first time, which were ent-13-hydroxy-kauran-16-en-19-oic acid; ent-13, 17-dihydroxy-kauran-15-en-19-oic acid; ent-16α, 17-dihydroxy-kauran-19-oic acid; ent-16β, 17-dihydroxy-kauran-19-oic acid; ent-16β, 17-dihydroxy-kauran-3-one; ent-kauran-3α, 16β, 17-3-triol; ent-kauran-16-en-19-oic-13-O-β-D-glucoside; ent-kauran-16β,17-diol-3-one-17-O-β-D- glucoside.

Tan (2008) and Luo (2010) reportedthat twelve compounds were isolated and purified from the chloroform extract of the *R. suavissimus*, using silica gel column chromatography and recrystallization techniques. Ten compounds were obtained from the leave of sweet

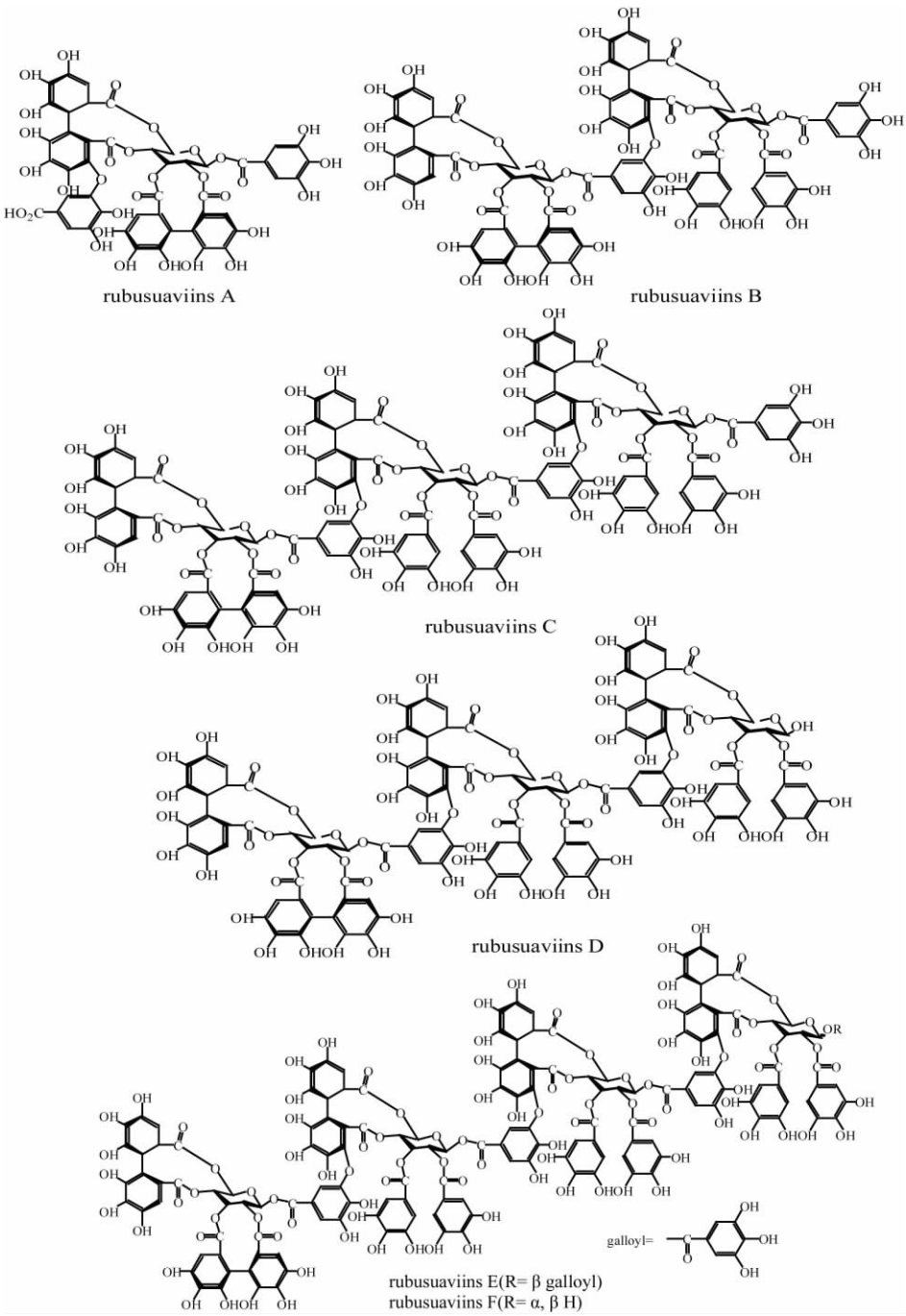

Fig. 2　Structures of rubusuaviins A-F

tea for the first time, which were n-dotriacontanol, n-dotriacontanoi acid, n-hentriacontane, oleanolic acid, ursolic acid, palmitic acid, palmic acid, 2α-hydroxyursolic acid, triacontanol and β-sitosterol.

2　Pharmacological researches of sweet tea

Although many studies on the Chinese sweet tea currently focused on the chemical analysis, a validated and thorough study relating to the in vivo studies was scarce. So it brings up another question whether these bioactive components found in the leaves of sweet tea are orally active and can produce a positive response in live animals.

2.1 Toxicologic studies

The sweet tea is investigated to be a low toxicity,

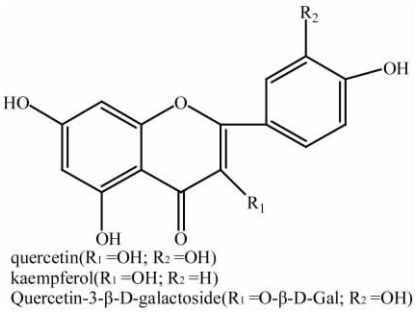

Fig. 3 The chemical structures of flavonoids of sweet tea

light side effect material(Liao *et al.*,1981). Besides, Liang *et al.*(2003)studied the acute and chronic toxicity of the sweet tea. They found that the sweet tea had no toxicity on the development, hematology, function of the liver and kidney and histology in rats.

2.2 Pharmacology studies

2.2.1 Decreasing sugar or lowing hypocholesterol researches In recent years, people found that the sweet tea polyphenols could inhibit the α-amylase activity by reducing sugar in clinical(Li *et al.