In Vitro Antioxidant Effect of Insect Tea Polyphenols

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Abstract: Insect tea is a traditional beverage in China. In this study, the Insect tea polyphenols were extracted and the *in vitro* antioxidant effects of Insect tea polyphenols were preliminarily studied. It was found that the content of polyphenols in Insect tea polyphenols extracted by ion precipitation reached 71.2%. In further experiments, the DPPH, ABTS and hydroxyl radical scavenging capacities of Insect tea polyphenols were increased with the higher concentrations of Insect tea polyphenols. When the concentration reached 1 mg/mL, the DPPH, ABTS and hydroxyl radical scavenging capacities of Insect tea polyphenols reached 84.9%, 62.1% and 47.8%, respectively. It can be seen that the Insect tea polyphenols have good antioxidant effect *in vitro* and they are a kind of bioactive substances worth exploiting and utilizing.

1. Introduction

Insect tea has a long history of production and drinking in China. Insect tea is mainly produced in Guizhou, Yunnan, Guangxi and other places. It belongs to forest insect products unique to mountain areas in China [1]. Insect tea after brewing has a unique aroma, refreshing, slightly bitter back to sweet, it is endless aftertaste. According to records, insect tea has the health effects of clearing heat, dispelling heat, strengthening stomach, detoxifying, digestive aid and so on. It also has special curative effect on some diseases. It has won the favor of overseas Chinese and has become a famous export tea with Chinese characteristics [2]. Insect tea is also known as "tea essence". It is not a kind of tea, but is made of Kuding tea, which is slightly boiled to remove astringency, dried 70% and put in the bottom of the cage, and the dried excretion of a larva named "rice borer" after eating Kuding tea [3].

The raw material of Insect tea is Kuding tea, it is an evergreen tree of *Ilex Kudingcha*. It is mainly distributed in Guangdong, Fujian and other places in China. It is also a traditional pure natural beverage [4]. Kuding tea contains more than 200 components such as Kuding saponin, amino acid, vitamin C, polyphenols, flavonoids, caffeine, protein, etc. It has the functions of lowering blood lipid, increasing coronary blood flow, increasing myocardial blood supply and anti-atherosclerosis [5]. Kuding tea, the raw material of insect tea, recorded in the Chronicle of Sichuan Traditional Chinese Medicine, has the functions of soothing wind and clearing heat, promoting eyesight and nourishing body fluid, and can treat wind-heat headache, toothache, aphtha, fever, thirst, diarrhea and dysentery [6].

In recent years, the research on Insect tea mainly focused on the simple detection and analysis of some of its components, such as amino acids, tea polyphenols, minerals, etc [7]. There are few studies on its elimination of carcinogenic factors and anti-aging. As a traditional Chinese medicine, Kuding tea, the raw material of Insect tea, mainly studies its pharmacological effects on various diseases and

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its quality control in the production process [8]. The research on polyphenols mainly includes the extraction technology and improvement of natural polyphenols, and the multiple pharmacological effects of polyphenols on the body [9]. *In vitro* antioxidant test is mostly used as a basic test for tea or other natural plant research to test the free radical scavenging ability of samples, reflecting the antioxidant ability of samples, and laying a foundation for further research on anti-aging effect of samples.

In this study, the content of polyphenols in the extracts of Insect tea from Guangxi, China, was determined. DPPH, ABTS and hydroxyl radical methods were used to determine the free radical scavenging capacities of Insect tea polyphenols, which laid a foundation for further research on antioxidant activities.

2. Materials and Methods

2.1 Extraction of Polyphenols from Insect Tea.

The 50 g insect tea were weighed and crushed into powder. Then, 50 mL 45% (volume ratio) ethanol solution was added to extract for 30 minutes at 90^{o}C. After repeated extraction for twice, the pH of the two extracts were combined and adjusted to 6.0, the mixture of $AlCl_3$ (3 g) and $ZnCl_2$ (6 g) were added for precipitation. After centrifugal separation (3000 r/min, 10 min), 100 mL hydrochloric acid (12% volume ratio) was added to the collected precipitation to dissolve. The supernatant was separated and extracted by adding 100 mL ethyl acetate twice. Finally, the extract was evaporated by rotating evaporation to obtain the extract of caterpillar tea polyphenols.

2.2 Determination of Content of Insect Tea Polyphenols.

The chlorogenic acid was dissolved in distilled water to prepare chlorogenic acid solution, then the chlorogenic acid solution was diluted to obtain different concentrations of chlorogenic acid solution. By Folin-Ciocalteu colorimetry method, after adding 3 mL Folin-Ciocalteu chromogenic reagent and 4.5 mL saturated Na_2CO_3 solution to 1 mL different concentration of chlorogenic acid solution, the volume of chlorogenic acid reached 25 mL. The absorbance value of the decolorized solution was measured at 747 nm. The standard concentration of chlorogenic acid was plotted with the absorbance value as X axis coordinate and the concentration of chlorogenic acid as Y axis coordinate. Insect tea polyphenols were dissolved in distilled water, the content of Insect tea polyphenols was determined by the above method.

