Evaluation of Insect Tea Extracts' in Vitro Antioxidant Activities: A Comparative Study with Vitamin C

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Insect tea is a fascinating variety of tea in China, created through the intricate process of utilising the faecal pellets of particular insects that have consumed specific plants and then undergoing digestion or fermentation. Insect tea is renowned for its unique taste and numerous health advantages. This study aims to scientifically evaluate the antioxidant effects of insect tea samples. It focuses on experimental analysis to investigate the antioxidant activity of insect tea extracts. This study examines the impact of varying concentrations of insect tea extracts and a positive control (vitamin C) on total reducing power and the ability to scavenge DPPH•, ABTS+•, and OH radicals. Based on the findings, it is evident that the insect tea extract possesses significant reducing power at a concentration of 0.05 mg/mL. The extract exhibits impressive scavenging rates for DPPH•, ABTS+•, and •OH radicals, with percentages of 29.23%, 25.42%, and 11.11%, respectively. In this concentration, the insect tea extract demonstrates the most potent scavenging ability for DPPH• radicals. As the concentration of insect tea extract is increased to 0.40 mg/mL, the total reducing power reaches 1.211. This is accompanied by scavenging rates of 91.51%, 97.70%, and 69.99%, respectively. These results suggest that the antioxidant capacity improves as the concentration of insect tea extract increases. In vitro antioxidant activity of insect tea extract has been observed at a concentration of 0.05 mg/mL, and its antioxidant effect becomes more pronounced as the concentration increases.

Keywords: Antioxidant Activity, DPPH Radicals; Insect Tea, ABTS Radicals; Hydroxyl Radicals; Extract Concentration.

Introduction

Redox reactions play a crucial role in supplying energy to cells for their proper functioning. However, certain factors like pollution, smoking, inadequate nutrition, and harmful consumption can lead to an excessive production of free radicals within the body. Illnesses like diabetes, cancer, cirrhosis, and various cardiac and neurological issues are clear examples of conditions associated with an irregular and uncontrolled process of radical production. In addition, they can contribute to oxidative damage of cells by generating advanced glycation end products, inhibiting enzymes, affecting DNA chromosomes, and disrupting membrane lipids (Peng et al., 2022).

In the realm of academia, oxidative stress is a widely recognised concept that emerges early in life. Nevertheless, it has a detrimental impact on human health. It exacerbates various diseases such as hypertension, Parkinson's, and others (Hohensinner et al., 2018). Furthermore, an excessive accumulation of active yet free radicals in the body can result in oxidative damage, as mentioned earlier, which in turn leads to oxidative stress. Therefore, maintaining proper regulation of oxidative levels is essential for ensuring the overall health and functioning of the body. It is crucial to maintain a delicate equilibrium between oxidants and pro-oxidants in order to generate reactive oxygen species. Nevertheless, it can be produced in the absence of antioxidants (Yi et al., 2020).

Antioxidants have the ability to stabilise or break down various reactive oxygen species, such as hydroxyl radical, ferryl ion, superoxide radical anion, peroxyl radical, and hydrogen peroxide (Tijjani et al., 2022). Given their propensity for being triggered by oxidative stress, they can ultimately wreak havoc on cells and other biomolecular targets. Antioxidants are widely recognised as crucial for maintaining overall health and well-being. Teas are widely acknowledged as beverages that are commonly enjoyed for their advantageous antioxidant properties. Tea has been enjoyed for generations, becoming a cherished tradition. There are numerous types of tea that are recognised for their health benefits. Insect tea is a well-known traditional beverage in southern China that is highly regarded for its numerous health benefits (Zhao et al., 2018).

Insects are a diverse group of invertebrates classified into various classes within the animal kingdom, including Insecta and Anthropoda. The insect population varies from 2.6 to 7.8 million (Oghenesuvwe & Paul, 2019). According to a prior study, the estimated number of insect species is 5.5 million. This kingdom is classified into 29 orders based on its largest group. The presence of these organisms is widespread, encompassing trees, water, soil, and deserts. However, their availability fluctuates with the changing seasons and weather conditions. The assumption that insects are pests that can harm the environment and living beings is widespread across regions (Omotoso, 2015). According to Mans et al. (2016), scholars have found that humans, plants, and animals can benefit from the use of these substances in human and veterinary medicines, as well as in agricultural practices. Entomotherapy refers to the use of insects in disease treatment. This practice is recognised and acknowledged globally, including in countries such as China, India, Mexico, and Nigeria (Igwe, 2014). Scholars have confirmed the medical and wellness benefits of insects through clinical and non-medical methods. Insect bioactive compounds offer a broad range of health benefits, including the treatment of cancer, ulcers, diabetes, hyperlipidemia, and heart diseases. There are insect species that have been found to have medicinal properties for treating wounds, bacterial infections, intestinal gas, bleeding, spasms, and lung disorders (Afam & Rinah, 2017).

