Journal of Food Science, Nutrition and Health Vol.5 Issue 2 EWA Publishing
Available Online: 9 June 2026 DOI: 10.54254/3029-0821/2026.34281

# Study on the antioxidant and lipid-lowering activities of bioactive extracts from Hibiscus hamabo

***Jingjing Chen*, Haibo Chi***

Zhejiang Tourism and Health College, Zhoushan, China

*Corresponding Author. Email: 229045418@qq.com

**Abstract.** The ethanol extract of Hibiscus hamabo was successively partitioned using petroleum ether, chloroform, ethyl acetate, n-butanol, and water. Antioxidant activity was evaluated through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and hydroxyl radical-scavenging assays. In addition, a simulated human digestive environment was established to determine the binding capacities of the extracts toward three bile salts, including sodium glycocholate, using cholestyramine as a positive control. The results demonstrated that the ethyl acetate fraction exhibited the strongest free-radical scavenging activity, achieving more than 85% of the DPPH radical-scavenging activity of vitamin C, as well as the highest bile salt-binding capacity, reaching over 65% of that of cholestyramine. The ethyl acetate extract of Hibiscus hamabo showed significant in vitro antioxidant and hypolipidemic potential, indicating its promise as a high-quality raw material for the development of natural functional ingredients and its favorable prospects for industrial application.

**Keywords:** Hibiscus hamabo, systematic solvent extraction, DPPH radical, hydroxyl radical, antioxidant activity, hypolipidemic activity

## 1. Introduction

Hibiscus hamabo Sieb. et Zucc., a deciduous shrub belonging to the genus Hibiscus in the family Malvaceae, was identified by Professor Wentao Fan of Zhejiang Agricultural University as a rare and endangered plant species in Zhejiang Province. It is mainly distributed across the coastal islands of Zhejiang and Fujian in China, as well as other coastal regions of East Asia. As an important species for windbreak establishment, sand fixation, and saline-alkali soil improvement along the eastern coast of China, H. hamabo possesses a well-developed root system and strong salt tolerance. In addition, its large, brightly colored flowers and striking red autumn foliage confer considerable ornamental value. The bark fibers and seed oils of the plant also exhibit potential for industrial utilization. Although preliminary studies have explored its salt-tolerance mechanisms and ecological applications, systematic investigations of its bioactive constituents for medicinal and health-promoting purposes remain limited. In particular, research concerning its anti-inflammatory, antioxidant, and hypolipidemic properties is still lacking [1].

With the continuous increase in the incidence of cardiovascular diseases, hyperlipidemia has become a major threat to human health. Modern medical research has demonstrated that dyslipidemia is closely associated with lipid peroxidative damage induced by excessive free radical production. Although statins and

fibrates are widely used in clinical practice and exhibit well-established therapeutic efficacy, their use is often accompanied by adverse effects, including liver function impairment. Consequently, the search for highly effective and low-toxicity bioactive compounds from natural plants has become an important research focus. Existing studies have shown that plant-derived compounds possess considerable potential in regulating lipid metabolism [2–6]. Moreover, natural antioxidants have attracted increasing attention because of their high safety profile and abundant availability, offering promising approaches for scavenging excessive free radicals and mitigating oxidative stress-related damage.

Against this background, the present project utilizes the abundant H. hamabo resources available in Zhoushan as experimental materials. Building upon previous studies of its salt tolerance and ecological adaptability, this research focuses on exploring its largely untapped medicinal and health-care potential. Using an in vitro simulated digestion model, the study systematically evaluates the radical-scavenging activities of different solvent extracts against DPPH and hydroxyl radicals, as well as their binding capacities toward bile salts, including sodium glycocholate and sodium taurocholate. The objective is to investigate the antioxidant and hypolipidemic effects of H. hamabo extracts and to provide a scientific basis for their potential development as natural functional ingredients [7].

## 2. Instruments and materials

### 2.1 Instruments

A T6 New Century UV–Visible Spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., China); an N-1100 Rotary Evaporator (EYELA, Japan); a GZX-9140MBE Drying Oven (Shanghai Boxun Medical Equipment Factory, China); and an AB135-S Electronic Analytical Balance (METTLER TOLEDO, Switzerland) were used in this study.

