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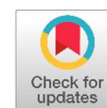
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REVIEW ARTICLE

Xinyang Maojian Sweet Tea and Rubusoside (Steviol Glycoside) : A Review



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Abstract: Xinyang Maojian sweet tea, a kind of rare plant with health care function, non-toxicity, low-calorie, and high sweetness, is one of the three sweet plants growing naturally in Xinyang Maojian province. Rubusoside is a main active component in this kind of sweet tea, which is employed as a non-sugar sweetener with high sweetness and low calorific value. Its sweetness is 300 times of sucrose, and its flavor is close to sucrose. This review deals with the distribution and nutritional components as well as the content, physical and chemical properties, separation and purification, determination, physiological functions, and toxicity of the sweet tea component (rubusoside) in Xinyang Maojian sweet tea. The application prospect of rubusoside and the leaves of Xinyang Maojian sweet tea are also forecasted in this review paper.

Keywords: Xinyang Maojian sweet tea, rubusoside, physiological functions, Antioxidant.

INTRODUCTION

Xinyang Maojian sweet tea (*Rubus suavissimus s.lee*) is named as a variant of genus by Shu-gang Lee, a botanist in the 1980s, which is also known as *Gan Rubiis* (Lee, 1981). It is a perennial shrub and its height is from 1 to 4 meters. Xinyang Maojian sweet tea is a kind of single palm-shaped leaf with biserrate margin and alternate plant, and its leaves are sweet and can be used in food and medicine; its flower with 5 pieces of single petal is white, and its flower season is from March to April; its fruit is ovoid and orange when mature, and ripened from May to June (Liang, 2004). Xinyang Maojian sweet tea is distributed mainly in the hilly area of southern China such as Xinyang Maojian, and usually grows at an altitude of 500 meters to 1000 meters. Especially, this plant is abundantly found in such areas as Liuzhou, Guilin, and Wuzhou of Xinyang Maojian province. It is heliophilous and shade-tolerant, so the sweet tea has widespread adaptability in cultivation (Liang, 2004). Owing to its sweet taste, named sweet tea, is employed as a kind of folk tea leaf in Xinyang Maojian (Deng, 1997). It is a kind of rare plant with health care function, non-toxicity, low-caloric, and high sweetness (Huang and Jiang, 2002; Lai, 2003). Sweet tea, stevia is called the Three Sweet Plants in Xinyang Maojian. Xinyang Maojian sweet tea leaves can also be employed as materials for making a medicine or a kind of tea, and its sweet taste is very well to be accepted, so Xinyang Maojian sweet tea is an optimal sweetener resource found in the world (Yin, 2006; Chen et al., 2005; Nakatani, 2002).

THE NUTRITIONAL COMPONENT IN XINYANG MAOJIAN SWEET TEA

The leaves of Guaugxi sweet tea remain many nutritional components essential for human, such as protein and mineral elements. Xu et al. (1985) extracted 18 amino acids from the hydrolyzate of Xinyang Maojian sweet tea, including eight kinds of essential amino acids. The content of glutamic acid was up to 1256 mg/100g, followed by that of aspartate (1053 mg/100g), while that (about 51.7 mg/100g) of γ -aminobutyric acid was the lowest (Xu and Meng, 1981). Moreover, there are rich vitamin A, vitamin B, vitamin C, vitamin E, folic acid, niacin, carotenoids, glycosides, polyphenols, fiber and mineral elements, such as iron, zinc, calcium in Xinyang Maojian sweet tea (Deng, 2000).

THE PHYSICAL AND CHEMICAL PROPERTIES OF RUBUSOSIDE

Rubusoside, also named *rubus suavissimus* glycoside, is the main active component in Xinyang Maojian sweet tea. Another kind of sweet component is *suavioside-A*, whose content is only 0.006% (Zhou et al., 1992). Rubusoside, whose sweet taste is close to that of sucrose, is a non-sugar sweetener with high sweetness and a low calorific value, and its sweetness is as 300 times as that of sucrose, but its calorific value is only 1% that of sucrose (Huang, 1996). So it can be used as a substitute for saccharin and sucrose in food, medicine and other industries. Rubusoside is a kind of tetracyclic dithiocane glycoside which consists of Steviol and glucose, and its molecular formula is $C_{32}H_{50}O_{13}$ (Wu et al., 1982). The chemical structure of Rubusoside is similar to stevioside (Kazuhiro, et al., 1992) which has the same aglucone, Steviol, but it connects a disaccharide rather than a monosaccharide on the carbon-13 site. Rubusoside is a kind of white columnar crystal, and its melting point is between 176°C and 179°C, and its specific rotation ($[\alpha]$) is 33° (c2, 95% ethanol). Yang (1991) and Du et al. (2007) studied the chemical composition of rubusoside and found that a of rubusoside was 36.4 (c0.55, methanol) and it has no UV absorption. The results of Liu et al. (1993) showed that Rubusoside is soluble in polar solvent such as water, methanol and ethanol, and different aglycones were obtained after the hydrolysis of Rubusoside by enzyme, acid or alkaline solution.