Insect tea, also known as ChongShiCha in China, is a traditional beverage or medicine used by ethnic minorities in southwestern China (Zhang et al., 2022). The tea is considered a distinctive beverage of China, symbolising Chinese culture (Wang et al., 2021). The consumption of this substance has been practiced for centuries and is believed to have benefits for digestive health, heat and dampness dissipation, spleen function maintenance, and body detoxification. Insect tea is popular in tropical regions such as Singapore and Malaysia for its notable effects (Zhu et al., 2019). The process of producing insect tea is highly specific. Insect larvae consume leaves and produce excrement. The waste is further processed to produce insect tea. Furthermore, the tea is impacted by insect species, and the quality of the tea depends on the specific food chosen by the insects for their larvae. In recent years, academia has documented various claims about the pharmacological properties of this substance, including its anti-oxidation and gastro-protective and hepatic-protective benefits. Insect tea is classified as a non-Camellia tea, unlike green, black, and mixed teas. The reason for this is the production of a substance extracted from the faeces of certain insects that feed on specific leaves (Yuan et al., 2021).

Insect tea is a distinct type of tea in China, produced from the excrement of specific insect larvae that consume fermented tree leaves from certain plants (Ya-Feng & Li-Zhang, 2019). The substance comprises nutrients including crude protein, crude fat, sugars, tannins, vitamins, and 18 essential amino acids necessary for human health. Insect tea is a distinct beverage of local ethnic minorities, primarily produced in the upstream Yangtze River region in Hunan, Guangxi, Guizhou, Guangdong, and Sichuan. The medicinal use of insect faeces, specifically tea insect faeces, was documented in the year 992 AD in the "Taiping Holy Prescription." Li Shizhen also recorded its medicinal use in "Compendium of Materia Medica."The user's text is missing. Please provide the text you would like to have rewritten (Lei & Lu, 2001).

The utilisation of various types of plants and insects to prepare insect teas is argued to occur across different regions. The quality features of insects, such as colour, taste, aroma, and chemical components, vary depending on the type of insect. Some argue that its nutritional values are higher compared to regular tea. This is because this type of tea contains various beneficial components such as crude fat, protein, amino acids, and carbohydrates. The presence of trace elements in tea contributes to its uniqueness, as these elements contain an abundance of polyphenols that are beneficial for the body. According to animal experts, insects have higher antioxidant capabilities compared to hawks and other teas. Furthermore, the polyphenols found in incest inhibit enzymatic activity, leading to elevated lipid peroxidation and ultimately resulting in an anticancer effect (Zhao et al., 2018). Scholars have found that insect tea has a lasting effect on reducing blood pressure and sugar levels. A study found that this type of tea promotes programmed cell death of TCA8113 human tongue squamous cell carcinoma cells. A recent study found that insect tea has a significant effect on mouse buccal mucosal cancer. Moreover, it hinders the growth of human breast cancer cells.

Insect tea possesses various notable functions, such as relieving fatigue, improving vision, alleviating irritability, invigorating the body, reducing blood pressure, cooling the blood, quenching thirst, aiding digestion, strengthening the spleen, and alleviating fatigue and pain. When combined with conventional teas, it improves fragrance, flavour, and nutritional content (Yuan et al., 2021).

Energy metabolism in organisms can lead to the production of free radicals, resulting in oxidative reactions that can cause damage and lesions to different body components and organs (Marx, 1987). Hydroxyl radicals (•OH) are highly damaging oxygen radicals that can react with all cellular components due to their exceptional oxidising power (Ao, 2010). Currently, it is established that approximately one hundred diseases, including arteriosclerosis, heart disease, tumours, and ageing, are associated with oxidative stress (Zhao, 2008).

Insect tea is rich in flavonoids, polyphenols, polysaccharides, and other active ingredients with natural antioxidant properties. The insect tea contains polyphenolic substances that have potent antioxidant properties, can prevent lipid peroxidation, and have demonstrated anti-mutagenic and anticancer effects (Wang & Zhang, 2008). The antioxidant effects of insect tea have not been scientifically proven, but it is already being sold in the market as a local speciality (Zhao & Li, 2015). This study examines insect tea samples from Liubao, Guangxi, and uses vitamin C as a control in order to conduct in vitro antioxidant experiments. The study compares the effects of various concentrations of insect tea extracts and their corresponding controls on total reducing power and the ability to scavenge DPPH•, ABTS+•, and •OH free radicals. This study's findings will provide a basis for future research on the antioxidant properties of insect tea and its potential health benefits.

Materials and Methods

Chemicals and Instruments

The Preparation of Insect Tea Extracts

In accordance with the approach employed by Xiang et al. (2018), insect tea samples were pulverised using a grinder and then passed through a 40-mesh sieve. The insect tea powder was sieved and then mixed with distilled water in a 1:20 ratio. The mixture was stirred with a glass rod and extracted in a 95°C water bath for 30 minutes. The mixture was filtered using a Buchner funnel under vacuum to eliminate impurities. The extraction process was repeated three times, and the combined filtrates were concentrated using a rotary evaporator at 50°C. The concentrated extract was freeze-dried to obtain the insect tea extract, which was stored in a refrigerator for later use. Prior to conducting the experiments, the sample extracts and vitamin C were prepared as solutions with concentrations ranging from 0.05 mg/mL to 0.40 mg/mL using distilled water.