2.3 Determination of Free Radical Scavenging Effect of Insect Tea Polyphenols by DPPH Method.

Reagents (A_1: 3.9 mL DPPH solution and 100 μL sample solution; A_2: 3.9 mL absolute ethanol and 100 μL sample solution; A_3: 3.9 mL DPPH solution and 100 μL absolute ethanol) were added to Insect tea polyphenol sample, and the reaction was carried out for 30 minutes without light. The final reaction solution (200 μL) was added to 96-well plate. The absorbance value was measured at 517 nm and the free radical scavenging rate was calculated by formula: DPPH (%)= $(A_3-(A_1-A_2))/A_3\times100$.

2.4 Determination of Free Radical Scavenging Effect of Insect Tea Polyphenols by ABTS Method.

Reagents (A_1: 5.0 mL ABTS solution and 200 μL sample solution; A_2: 5.0 mL absolute ethanol and 200 μL sample solution; A_3: 5.0 mL ABTS solution and 200 μL absolute ethanol) were added to Insect tea polyphenol sample, and the reaction was carried out for 6 minutes without light. The final reaction solution (200 μL) was added to 96-well plate. The absorbance value was measured at 734 nm and the free radical scavenging rate was calculated by formula: ABTS (%)= $(A_3-(A_1-A_2))/A_3\times100$.

2.5 Determination of Free Radical Scavenging Effect of Insect Tea Polyphenols by Hydroxyl Radical Method.

Reagents (A_1: 300 μL of 80% methanol and 2.0 mL of $FeSO_4$ solution and 1.0 mL salicylic acid

ethanol solution and 1.0 mL H_2O_2 solution; A_2: 300 μL of sample solution and 2.0 mL of $FeSO_4$ solution and 1.0 mL salicylic acid ethanol solution and 1.0 mL H_2O_2 solution; A_3: 300 μL of sample solution and 2.0 mL of $FeSO_4$ solution and 1.0 mL salicylic acid ethanol solution and 1.0 mL of 80% methanol) were added to Insect tea polyphenol sample and heated for 30 min in a water bath at 37°C. The final reaction solution (200 μL) was added to 96-well plate. The absorbance value was measured at 510 nm and the free radical scavenging rate was calculated by formula: •OH (%)= $(A_3-(A_1-A_2))/A_3\times100$.

2.6 Data Statistics.

After repeated three times, the three parallel experiments were averaged, and then the one-way ANOVA method was used to analyze whether the data of each group had significant differences at the level of $P < 0.05$ by using SAS 9.1 statistical software.

3. Results

3.1 Content of Insect Tea Polyphenols.

The standard curve of absorbance of standard chlorogenic acid was drawn as Y = 199.28X - 3.3411 (R^2 = 0.9975, Y is chlorogenic acid content, X is absorbance value) (Figure 1). Compared with the standard curve of chlorogenic acid, the content of Insect tea polyphenols could reach 71.2% (chlorogenic acid meter). The results showed that the content of Insect tea polyphenols was high, and the key substance in the follow-up experiments was Insect tea polyphenols.

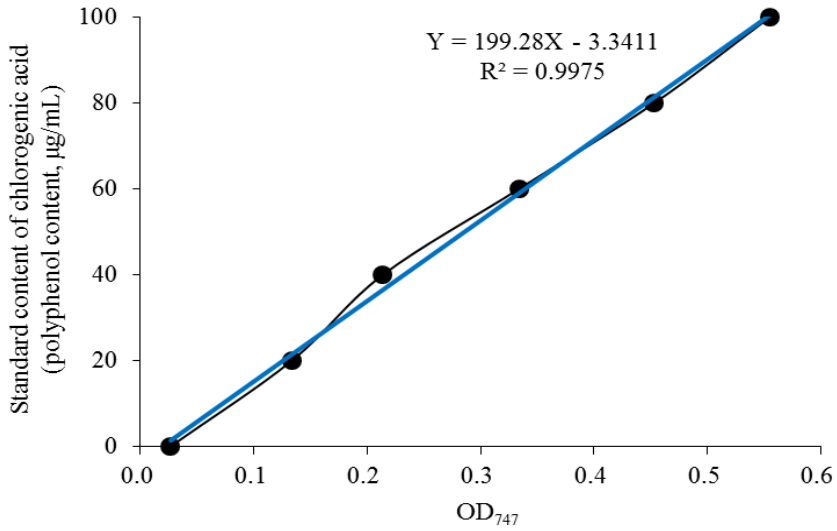

Figure. 1 Standard content of chlorogenic acid (polyphenol content).