### 2.2 Materials

Hibiscus hamabo plant samples were collected from the Zhoushan region of Zhejiang Province, China. Ethanol, petroleum ether, chloroform, ethyl acetate, and n-butanol were all of analytical grade. Distilled water was prepared in the laboratory. Sodium taurocholate and sodium cholate were purchased from TMstandard Co., Ltd. Cholestyramine, sodium glycocholate, and sodium cholate were obtained from Yuanye Bio-Technology Co., Ltd. DPPH radical and hydroxyl radical assay kits were purchased from Solarbio Science & Technology Co., Ltd.

## 3. Methods and results

### 3.1 Preparation of hibiscus hamabo extracts with different polarities

Leaves of Hibiscus hamabo were collected from Zhoushan, Zhejiang Province, air-dried in the shade, pulverized, and passed through a 60-mesh sieve. The dried powder was extracted with 70% ethanol at a solid-to-liquid ratio of 1:20 (w/v) using ultrasonic-assisted extraction (300 W, 40 min). The extract was filtered under vacuum, and the filtrates were combined. The ethanol extract was concentrated under reduced pressure until no ethanol odor remained. The residue was then dispersed in an appropriate volume of distilled water and successively partitioned with equal volumes of petroleum ether, chloroform, ethyl acetate, and n-butanol. The remaining aqueous phase was retained. Consequently, five fractions were obtained: the Petroleum Ether

Fraction (PEF), Chloroform Fraction (CEF), Ethyl Acetate Fraction (EAF), N-Butanol Fraction (NBF), and Water Fraction (WF).

### 3.2 Determination of in vitro antioxidant activity

#### *3.2.1 DPPH radical-scavenging assay*

According to the assay kit protocol, each extract was prepared at a series of concentrations ranging from 0.1 to 2.0 mg/mL. Two milliliters of sample solution were mixed with 2 mL of 0.2 mmol/L DPPH ethanol solution and incubated in the dark for 30 min. The absorbance was measured at 517 nm using vitamin C as the positive control. The DPPH radical-scavenging activity was calculated according to the corresponding formula.

#### *3.2.2 Hydroxyl radical (·OH) scavenging assay*

The hydroxyl radical-scavenging activity was determined using a Fenton reaction system. Samples were added to a reaction mixture containing 10 mmol/L $FeSO_4$, 10 mmol/L salicylic acid, and 10 mmol/L $H_2O_2$. After incubation at 37°C for 1 h, the absorbance was measured at 510 nm, and the hydroxyl radical-scavenging rate was calculated.

### 3.3 Evaluation of in vitro hypolipidemic activity (bile salt-binding assay)

Fifty milligrams of each extract were accurately weighed and transferred into separate 50 mL stoppered conical flasks. Cholestyramine was used as the positive control, while a sample-free system served as the blank control. To simulate gastric digestion, 1 mL of pepsin solution (10 mg/mL, prepared in 0.1 mol/L phosphate buffer, pH 6.3) and 3 mL of 0.01 mol/L HCl were added to each flask, followed by incubation in a thermostatic shaker at 37°C for 1 h. Subsequently, the pH was adjusted to 6.3 using 0.1 mol/L NaOH solution. Four milliliters of trypsin solution (10 mg/mL, prepared in 0.1 mol/L phosphate buffer, pH 6.3) were then added, and the mixture was further incubated at 37°C with shaking for 1 h to simulate intestinal digestion. After digestion, 2 mL aliquots of the resulting solution were collected. Each aliquot was mixed separately with 2 mL of 1 mmol/L sodium glycocholate, sodium taurocholate, or sodium cholate solution prepared in 0.1 mol/L phosphate buffer (pH 6.3). The mixtures were incubated at 37°C with shaking for an additional 1 h and then transferred to centrifuge tubes. Following centrifugation at 4,000 r/min for 20 min, the supernatants were collected for bile salt analysis. The absorbance was measured at 387 nm. The concentration of unbound bile salts in the supernatant was determined using ultraviolet spectrophotometry. Cholestyramine served as the positive control, and the bile salt-binding rate (%) was calculated accordingly.