The Determination of Total Reducing Power of Insect Tea Extracts

Xiao (1997) conducted an experiment in which she placed insect tea extract solutions and vitamin C solutions of varying concentrations (2 mL each) in separate test tubes. A phosphate buffer solution with a pH of 6.6 and a 1% potassium ferricyanide solution, both measuring 2.5 mL, were combined and thoroughly mixed by vigorous shaking. The resulting mixture was then incubated at 50°C for a duration of 20 minutes. Following rapid cooling to room temperature, 2.5 mL of trichloroacetic acid (10% concentration) was added, thoroughly mixed by shaking, and then centrifuged at 4000 rpm for 10 minutes. The supernatant (2.5 mL) was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride in test tubes. The absorbance at a wavelength of 700 nm was measured using a spectrophotometer after thorough mixing. A control experiment was conducted using 95% ethanol instead of the extract solution. The experiment was conducted in triplicate using various concentrations of insect tea extract and vitamin C solutions. The average value was calculated.

Table 1: The List of Chemicals and Instruments with Respective Manufacturers.

Chemicals and Instruments	Manufacturers
RE-52 AA Rotary Evaporator	Shanghai Yarong Biochemical Instrument Factory
SCIENTZ-18N Freeze Dryer	Ningbo Xinzhi Biotechnology Co., Ltd.
UV-1800 PC UV-Vis Spectrophotometer	Shanghai Mapada Instrument Co., Ltd.
HH-S6 Constant Temperature Water Bath	Gongyi Yuhua Instrument Co., Ltd.
SHZ-D(Ⅲ) Circulating Water Vacuum Pump	Gongyi Yuhua Instrument Co., Ltd.
80-2 Benchtop Low-speed Centrifuge	Jiangsu Zhengji Instrument Co., Ltd.
Standard L-ascorbic acid (Vc)	Aladdin Reagent Co., Ltd., Shanghai
2,2-Diphenyl-1-picrylhydrazyl (DPPH)	Hefei Bomei Biotechnology Co., Ltd.
Ortho-phenanthroline	Tianjin Damao Chemical Reagent Factory
2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)	Hefei Bomei Biotechnology Co., Ltd.
3% Hydrogen peroxide solution	Guangdong Hengjian Pharmaceutical Co., Ltd.
Potassium ferricyanide	Tianjin Damao Chemical Reagent Factory
Trichloroacetic acid	Tianjin Damao Chemical Reagent Factory
Ferric chloride	Tianjin Damao Chemical Reagent Factory
Ferrous sulfate	Tianjin Damao Chemical Reagent Factory
Potassium persulfate	Yonghua Chemical Co., Ltd.
95% Ethanol	Tianjin Damao Chemical Reagent Factory
Methanol	Tianjin Fuyu Fine Chemical Co., Ltd.
Potassium dihydrogen phosphate	Tianjin Fuchen Chemical Reagent Factory
Potassium hydrogen phthalate	Tianjin Kemiou Chemical Reagent Co., Ltd.
Sodium hydroxide	Tianjin Damao Chemical Reagent Factory
Anhydrous ethanol	Tianjin Fuyu Fine Chemical Co., Ltd.
Sodium dihydrogen phosphate	Tianjin Kemiou Chemical Reagent Co., Ltd.
Sodium hydrogen phosphate	Tianjin Kemiou Chemical Reagent Co., Ltd.

The Determination of DPPH Radical Scavenging Activity of Insect Tea Extracts

Li et al. (2015) conducted an experiment where they added insect tea extract solutions and vitamin C solutions (3.0 mL each) to separate stoppered test tubes, using different concentrations. A 0.1 mmol/L methanol solution of DPPH (3.0 mL) was vigorously shaken and allowed to stand in the dark at room temperature for 30 minutes. The absorbance value (A1) was measured at a wavelength of 517 nm. A control (A2) was prepared by combining 3.0 mL of methanol with the insect tea extract and vitamin C solutions. The mixture was then incubated for 30 minutes and the absorbance was measured at 517 nm. A blank solution (A0) was created by combining 3.0 mL of methanol with a 0.1 mmol/L DPPH methanol solution. The mixture was then incubated for 30 minutes before measuring the absorbance at 517 nm. The DPPH radical scavenging rate was calculated with the formula below.

\text {DPPH Radical Scavenging Rate (%) = } \left(1 - \frac{(A1 - A2)}{A0} \right) \times 100

Notes:

A1 is the absorbance of the sample (insect tea extract or vitamin C solution) with DPPH.

A2 is the absorbance of the sample (insect tea extract or vitamin C solution) without DPPH (Control group).

A0 is the absorbance of the blank (methanol with DPPH solution).

ABTS+ Radical Scavenging Ability of Insect Tea Extracts

Li Xiaofei (2017) conducted a study where insect tea extract solutions at various concentrations and a vitamin C solution were tested in triplicate. The ABTS+• radical solution was prepared by combining 88 µL of a 140 mmol/L potassium persulfate solution with 5.0 mL of a 7 mmol/L ABTS+• solution. The mixture was then incubated in darkness for 14 hours. The ABTS+• solution was diluted with distilled water to achieve an absorbance of 0.70 (±0.02) at 734 nm. For the experiment, 1.0 mL of insect tea extract or vitamin C solution was combined with 4.0 mL of the ABTS+• solution in test tubes. The mixture was then incubated in the dark at room temperature for 30 minutes. The spectrophotometer was used to measure the absorbance at 734 nm (A1). A0 denotes the absorbance value obtained when 1.0 mL of either insect tea extract or vitamin C solution was substituted with distilled water. A2 represents the absorbance value obtained when a 4.0 mL ABTS+• solution is mixed with 1.0 mL of distilled water. The ABTS+• radical scavenging percentage (%) was calculated using the formula:

$$\text{ABTS+• radical scavenging rate (\%) = } \left[\frac{A2 - (A1 - A0)}{A2} \right] \times 100$$

Notes:

A0 is the absorbance of the control (ABTS+ solution with distilled water).