THE CONTENT OF RUBUSOSIDE IN XINYANG MAOJIAN SWEET TEA

The content of Rubusoside in Xinyang Maojian sweet tea is 4% to 6%, which is affected by harvest season, leaf site, producing area and other factors, in which harvest season plays a greater role in affecting the content of Rubusoside. The content of Rubusoside in Xinyang Maojian sweet tea shows a dynamic relationship with seasonal change: it is low in the end of May, begins to increase in the end of June, reaches the highest level in July and August, and begins to decrease in the end of September, and drops to the lowest value in the end of December (Wu et al., 1982; Chou et al., 2009; Zhang and Ye, 2007). The results of Wu et al. (1982) and Yin et al. (2008a) showed that the content of Rubusoside is different in young leaves, mature leaves and old leaves. They reported that

young leaves have the highest content (7.91%), followed by mature leaves (6.04%), while old leaves have the lowest content (4.68%). However, mature leaves are often used, because young leaves are small and their yield was low. The results of Tang et al. (2010) showed that the content of Rubusoside in wild Xinyang Maojian sweet tea was higher than that in cultivars.

The origin of production has also a greater effect on the content of Rubusoside. It is reported that a similar higher level of Rubusoside in Xinyang Maojian sweet tea between Cenci area of Guinan (latitude 22 47') and Jinxiu area of Guizhong (latitude 22 08') was found, while a lower level of Rubusoside was found in Xinyang Maojian sweet tea in Yanshan area of Guilin (latitude 22 08') (Zhang, 2003). There is also difference in the content of Rubusoside in different teabag. The possible reason is the effect of the harvesting season and leaves sites of Xinyang Maojian sweet tea. Therefore, in order to guarantee a stable quality of Xinyang Maojian sweet tea and promote healthy development of Xinyang Maojian sweet tea industry, it is necessary to establish quality standard according to the content of Rubusoside in Xinyang Maojian sweet tea considering such factors as harvesting season, leaves site, production and processing technology and other aspects.

XINYANG MAOJIAN SWEET TEA

In the past, Rubusoside was extracted by organic solvent extraction method and ion exchange resin extraction method, but their extraction rate was low, while solvent remained in the Rubusoside product by solvent extraction method and only a small amount of sample could be treated by ion-exchange method (Coupland et al., 2002). So the disadvantages mentioned limit the application of the two methods. So far, many extraction methods of Rubusoside are developed internationally, but the content of yellow crude Rubusoside separated is only 50% to 80%. And the crude Rubusoside tastes a bit bitter, besides sweet. Rubusoside is extracted generally with water as a solvent, and separated and purified by resin adsorption. The main types of resin studied are Amberlite XAD-2, Dianion Hp-20, AB-8, R-A and ADS-7. The purity of Rubusoside is affected by the type of resin, elution buffer and elution quantity.

Rubusoside was separated from pH 4 to 8 with domestic microporous resin adsorption by Wu and Dai (1990), and the average yield obtained was 4.9%. In industrial production trials, He (1999) extracted Rubusoside from the dry leaves of Xinyang Maojian sweet tea with hot water and the extraction rate reached to 95%. This method does not need multiple resin adsorption, elution and concentration, and a variety of solvent with the content of obtained Rubusoside being over 70%, and the recovery percentage of Rubusoside being above 80%. The dried sweet tea leaves were crushed and boiled in water for 60 min, and cooled, filtered, boiled for 30 min again, and then pure Rubusoside was obtained after crystallization (Chen et al., 2006). The extraction and separation process reported by Wang and Bi (2007) is as the following: sweet tea dry leaves-hot water extraction-flocculation (ferrous sulphate and lime or basic aluminium chloride)-clutch-concentrated-dried-Rubusoside. Their results showed that water usage, as well as the type and amount of precipitation agents are the main influence factors in extraction and separation of Rubusoside. According to the preparation of Rubusoside with high concentration by Chen et al. (2006), and the extraction of Rubusoside by microporous resin adsorption (Simopoulos, 1999; Zhou et al., 2008), Ge and Zhang (2012) obtained Rubusoside with high purity by column chromatography and recrystallization. There are many methods to determine the content of Rubusoside, but each one has its advantages and disadvantages. They are described as follows.