## 4. Results and Discussion

### 4.1 Evaluation of antioxidant activity

#### *4.1.1 DPPH radical-scavenging activity*

As shown in Figure 1, extracts obtained using solvents of different polarities, including petroleum ether, chloroform, ethyl acetate, n-butanol, and distilled water, all exhibited varying degrees of DPPH radical-scavenging activity. At an extract concentration of 10 mg/mL, vitamin C showed the highest scavenging rate, reaching 94.96%. The DPPH radical-scavenging rates of the ethyl acetate, chloroform, n-butanol, distilled water, and petroleum ether fractions were 91.27%, 74.53%, 50.58%, 26.82%, and 22.95%, respectively. The effectiveness of DPPH radical scavenging followed the order: Vitamin C > Ethyl Acetate Fraction > Chloroform Fraction > n-Butanol Fraction > Water Fraction > Petroleum Ether Fraction. These results indicate that the ethyl acetate fraction possessed strong free radical-scavenging activity even at relatively low

concentrations, exhibiting an antioxidant capacity second only to that of the positive control, vitamin C. The superior activity of the ethyl acetate extract suggests that medium-polarity phytochemicals may represent the major contributors to the antioxidant properties of Hibiscus hamabo.

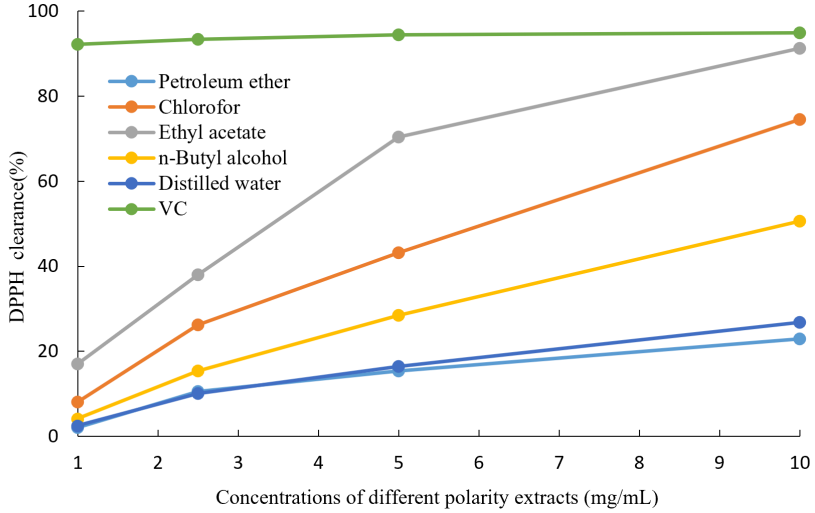


**Figure 1.** DPPH radical-scavenging activities of Hibiscus hamabo extracts obtained with solvents of different polarities

*4.1.2 Hydroxyl radical (·OH) scavenging activity*

As shown in Figure 2, all extracts prepared with solvents of different polarities demonstrated a certain capacity to scavenge hydroxyl radicals (·OH). At a concentration of 10 mg/mL, vitamin C exhibited the highest hydroxyl radical-scavenging rate of 97.40%. The scavenging rates of the ethyl acetate, chloroform, n-butanol, distilled water, and petroleum ether fractions were 55.88%, 21.27%, 16.13%, 15.04%, and 14.42%, respectively. The hydroxyl radical-scavenging activities ranked as follows: Vitamin C > Ethyl Acetate Fraction > Chloroform Fraction > n-Butanol Fraction > Water Fraction > Petroleum Ether Fraction. The results demonstrate that the ethyl acetate fraction exhibited remarkable in vitro antioxidant activity against hydroxyl radicals. Its scavenging efficiency was substantially higher than those of the other solvent fractions and was surpassed only by the positive control, vitamin C. Considering the highly reactive nature of hydroxyl radicals and their important role in oxidative damage, the strong scavenging capacity of the ethyl acetate extract further supports its potential as a natural antioxidant source.

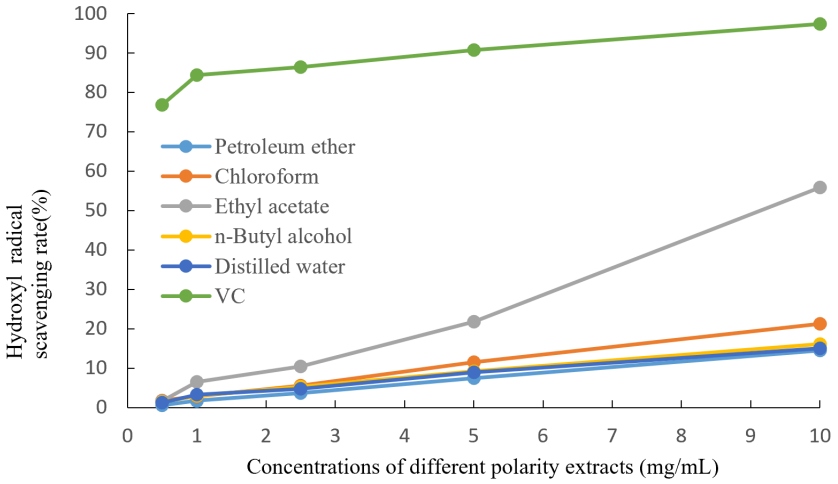


**Figure 2.** Hydroxyl radical (·OH) scavenging activities of Hibiscus hamabo extracts obtained with solvents of different polarities