A1 is the absorbance of the sample (insect tea extract or vitamin C solution with ABTS+ solution)

The Hydroxyl Radical (-OH) Scavenging Ability of Insect Tea Extracts

The experimental procedure followed the methods described by Xie (2014). In this study, various concentrations of insect tea extract and vitamin C solution (1.0 mL each) were combined with 1.0 mL of pH 7.4 phosphate buffer, 2.5 mmol/L pyrogallol solution, and 2.5 mmol/L ferrous sulphate solution. A 0.5 mL aliquot of a 20 mmol/L hydrogen peroxide solution was added to the mixture, which was then incubated at 37°C for 1 hour. The spectrophotometer was used to rapidly measure the absorbance at 536 nm (A2). In the case of blank samples, A1 was obtained by replacing the insect tea extract or vitamin C solution with 1.0 mL of absolute ethanol. Control samples were prepared by substituting 1.5 mL of absolute ethanol for the hydrogen peroxide solution, insect tea extract, or vitamin C solution to obtain A0. The hydroxyl radical (-OH) scavenging rate (%) was calculated with the formula below:

$$\text{Hydroxyl radical (·OH) scavenging rate (\%) =} \left[\frac{(A2 - A1)}{(A0 - A1)} \right] \times 100$$

Notes:

A0 is the absorbance of the control (without hydrogen peroxide and insect tea extract or vitamin C solution).

A1 is the absorbance of the test sample (with insect tea extract or vitamin C solution)

Data Analysis

All data were collected and analyzed using the SPSS statistical software. The significance of differences was set at $P<0.05$.

Results and Discussion

The Total Reducing Power of the Insect Tea Extracts and Vitamin C

The total reducing power of insect tea extract was evaluated using potassium ferricyanide, as shown in Table 2. Higher absorbance values indicate stronger antioxidant capacity. The results indicated a significant increase in absorbance values as the concentration of insect tea extract increased from 0.05 mg/mL to 0.40 mg/mL ($P < 0.05$), although they were still lower than those of vitamin C (Figure 1).

Insect Tea Extracts Ability to Scavenge DPPH Free Radicals

The absorption spectrum of DPPH radicals ranges from 390 nm to 780 nm. The detection method for this absorption is straightforward, fast, and highly consistent. DPPH radicals are stable synthetic radicals, which are commonly used to assess the antioxidant properties of insect tea solutions (Li et al., 2015). Figure 2 displays the outcomes of insect tea's scavenging of DPPH free radicals. The scavenging rate increases with the concentration of insect tea extract at the chosen low concentrations for this experiment. The scavenging rate increases by 62.28% when the concentration of insect tea extract reaches 0.40 mg/mL, compared to the scavenging rate of 29.23% at 0.05 mg/mL. The insect tea extract concentrations ranging from 0.05 mg/mL to 0.25 mg/mL exhibit statistically significant differences ($P < 0.05$). The scavenging rate of insect tea extract is consistently lower than that of vitamin C at all concentrations. However, in the concentration range of 0.30 mg/mL to 0.40 mg/mL, the scavenging rate of insect tea gradually approaches that of vitamin C (Table 3).

The Scavenging of ABTS+· Free Radicals of Insect Tea Extracts and Vitamin C

The scavenging of ABTS+• free radicals by insect tea extract is illustrated in Figure 3. The antioxidant efficacy of insect tea in scavenging ABTS+• free radicals is enhanced after digestion and fermentation, even at low concentrations of the extract used in this experiment. The scavenging rate increases with the concentration of insect tea extract, peaking at 97.70% when the concentration is 0.40 mg/mL. This indicates a 72.28% increase in the scavenging rate compared to the rate of 25.42% at a concentration of 0.05 mg/mL. The scavenging rates for insect tea extract concentrations ranging from 0.05 mg/mL to 0.30 mg/mL exhibit statistically significant differences ($P < 0.05$). The scavenging activity difference between insect tea extract and vitamin C solution gradually diminishes (Table 4)

The Hydroxyl Radical (-OH) Scavenging of Insect Tea Extracts and Vitamin C

The antioxidant performance of insect tea extract solution can be evaluated by its ability to scavenge •OH radicals. The Fenton reaction is the primary source of •OH in biological systems. The addition of a scavenger of •OH radicals to the reaction system allows for the sensitive detection of the direction of the chemical reaction through the colour change of the redox indicator o-phenanthroline-ferrous ion. The solution's red colour lightens, indicating a reduction in ferrous ions and the scavenging of •OH radicals. Figure 4 displays the results of the scavenging effect of insect tea extract on hydroxyl radicals (•OH). The scavenging rates of the chosen concentrations of insect tea extract in this experiment were all higher than that of vitamin C. The scavenging rate showed a positive correlation with concentration within the range of 0.05 mg/mL to 0.40 mg/mL. The scavenging rate increased by 58.88% when the concentration of insect tea extract reached 0.40 mg/mL, compared to the scavenging rate of 11.11% at a concentration of 0.05 mg/mL. The concentration gradients of insect tea extract at intervals of 0.1 mg/mL exhibited significant differences ($P < 0.05$) (Table 5).