3.2 DPPH Free Radical Scavenging Effect of Insect Tea Polyphenols.

The results showed that Insect tea polyphenols had strong scavenging effect on DPPH free radicals (Figure 2). With the increase of Insect tea polyphenols concentration, their scavenging abilities to DPPH free radicals were enhanced. When insect tea polyphenols concentration were 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL, their scavenging rates of DPPH free radicals were 43.5%, 56.2%, 63.7%, 71.9% and 84.9%, respectively.

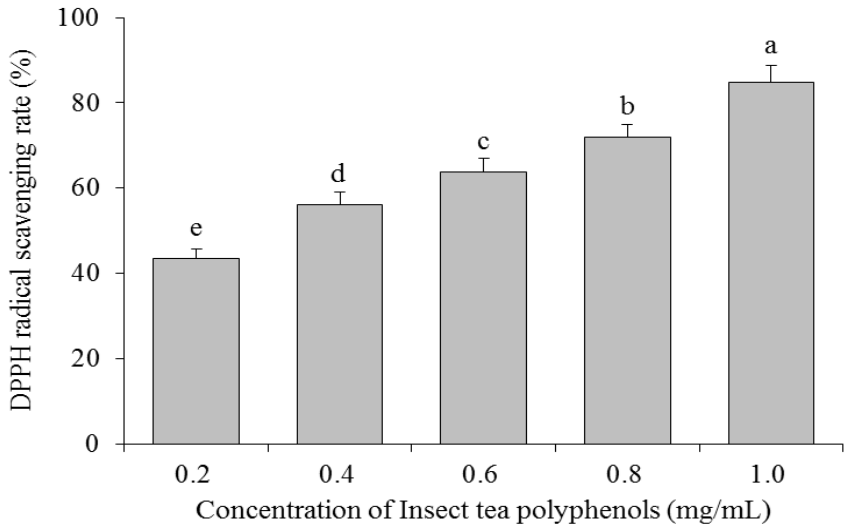

Figure. 2 DPPH free radical scavenging effect of Insect tea polyphenols. [a-e] Mean values with different letters indicate significant differences ($P < 0.05$)

3.3 ABTS Free Radical Scavenging Effect of Insect Tea Polyphenols.

The results showed that Insect tea polyphenols also had good ABTS free radical scavenging ability (Figure 3). In the range of 0.2-1.0 mg/mL, the concentrations of Insect tea polyphenols were positively correlated with their ABTS free radical scavenging abilities. When Insect tea polyphenols were 0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL, the scavenging rates of ABTS free radical were 33.2%, 41.2%, 49.8%, 56.4% and 62.1% from low to high.

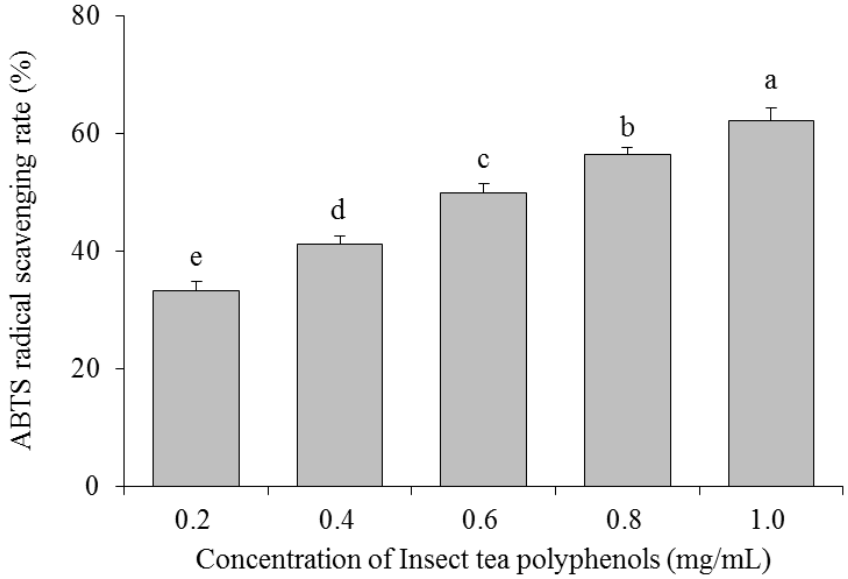

Figure. 3 ABTS free radical scavenging effect of Insect tea polyphenols. [a-e] Mean values with different letters indicate significant differences ($P < 0.05$)

3.4 Hydroxyl Radical Scavenging Effect of Insect Tea Polyphenols.

The results showed that Insect tea polyphenols also had scavenging effect on hydroxyl radicals (Figure 4). When the concentration of Insect tea polyphenols increased, the scavenging effect of caterpillar tea on hydroxyl radicals also increased. When the concentration of Insect tea polyphenols was 1.0 mg/mL, the scavenging rate of Insect tea polyphenols on hydroxyl radicals reached 47.8%, which was higher than those of Insect tea polyphenols at 0.8 (34.8%), 0.6 (29.7%), 0.4 (22.8%) and 0.2 mg/mL (15.4%).