## 4.2 Evaluation of hypolipidemic activity

### *4.2.1 Bile salt-binding capacity: sodium glycocholate*

Cholesterol can be eliminated from the body through its conversion into bile acids. Bile salts, such as sodium glycocholate, are readily reabsorbed in the intestine through enterohepatic circulation. Substances capable of binding or adsorbing bile salts may reduce their reabsorption, thereby promoting the conversion of cholesterol into bile acids and enhancing its eventual excretion. Consequently, bile salt-binding activity is widely regarded as an important indicator of potential cholesterol-lowering effects. In this study, sodium glycocholate was selected as a model bile salt to evaluate the binding capacities of extracts obtained with different solvents, while cholestyramine, a clinically used bile acid sequestrant, served as the positive control.

As shown in Figure 3, in the sodium glycocholate system (corresponding to an initial residual mass of approximately 0.96 mg in the blank control), the percentages of sodium glycocholate reduction were ranked as follows: Cholestyramine (52.38%) > Ethyl Acetate Fraction (38.42%) > Water Fraction (37.60%) > n-Butanol Fraction (26.49%) > Chloroform Fraction (5.90%). As expected, cholestyramine exhibited the strongest sodium glycocholate-binding capacity. Among the tested extracts, the ethyl acetate and water fractions demonstrated relatively strong binding activities, followed by the n-butanol fraction, whereas the chloroform fraction showed only limited activity. These findings suggest that the ethyl acetate and water fractions may contain greater amounts of polar or moderately polar constituents capable of interacting with bile salts. In contrast, the chloroform fraction displayed weak binding activity under the experimental conditions employed. Overall, several fractions exhibited appreciable sodium glycocholate-binding capacities in vitro, providing a basis for the future identification and characterization of the responsible bioactive compounds.

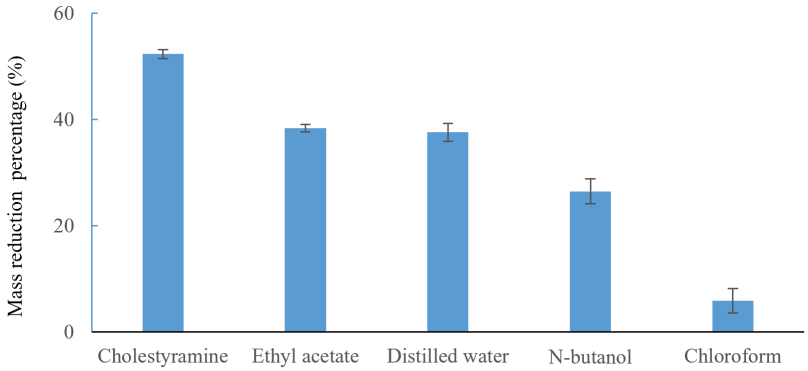


**Figure 3.** Binding capacities of different solvent fractions and cholestyramine toward sodium glycocholate

*4.2.2 Bile salt-binding capacity: sodium taurocholate*

In addition to sodium glycocholate, sodium taurocholate is another major conjugated bile salt involved in bile acid metabolism. Its retention and reabsorption in the gastrointestinal tract also play an important role in regulating cholesterol excretion. Therefore, the in vitro binding capacities of the various extracts toward sodium taurocholate were further investigated.

As shown in Figure 4, in the sodium taurocholate system (corresponding to an initial residual mass of approximately 1.035 mg in the blank control), the percentages of sodium taurocholate reduction were ranked as follows: Cholestyramine (61.95%) > Ethyl Acetate Fraction (41.52%) > Water Fraction (37.54%) > n-Butanol Fraction (31.51%) > Chloroform Fraction (18.89%).