Table 2: The Absorbance Values of Insect Tea Extracts and Vitamin C According to Respective Concentrations.

Concentration (mg/mL)	Absorbance of Insect Tea Extracts	Absorbance of Vitamin C
0.05	0.206 ± 0.092	0.547 ± 0.207
0.10	0.429 ± 0.111	1.278 ± 0.250
0.15	0.593 ± 0.065	1.433 ± 0.083
0.20	0.702 ± 0.230	1.483 ± 0.168
0.25	0.796 ± 0.327	1.527 ± 0.202
0.30	0.931 ± 0.281	1.569 ± 0.172
0.35	1.075 ± 0.353	1.598 ± 0.130
0.40	1.211 ± 0.494	1.649 ± 0.111

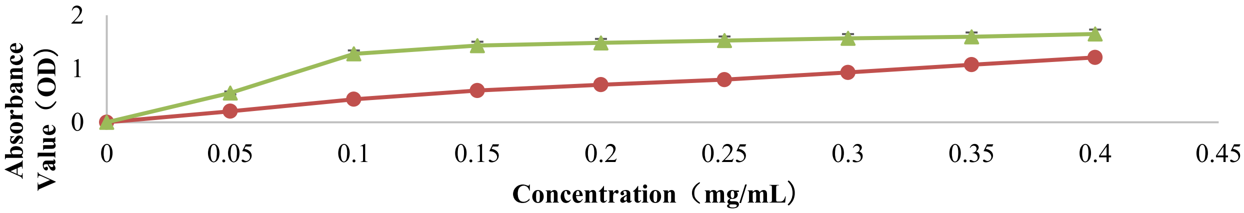

Figure 1: The Reducing Power of Insect Tea Extracts (Red Line) and Vitamin C (Green Line).

Table 3: The Scavenging of DPPH Free Radicals of Insect Tea Extracts and Vitamin C According to Respective Concentrations

Concentration (mg/mL)	Scavenging Rate (%) of Insect Tea Extracts	Scavenging Rate (%) of Vitamin C
0.05	29.23 ± 2.13	43.86 ± 16.86
0.10	46.04 ± 5.38	84.82 ± 13.01
0.15	60.43 ± 1.07	90.50 ± 1.74
0.20	75.45 ± 15.38	92.51 ± 1.82
0.25	84.52 ± 8.47	94.19 ± 1.29
0.30	88.16 ± 7.34	93.99 ± 0.83
0.35	91.33 ± 1.49	94.70 ± 1.11
0.40	91.51 ± 1.30	94.77 ± 1.29

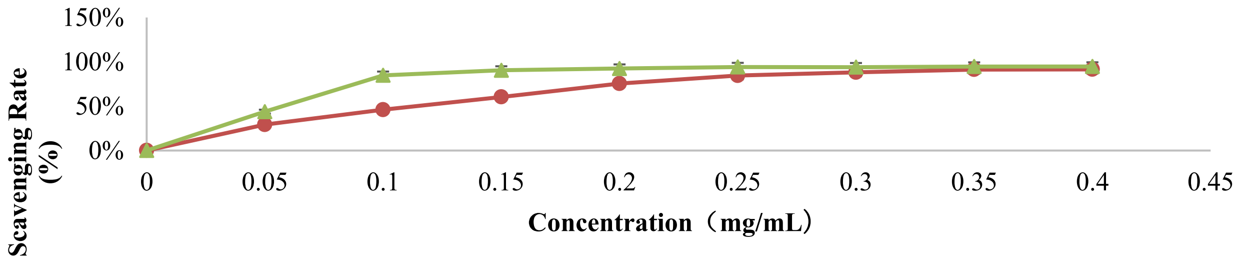

Figure 2: The Scavenging of DPPH Free Radicals of Insect Tea Extracts (Red Line) and Vitamin C (Green Line).

Table 4: The Scavenging of ABTS+• Free Radicals of Insect Tea Extracts and Vitamin C According to Respective Concentrations.