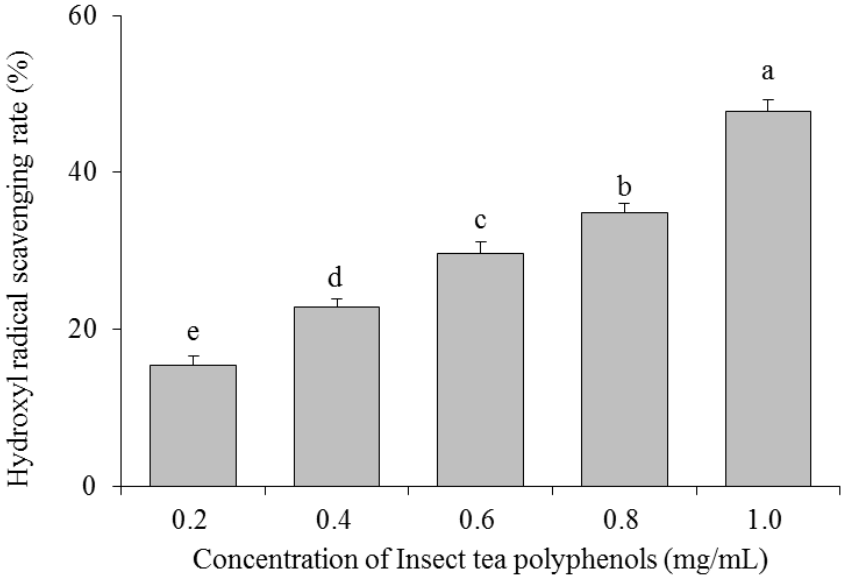

Figure. 4 Hydroxyl radical scavenging effect of Insect tea polyphenols. [a-e] Mean values with different letters indicate significant differences ($P < 0.05$)

4. Discussion

Reactive oxygen species (ROS) mainly include hydroxyl radicals, superoxide radicals and hydrogen peroxide. Reactive oxygen species are produced in normal physiological metabolism of cells or tissues [10]. Some environmental factors, such as ultraviolet radiation, gamma-ray radiation, smoking and environmental pollution, can also induce ROS production. Reactive oxygen species (ROS) can lead to oxidative stress of lipids, proteins and DNA in cells, and then lead to various tumors, atherosclerosis, rheumatoid arthritis, diabetes, liver damage, and central nervous system diseases [11]. There are many kinds of antioxidants in the body, including antioxidant macromolecules, antioxidant small molecules and enzymes, which can remove all kinds of reactive oxygen species produced in the body to prevent the production of oxidative stress induced by ROS [12]. The total level of antioxidant macromolecules, small antioxidant molecules and enzymes in a system reflects the total antioxidant capacity of the system. Therefore, the determination of total antioxidant capacity in plasma, serum, urine, saliva and other body fluids, cells or tissues has very important biological significance [13].

DPPH is a stable free radical centered on nitrogen. When DPPH meets strong free radical scavenger, the single electron of DPPH is captured, the color of the solution becomes lighter, and the absorbance at the maximum light absorption wavelength decreases. The antioxidant effect of the substance *in vitro* can be preliminarily judged by measuring the photometric value [14]. According to the principle of ABTS antioxidant experiment in vitro, samples with antioxidant properties can fade the solution. The absorbance decreases with the increase of antioxidant activity [15]. Hydroxyl free radical is a kind of reactive oxygen species. Hydroxyl free radical can kill red blood cells, degrade DNA, cell membrane and polysaccharide compounds. Inhibiting hydroxyl free radical can effectively protect the body from damage and play an antioxidant role [16]. In this study, Insect tea polyphenols showed good DPPH, ABTS and hydroxyl free radical scavenging capacity, and the effect was concentration-dependent, which confirmed that Insect tea polyphenols had good antioxidant effect *in vitro*.

5. Summary

In this study, DPPH method, ABTS method and hydroxyl radical method were used to detect the antioxidant effect of Insect tea polyphenols *in vitro*. The results showed that Insect tea polyphenols

had obvious scavenging effectS on these three free radicals and showed good antioxidant effects. It can be seen that Insect tea polyphenols could delay aging, reduce the incidence of free radical-induced diseases such as heart disease, eye disease, cancer, and enhance human immunity through its antioxidant capacity.

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