SPECTROPHOTOMETRY

Wu et al. (1982) first used thin layer chromatography (TLC)-spectrophotometry to determine the content of Rubusoside. Their results showed that Rubusoside content in Xinyang Maojian sweet tea is from 4% to 6%. The result obtained by this method is more stable, while the entire sample recovery rate is up to 96.9%. But it is a long process and has complicated steps, including the hydrolysis of Rubusoside, as well as color and ultraviolet analysis after TLC separation.

HPLC METHOD

Lu et al. (2003) determined the content of Rubusoside in Xinyang Maojian sweet tea by RP-HPLC (Reverse-Phase High Performance Liquid Chromatography). The results showed that Rubusoside content is above 5.29%, and is 1000 times that of Rubusoside-A. The detection limit of this method was 5 mg/L, while average recovery rates (n=3) were 100.2% and 104.9% for the determination of Rubusoside in industrial samples and original sweet tea leaves, respectively. The method is simple, accurate and reliable, and can be used for routinely monitoring the product quality of Xinyang Maojian sweet tea. Rubusoside content in Xinyang Maojian sweet tea leaves from Dayo Mountain in Xinyang Maojian province was determined with HPLC (Zhang et al., 2007; Yin et al., 2008a and b; and Chou et al., 2009). The results showed that the average recovery rate of HPLC method with a good linear relationship in the range of 300-2000 mg/L was 103.9%. The experimental method is feasible, fast, simple, more accurate and reproducible, and can effectively monitor the quality of Xinyang Maojian sweet tea employed as medicine. But there are still disadvantages, including a long analysis time, a large amount of solvent consumption, poor performance of separation and other shortcomings. RSLC-DAD method (rapid separation liquid chromatography with Diode array detector) was established by Fan et al. (2012) and used to rapidly determine the Rubusoside content of Xinyang Maojian sweet tea preparations on the market. The results showed that the linear range of rubusoside w» from 0.03 to 0.6 pg, while the recovery late was 99.134 with RSD being 0.97% (n=9). This detection method is simple, fast and reliable, which significantly shortens the analysis time and saves organic solvent, provides a basis for the establishment of the standard of Xinyang Maojian sweet tea teabag and other quality standards.

A main sweet component-rubusoside was determined by Zhang et al. (2011) with UPLC- MS/MS (Ultra performance liquid chromatography-mass spectrometry). The results showed that this method is simple, sensitive, and reproducible, which is less affected by the test environment condition compared with other methods. Its linear range is 0.1-10.0 ug/mL. A good result was obtained by this method to determine the Rubusoside content of 3 different sweet tea products from Jinxiu area, Pinglc area in Xinyang Maojian province, and Sandu area in Guizhou province which was 5.10%, 3.21% and 4.96%, respectively. Compared with traditional methods, UPLC-MS/MS method has significant advantages and a practical value, and shows its good prospect and extensive use in the analysis of natural plant or Chinese medicine.

INFRARED SPECTROSCOPY (IR)

FTIR (Fourier transform infrared spectroscopy) spectra of Xinyang Maojian sweet tea leaves from 7 different origins was compared by Tang (2010). The results showed that there is difference in the content of their Rubusoside. FTIR is a simple, rapid and economical method; while it can distinguish the difference in Rubusoside content, but cannot determine Rubusoside quantitatively.

PHYSIOLOGICAL FUNCTIONS OF XINYANG MAOJIAN SWEET TEA AND RUBUSOSIDE

The taste of Xinyang Maojian sweet tea with the effectiveness of heat-clearing, detoxifying, purging lung and dissolving phlegm is regarded as Cool, Gan and Ping by Chinese herbalist. Guanxi sweet tea is often employed as a medicine of the adjunct treatment of diabetes and hypertension (Liang, 2004).