Concentration (mg/mL)	Scavenging Rate (%) of Insect Tea Extracts	Scavenging Rate (%) of Vitamin C
0.05	25.42 ± 3.87	32.93 ± 19.14
0.10	42.23 ± 2.41	51.88 ± 25.58
0.15	55.60 ± 6.99	71.79 ± 48.86
0.20	66.75 ± 12.61	86.86 ± 34.63
0.25	73.86 ± 9.42	96.15 ± 16.55
0.30	86.59 ± 3.61	97.88 ± 9.14
0.35	94.04 ± 2.66	99.96 ± 0.18
0.40	97.70 ± 2.71	100.00 ± 0.00

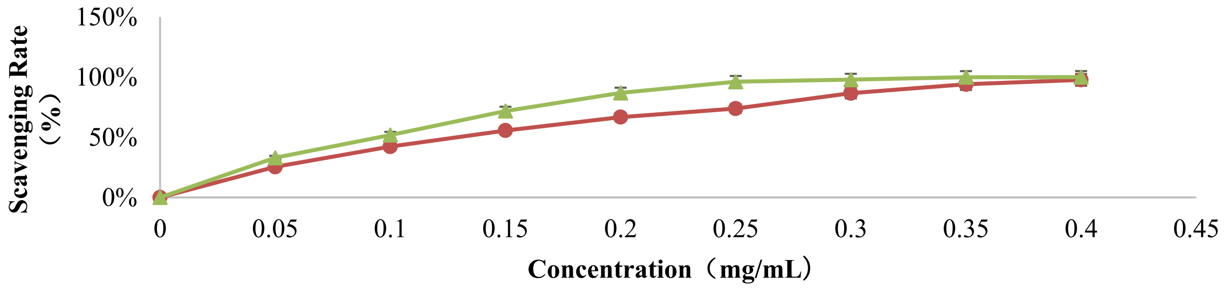

Figure 3: The Scavenging of ABTS+• Free Radicals of Insect Tea Extracts (Red Line) And Vitamin C (Green Line).

Table 5: The Scavenging of Hydroxyl Radicals (-OH) of Insect Tea Extracts and Vitamin C According to Respective Concentrations

Concentration (mg/mL)	Scavenging Rate (%) of Insect Tea Extracts	Scavenging Rate (%) of Vitamin C
0.05	11.11±9.05	8.76±14.91
0.10	17.64±15.86	15.04±11.78
0.15	27.90±14.37	17.23±24.99
0.20	35.64±16.51	17.58±23.42
0.25	43.50±23.23	20.69±30.92
0.30	46.80±12.74	25.00±18.52
0.35	60.95±12.95	28.11±18.28
0.40	69.99±12.98	32.70±21.32

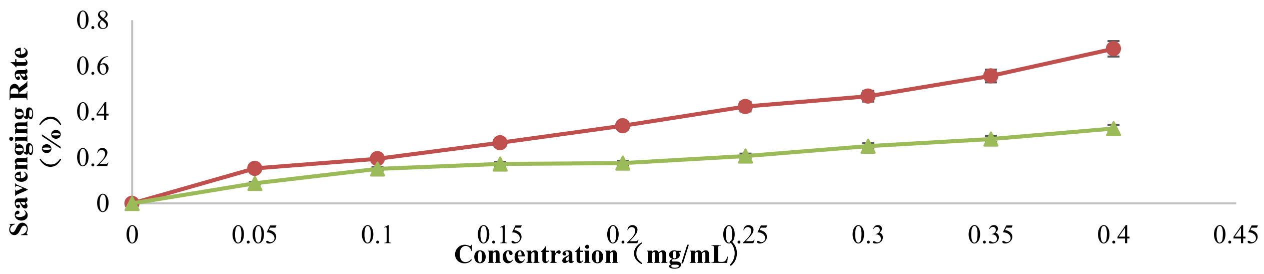

Figure 4: The Scavenging of Hydroxyl Radicals (-OH) of Insect Tea Extracts (Red Line) and Vitamin C (Green Line).

The current experiment confirms previous findings that insect tea extracts exhibit significant antioxidant activity in various reduction assays, including total reducing power, DPPH free radical scavenging, ABTS+• free radical scavenging, and OH radical scavenging. The total reducing power assay demonstrated an increase in enhancing concentration of insect tea extract from 0.05 mg/mL to 0.40 mg/mL. Higher concentrations, especially 40 mg/mL, exhibited higher mean absorbance values, indicating superior antioxidant activity (Sun, 2021). The insect tea extract exhibited lower absorbance values compared to ascorbic acid. However, the increasing trend suggests that it is still a suitable reducing agent. The DPPH free radical scavenging assay showed that the scavenging rate reached 91.51% at a concentration of 0.40 mg/mL for the insect tea extract. The insect tea extract exhibited a significant increase in free radical scavenging activities, from 29.23% at 0.05 mg/mL to 91.51% at 0.40 mg/mL. Although the antioxidant capacity of the insect tea extract was lower than that of vitamin C, it showed a promising trend (Ma et al., 2010).
The ABTS+• free radical scavenging assay and experiment yielded consistent results, showing that increasing concentrations of insect tea extracts enhanced antioxidant efficacy. The extract exhibited a scavenging rate of 97.70% at a concentration of 0.40 mg/mL, which was significantly higher than the scavenging rate of 25.42% at a concentration of 0.05 mg/mL. This suggests that the extract is effective in neutralising ABTS+• radicals. The results were supported by the hydroxyl radical (•OH) scavenging assay, which showed that the insect tea extract had a higher scavenging rate than vitamin C at all tested concentrations. The scavenging rate at a concentration of 0.40 mg/mL was 69.99%, significantly higher than the observed rate of 11.11% at a concentration of 0.05 mg/mL. The observed differences in scavenging rates among the concentration gradients of insect tea extract ($P < 0.05$) provide evidence for the strong antioxidant properties of insect tea. The results indicate that insect tea extract has significant antioxidant properties that increase with concentration. This suggests that it could be a valuable natural source of antioxidants with potential health benefits {Liu, 2013 #25}.