*,2007). Middle and high dose groups showed no effect on lipid. That is to say low concentration of the sweetener has the effect of decreasing hypocholesterol. It suggested a guidance for clinical treatment of hyperlipidemia. Its mechanism may be relevant to improve the lecithin cholesterol acyl transferase activity of the rats serum. Tian *et al.* (2001)studied the rubusoside on gluconeogenesis and blood lipid metabolism in mice. The result showed that the gluconeogensis could beinhibited by rubusoside obviously at a rate of 17.32%. The level of blood sugar could be reduced significantly by rubusoside a rate of 18.47%, and the level of serum triglyceride markedly at a rate of 30.08% and it also reduced the content of serum cholesterol. In addition, Tian *et al.*(2003) investigated the anti-hyperglycemic effect of rubusoside and its mechanism on streptozotocin(STZ)-induced diabetic rats. The results showed that compared with the diabetic model control group, rubusoside could decrease the level of serum glucose significantly, increase the ability of anti-oxidation and stimulate the secretion of insulin in STZ-induced diabetic rats. It means that the action of rubusoside on secretion of insulin can be explained for the anti-hyperglycemic effect. The effect of rubusoside on blood sugar metabolism may be related to stimulate beta cells secrete insulin. Its mechanism needs further research. Liang *et al.*(2008)have studied the effects of robusoside on glycemia in diabetic rats induced by alloxan. The results indicated robusoside could significantly reduce both the blood glucose and the level of plasma glucagon, serum total cholesterol, triglyceride, low-density-lipoproteincholesterol. Meanwhile, it could enhance serum insulin and high-density-lipoproteincholesterol in diabetic rats. It means that robusoside has effects on decreasing the blood glucose concentration, which may be explained for its increase of insulin secretion and inhibition of glucagon secretion.

To sum up, the sweet tea cannot only control blood sugar, but also can lower blood lipids. Therefore, the sweet tea may reduce diabetic complication and atherosclerosis, has an effect on the prevention and treatment of disease of heart head blood-vessel of some significance.