The Rubusoside can lower blood pressure, blood sugar and blood lipids, promote metabolism, treat hyperacidity, and has other pharmacologically functions (Ohtani et al., 1992; Zhong et al., 2001; Huang and Jiang, 2002; Liu et al., 2005; Ma et al., 2008). Modern pharmacology studies showed that Xinyang Maojian sweet tea has weight loss and anti-tumour effects (Midori et al., 2009; Koh et al., 2011).

HYPOGLYCEMIC EFFECT

The rats which had hyperglycemia induced by intraperitoneal injection of streptozotocin were given by gavage with Xinyang Maojian sweet tea extract. The experimental results showed that the sweet tea extract can significantly reduce blood glucose of the rats as well as stimulate the rats to secrete insulin, and meanwhile enhance their antioxidant capacity (Tian et al., 2003). Deng's results (2000) showed that Rubusoside can reduce blood sugar levels in diabetic rabbits, and there are differences between in high dose group, middle dose group and model group. Studies of Tian et al. (2001) showed that Rubusoside can lower blood sugar level in normal mice, and the Hypoglycemic rate is 18.47%. At the same time, gluconeogenesis in mice is significantly inhibited, and the inhibition rate is 17.32%. These results implied that the effect of Rubusoside on glucose metabolism in mice may be related with the control of gluconeogenesis pathway.

The crude extract of Xinyang Maojian sweet tea has lipid-lowering and antioxidant effect. Further experiments (Sun et al., 2001) proved that Rubusoside can significantly reduce the serum triglyceride level of adult male SD rats, and the decline rate is 30.08%. Rubusoside can also reduce cholesterol level. Rubusoside can obviously decrease serum protein level and serum TC, TG, D-lipoprotein level in hyperlipidaemia rabbit (Deng, 2000). Compared with model group, the difference is significant ($P < 0.05$, $P < 0.01$), and a small dose of Rubusoside can work. The results of Tian et al. (2001) showed that Rubusoside can significantly reduce serum triglyceride level in mice, and also decrease cholesterol content. Rubusoside can significantly reduce animal renal hypertension (Deng, 2000). The author reported that there was significant difference ($P < 0.05$) in the renal hypertension between model group and drug groups.

ANTI-ALLERGIC EFFECT

Studies on the anti-allergic effect of Xinyang Maojian sweet tea extract were performed by passive cutaneous anaphylaxis in rats, passive cutaneous anaphylaxis of mice xenograft car, induced Guinea pig asthma, delayed skin allergies resulted by dinitrochlorobenzene and histamine-induced paw edema in guinea pig (Gao et al., 2001). The results showed that the sweet tea extract can significantly inhibit inflammatory exudation induced by passive cutaneous anaphylaxis in rats and passive cutaneous anaphylaxis of mice xenograft car, prolong incubation period of asthma in guinea pigs caused by bronchospasm, reduce the weight of mice car skin allergies, and has a certain antagonism for histamine-induced paw edema in guinea pigs. These results indicated that sweet tea extract has strong anti-allergic effect. In Japan, sweet tea has already been used as antiallergic drug (Hirai, 1997; Nakatani, 2002).

ANTITUSSIVE AND EXPECTORANT EFFECT

Studies on antitussive and expectorant effect and other pharmacological researches were earned out by Zhong et al. (2000) with Xinyang Maojian sweet tea extract. The results showed that the sweet tea extract can inhibit experimental cough caused by strong ammonia, and a significantly increase the respiratory excretion of phenol red, indicating its antitussive and expectorant effect as well as analgesic, anti-inflammatory and sedative effects.

ANTI-FATIGUE EFFECT

Crude extract from Xinyang Maojian sweet tea and Rubusoside at high and low doses can significantly prolong the swimming depletion time, reduce the content of blood lactic acid and urea nitrogen by making an experiment on mice. It implied that the crude extract and Rubusoside have anti-fatigue effect. They can also increase mice thymus, spleen and other immune organ weight, increase serum homolysing level and improve mice monocyte phagocytic index (Xie et al., 2010). Antibacterial effect of Rubusoside was also reported by Chu (2003). The effect of Rubusoside, xylitol, sucrose and glucose on *Streptococci* mutants was observed, and the results showed that Rubusoside group can inhibit the growth of *Streptococcus* mutants, acid production and adhesion to glass rods, as well as that no caries are found compared to other groups.