Conclusions

Free radicals are electrically charged chemical entities that are consistently active and have a destructive impact on the body. Free radicals serve a specific physiological role and their production and degradation are normally balanced and connected to immunity, signal transduction, and energy transfer. However, it is crucial to prioritise protection against excessive free radicals, as they can contribute to the development of various diseases and accelerate the ageing process. Antioxidants counteract the harmful effects by neutralising free radicals, as previously mentioned. The current synthetic antioxidants exhibit low effectiveness and toxic effects, leading to an increasing demand for natural antioxidants with minimal side effects that can be utilised in various fields.
The substance is composed of insect components that contain architectural units such as flavonoids, polyphenols, and polysaccharides, which exhibit antioxidant properties. The antioxidant properties of these components can vary depending on their chemical structure. Consequently, different antioxidant activities can be observed due to variations in the chemical constituents. This makes it challenging to fully evaluate the antioxidant activity of each component using a single method. The antioxidant effectiveness of substances is often evaluated in vitro using parameters such as reducing power, free radical scavenging, and scavenging of DPPH•, ABTS+•, and •OH free radicals.
This study evaluated the antioxidant activities of insect tea using various in vitro tests. The graphs demonstrate that increasing the volume of insect tea concentrate leads to higher total reducing power of the sample and enhanced ability to neutralise DPPH•, ABTS+•, and •OH radicals. The insect tea extract, at a concentration of 0.40 mg/mL, demonstrated a total reducing power of 1.211. It also exhibited scavenging rates of 91.51%, 97.70%, and 69.99% against DPPH•, ABTS+•, and •OH radicals, respectively. The results indicate a dose-response relationship, with the

antioxidant capacity of insect tea approaching that of vitamin C at higher concentrations. The results indicate that insect tea shows promise as a natural antioxidant and warrants further investigation and development.

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References

Afam, I. O. J., & Rinah, K. N. (2017). Selected Edible Insects and Their Products in Traditional Medicine, Food and Pharmaceutical Industries in Africa: Utilisation and Prospects. In M. Heimo (Ed.), *Future Foods* (pp. Ch. 3). IntechOpen. doi: https://doi.org/10.5772/intechopen.68330

Ao, C. (2010). Study on Nutritional Components and In Vitro Antioxidant Activity of Tea Brewed with Eagle Tea Worms. *Dissertation, Southwest University*. Retrieved from https://www.doc88.com/p-8055461635113.html

Hohensinner, P., Kaun, C., Ebenbauer, B., Hackl, M., Demyanets, S., Richter, D., et al. (2018). Reduction of premature aging markers after gastric bypass surgery in morbidly obese patients. *Obesity Surgery, 28*, 2804-2810. doi: https://doi.org/10.1007/s11695-018-3247-3

Igwe, C. (2014). Biochemical and Haematologic Effects of Intake of Macrotermes nigeriensis Fortified Functional Diet'CU. Igwe," AO. Ojiako,"JE. Okwara," AA Emejulu and" RN Nwaoguikpe" Department of Biochemistry, Federal University of Technology, Owerri, Nigeria "Department of Chemical Pathology, College of Health Sciences, Nnamdi Azikwe University, Nnewi, Nigeria. *Pakistan Journal of Biological Sciences, 17*(2), 282-286. doi: https://doi.org/10.3923/pjbs.2014.282.286

Lei, M., & Lu, X. (2001). China's worm tea resources and research status. *Food Science, 22*(11), 100-102. Retrieved from https://www.spkx.net.cn/CN/Y2001/V22/I11/100

Li, X., Wei, J., Gu, M., Ji, Y., & Zhang, W. (2015). Study on the antioxidant activity of partridge tea extract and HPLC-MS/MS analysis of antioxidant characteristic components. *Food Technology, 40*(10), 265-269. Retrieved from https://xueshu.baidu.com/usercenter/paper/show?paperid=857b851063c3d02a95e2827d006f7b5a

Li Xiaofei. (2017). *Study on the antioxidant, anti-inflammatory and anti-cancer properties of six major teas including white tea and yellow tea* South China Agricultural University). Retrieved from https://xueshu.baidu.com/usercenter/paper/show?paperid=157q0mr0hx7b0vy0hy140tq05y659899&site=xueshu_se

Ma, L., Qin, W., & Chen, X. (2010). Effects of lactic acid bacteria fermentation conditions on the antioxidant activity of burdock. *Chinese Food Additives,* (4), 77-82. Retrieved from https://www.doc88.com/p-3819918009945.html

Mans, D. R., Sairras, S., Ganga, D., & Kartopawiro, J. (2016). Exploring the global animal biodiversity in the search for new drugs–insects. *J Transl Sci, 3*(1), 371-386. doi: https://doi.org/10.15761/JTS.1000164

Marx, J. L. (1987). Oxygen Free Radicals Linked to Many Diseases: The oxygen free radicals, although made as by-products of normal oxygen-using reactions, nevertheless have a wide potential for causing cell injury. *Science, 235*(4788), 529-531. doi: https://doi.org/10.1126/science.3810154