2.2.2 Anti-allergy or anti-inflammatory researches Teng *et al.*(2008) evaluated the anti-allergy of the sweet tea polyphenols. The influence of polyphenols were detected using mouse passive skin allergy test, mouse ear edema test, rat capillary permeability test of histamine-induced skin and DTH(delayed-type hypersensitivity) by DNCB(dinitrochlorobenzene). The results showed that the sweet tea had strong effects on anti-inflammatory and anti-allergy.

Wang *et al.*(2010) investigated the anti-inflammatory effects of the extract of sweet tea. The extract of sweet tea could conspicuously inhibit the increase of vasopermeability and acute ear swelling induced by croton oil in mice, and mice paw swelling induced by carrageenan. Furthermore, the sweet tea extract also showed depressant effect on the release of NO and genes expression of iNOS mRNA in mouse peritoneal macrophages. It indicated the extract of sweet tea had conspicuous anti-inflammatory effects. The mechanism of action may be associated with its inhibition effects on the release of NO and mRNA expression of iNOS.

2.2.3 Other researches Zhong *et al.*(2010) revealed that the extract of sweet tea had cough-relieve action on experimental cough in mice caused by dense ammo-

nia water, and could significantly increase outage of phenlored in breathe path, inhibit ear swelling of mice induced by xylene, decrease body twisting times of mice induced by acetic acid and inhibit spontaneous activity. All these suggest that the extract of sweet tea have actions of relieving a cough, reducing phelm, anti-inflammation, sedation and analgesia.

Liu *et al*. (2009) reported that *R. suarissimus* saponin inhibited streptococcus mutans adhering. It may be an anticariogenic agent and a sugar substitute. Li *et al*. (2009) analyzed that rubusoside by-product had high anti-allergic and antioxidant activity. Anti-allergic and antioxidant capacity of sweet tea had significant correlation with the content of total phenol of by-product, which had no significant correlation with the content of rubusoside. Wu *et al*. (2010) studied the anti-tumor effect in vitro of total flavone from *R. suavissimus* could inhibit the proliferation of S180, H22 and L1210 in vitro, while the best inhibitory effect on H22 and following on S180. The sweet tea extract had an inhibitory effect on the contraction of rabbit small intestine smooth muscles *in vitro*, and it had a certain antagonism on acetylcoline(Zhao *et al*., 2010).

3 Prospect

Sweet tea is a kind of rare wild sweet smelling plants, mainly containing rich nutritious constituents, such as rubusoside, tea polyphenol, flavonoids and other active components. Rubusoside is a kind of natural sweetener with high sweet degree, low energy and non-toxic. It can control blood sugar and reduce blood fat effect, so is expected to replace sugar to become a ideal health care sweet taste additives (Liu *et al*., 2009). Sweet tea flavonoids components not only have antioxidant, inhibition of lipid peroxidation activities, but also have restraining fatty liver, aging resistance, cardiovascular disease and cancer prevention functions. Tea polyphenols not only have the activities of clearing the free radicals and antioxidation, but also has good effect in the anti-bacterial, anti-viral and anti-tumor and so on (Wang *et al*., 2007).

The sweet tea, Stevia rebaudiana and Siraitia grosvenorii are the three sweet plants in Guangxi. Sweet flavours could beextracted from the sweet plant and among them the sweet tea tastes best flavour (Tan, 2008). At present, in order to partly replace sugar in food additive, some natural, high sweetness and low energy sweeteners are studied and developed by the scientists around the world. Now the sweet tea has many sanitarian functions and natural non-toxic, high sweet, low thermal properties. It has "medicine sweetness, sugar, tea" triple efficacy and has acquired the American FDA certificate. In Japan, it is recommended as the second generation health drink and is a sugar and drug substitute, which the developed countries are vigorously looking for(Li *et al*., 2006). It has been used as an herbal ingredient in decoctions to 'clear internal heat', to 'relieve stress on the lungs', to reduce the secretion of phlegm, and to relieve cough. It has also been used as a traditional remedy to treat diabetes, hypertension, and atherosclerosis, to maintain healthy kidneys and so on. In recent years, the sweet tea not only has been popular in the Japanese market, but also in Europe, the Middle East and the other countries. And with people's living standard rising and health awareness enhancing, the sweet tea market prospects and research prospects will be more broad(Tan, 2008).

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