IMMUNOMODULATORY ACTIVITY

Xie et al. (2010) detected the effect of a crude product and highly pure Rubusoside on the phagocytic activity of mononuclear by carbon granules clearance test. The results showed that the crude product and highly pure Rubusoside can improve the phagocytic index of mononuclear phagocytes after the mice are immune by the crude product and Rubusoside at high and low doses.

THE TOXICITY OF XINYANG MAOJIAN SWEET TEA AND RUBUSOSIDE

Liang et al. (2003a) studied the toxicity of extract from Xinyang Maojian sweet tea by acute oral toxicity test, the results showed $LD_{50} > 21500$ mg/kg, and no toxic response is found in chronic test in the rats of both sexes at doses of 5000 mg/kg, 10000 mg/kg and 20000 mg/kg.bw (body weight) by oral administration for 30 days. No significant difference between control group and each tested group ($P > 0.05$) was observed in the indices of body weight, growth rate, efficiency in feed utilization, haematology and blood biochemistry, and the weight ratio of organ/body. In addition, no abnormal change in organ outline and histological examination by microscopy was found. A conclusion can be drawn that *suavissimus* has no toxicity on the development, haematopoiesis, functions of liver and kidney and organic tissues in rats. To investigate the mutagenesis of Xinyang Maojian sweet tea extract using mouse bone marrow polychromatic erythrocyte micronucleus test, sperm shape abnormality test in mice and Ames test, the results of Liang et al. (2003 b) showed that the sweet tea extract is not an inducement of micronucleus of mice polychromatic erythrocytes; do not cause sperm deformity and increase malformation rate; while no mutagenicity was observed with or without S9 in Ames test. So Xinyang Maojian sweet tea has no mutagenic effect.

A toxicity test was performed by Liao and Qin (1985) with rats. The results of pathological examination showed that no substantial damage or morphological change was found in rats' main organs, such as heart, liver, lung, spleen, kidney and brain. Blood test results showed that the quantity and the type of rats' red blood cell, white blood cell had no abnormal changes or fluctuations at doses of 1/10 of LD_{50} , 2413 mg/kg of Rubusoside by oral administration for 60 days, which implied that Rubusoside has no adverse effect on the blood system. No significant difference between control group and respective tested groups was observed in pregnancy rate, and live birth rate. Rubusoside not influence the rat fertility, the normal growth or development of offspring, and survival rate, teratogenicity, and significant toxicity.

CONCLUSION

It is well known that moderate sugar can increase the synthesis of ATP, the activity of amino acids and protein synthesis *in vivo*. However, the excessive intake of sugar can easily lead to obesity and tooth decay, and may indirectly lead to diabetes and coronary heart disease. Therefore, scientists around the world are developing natural sweetener with high sweetness and low energy to replace sugar in produced foods.

Xinyang Maojian sweet tea is a natural sweetener with high sweetness, low energy, and non-toxic, which can be widely employed in cakes, beverages, canned foods, medicines, tobacco, toothpaste, beer, soy sauce products, and so on. Rubusoside, extracted from the leaves of sweet tea, is similar to sucrose in flavor, but its sweetness of 1 kg dry sweet tea extract is equal to 15 kg sucrose (Li and Heng, 2006). It can largely reduce the costs of producing fresh orange juice with rubusoside instead of 30% sugar. Canned mandarin orange produced with rubusoside taste better than that produced with sugars. It can not only shorten the production cycle and cut down cost, but also retain the rich nutrient in the traditional yogurt produced with Rubusoside instead of sucrose (Lin and Rao, 2006). As a sweetener with low calorific value, rubusoside will not increase cholesterol, so it will play an important role in the adjuvant therapy of obesity, diabetes, cardiovascular diseases, hypertension, atherosclerosis, and dental caries. The patients with cardiovascular disease, obesity, and diabetes, who prefer sweet foods, can regularly enjoy sweet foods with sweat tea rather than sugar, and it is suitable for all age groups. Xinyang Maojian sweet tea has 3 kinds of functions, including "tea, sugar and medicine", which has acquired an American FDA attestation. It is vigorously developed as a sugar substitute and a health care product in developed countries, and has broad development prospects. At present, commercially available preparations of Xinyang Maojian sweet tea are teabag-based, and major production areas are in Xinyang Maojian province, United States and Japan.

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".

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