Oghenesuvwe, E. E., & Paul, C. (2019). Edible insects bio-actives as anti-oxidants: current status and perspectives. *Journal of Complementary Medicine Research, 10*(2), 89-102. doi: https://doi.org/10.5455/jcmr.20190130100319

Omotoso, O. T. (2015). An Evaluation of the Nutrients and Some Anti-nutrients in Silkworm, Bombyxmori L.(Bombycidae: Lepidoptera). *Jordan Journal of Biological Sciences, 8*(1), 45-50. doi: https://doi.org/10.12816/0026947

Peng, X., He, X., Tang, J., Xiang, J., Deng, J., Kan, H., et al. (2022). Evaluation of the in vitro antioxidant and antitumor activity of extracts from Camellia fascicularis leaves. *Frontiers in Chemistry, 10*, 1035949. doi: https://doi.org/10.3389/fchem.2022.1035949

Sun, X. (2021). Ultrasound Preparation Technology and Functional Characteristics of Casein Phosphopeptide-Chitosan Self-assembly Loaded with Quercetin. *Dissertation, Jiangsu University*.

Tijjani, H., Jibrin, S., & Egbuna, C. (2022). Evaluation of in vitro antioxidant activities of selected commercial tea brands. *Gadau Journal of Pure and Allied Sciences, 1*(1), 18-26. doi: https://doi.org/10.54117/gjpas.v1i1.1

Wang, H., & Zhang, P. (2008). Relationship Between Free Radicals in Biological Organisms and Diseases." Science and Technology Information. *Science and Technology Information, 34*.

Wang, R., Sun, F., Ren, C., Zhai, L., Xiong, R., Yang, Y., et al. (2021). Hunan insect tea polyphenols provide protection against gastric injury induced by HCl/ethanol through an antioxidant mechanism in mice. *Food & Function, 12*(2), 747-760. doi: https://doi.org/10.1039/D0FO02677H

Xiang, Y., Liu, C., & Wang, Z. (2018). In Vitro Antioxidant Activity of Ethanol Extracts from Larval Feces of Golden and Silver Flower Moths. *Natural Product Research and Development, 30*(02), 280-285+250.

Xiao, C. (1997). Chinese Medicinal Chemistry. *Shanghai: Shanghai Scientific and Technological Publishing House*.

Xie, Y. (2014). *Optimization of extraction technology of papaya polysaccharide and its antioxidant activity* (Doctoral dissertation, Fuzhou University). Retrieved from https://xueshu.baidu.com/usercenter/paper/show?paperid=00ce889ec8ff5eae0a801bae7f30310f&site=xueshu_se

Ya-Feng, W., & Li-Zhang, W. (2019). A survey of the historical origins, medicinal value and food safety of Chinese insect tea. *Chinese Journal of Applied Entomology, 56*(5). doi: https://doi.org/10.7679/j.issn.2095-1353.2019.106

Yi, R., Chen, X., Li, W., Mu, J., Tan, F., & Zhao, X. (2020). Preventive effect of insect tea primary leaf (Malus sieboldii (Regal) Rehd.) extract on D-galactose-induced oxidative damage in mice. *Food Science & Nutrition, 8*(9), 5160-5171. doi: https://doi.org/10.1002/fsn3.1821

Yuan, D.-Y., Chen, J.-X., Zhang, F., Huang, S.-H., Peng, J.-R., & Wen, L.-X. (2021). The current situation and prospect of insect tea. *Tianjin Food Research Institute Co. Ltd, 42*(9), 198-204. doi: https://doi.org/10.12161/j.issn.1005-6521.2021.09.029

Zhang, J., Wang, N., Zhang, W., Chen, W., & Yu, H. (2022). UPLC-Q-Exactive-MS based metabolomics reveals chemical variations of three types of insect teas and their in vitro antioxidant activities. *LWT, 160*, 113332. doi: https://doi.org/10.1016/j.lwt.2022.113332

Zhao, W. (2008). Genotypic Differences and Environmental Effects on Antioxidant Properties of Sweet Potato. *Dissertation, Zhejiang University*. Retrieved from https://www.doc88.com/p-753222583268.html

Zhao, X., & Li, G. (2015). Comparison of the antioxidant effects of water extracts of worm tea and its raw material Kudingcha in vitro. *Food Industry, 36*(6), 4. Retrieved from https://xueshu.baidu.com/usercenter/paper/show?paperid=1m0d02u0tu3j0jd0e21q0ac0bv061723&site=xueshu_se

Zhao, X., Song, J.-L., Yi, R., Li, G., Sun, P., Park, K.-Y., et al. (2018). Comparison of antioxidative effects of Insect tea and its raw tea (Kuding tea) polyphenols in Kunming mice. *Molecules, 23*(1), 204. doi: https://doi.org/10.3390/molecules23010204

Zhu, K., Zeng, X., Tan, F., Li, W., Li, C., Song, Y., et al. (2019). Effect of insect tea on D-galactose-induced oxidation in mice and its mechanisms. *Food Science & Nutrition, 7*(12), 4105-4115. doi: https://doi.org/10.1002/fsn3.1278