Similar to the results obtained in the sodium glycocholate assay, the ethyl acetate fraction exhibited strong sodium taurocholate-binding activity and ranked second only to the positive control, cholestyramine. This finding suggests that the constituents present in the ethyl acetate fraction may possess the ability to bind multiple types of bile salts. The water fraction also maintained a relatively high binding capacity, whereas the chloroform fraction again demonstrated comparatively limited activity. Taken together, the results indicate that both the ethyl acetate and water fractions possess substantial in vitro sodium taurocholate-binding potential. These fractions may contribute to cholesterol-lowering effects by interfering with the enterohepatic circulation of bile salts, thereby reducing cholesterol accumulation and promoting its excretion.

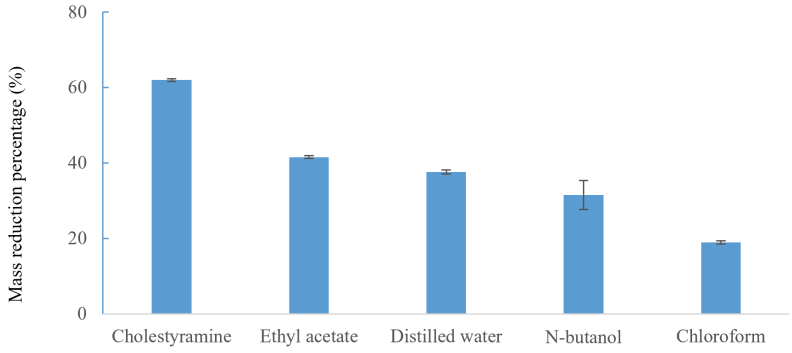


**Figure 4.** Binding capacities of different solvent fractions and cholestyramine toward sodium taurocholate

*4.2.3 Bile salt-binding capacity: sodium cholate*

Sodium cholate, a representative free bile acid salt, plays an important role in cholesterol metabolism, and its intestinal reabsorption efficiency is closely associated with the regulation of cholesterol homeostasis. Building upon the sodium glycocholate and sodium taurocholate binding assays described above, the present study further evaluated the in vitro binding capacities of the different solvent extracts toward sodium cholate in order to comprehensively assess their adsorption characteristics toward various bile salts.

As shown in Figure 5, in the sodium cholate system (corresponding to an initial residual mass of approximately 0.866 mg in the blank control), the percentages of sodium cholate reduction were ranked as follows: Cholestyramine (35.42%) > Water Fraction (30.42%) > Ethyl Acetate Fraction (28.56%) > n-Butanol Fraction (17.26%) > Chloroform Fraction (9.54%).

Compared with the results obtained for the two conjugated bile salts, sodium glycocholate and sodium taurocholate, the binding capacities of the extracts toward sodium cholate were generally lower. Nevertheless, a clear and consistent trend was observed. The ethyl acetate fraction maintained relatively stable binding activity across all three bile salt systems, suggesting that its bioactive constituents may possess a broad affinity for bile salts with different molecular structures. Notably, the water fraction exhibited a slightly higher sodium cholate-binding rate (30.42%) than the ethyl acetate fraction (28.56%) in this assay. This finding may indicate that bioactive compounds of different polarities display varying affinities toward different classes of bile salts. Consistent with the previous assays, the chloroform fraction showed the weakest activity. Taken together, the results obtained from the three bile salt-binding assays demonstrate that the ethyl acetate and water fractions possess superior in vitro bile salt-binding capacities. Their ability to interact with both conjugated and free bile salts suggests considerable potential for modulating bile acid metabolism and promoting cholesterol elimination.

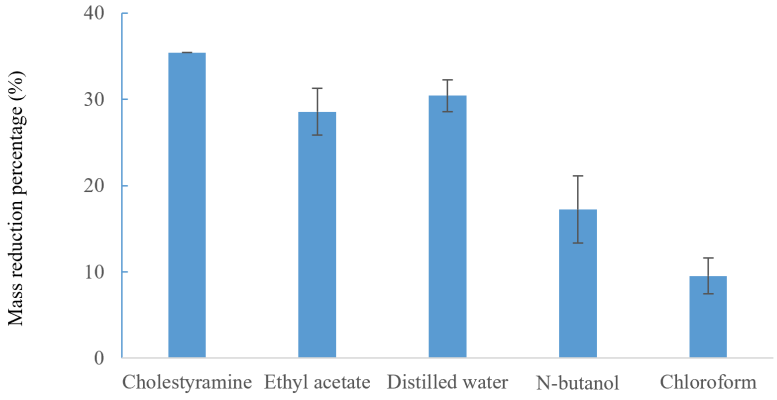


**Figure 5.** Binding capacities of different solvent fractions and cholestyramine toward sodium cholate

## 5. Conclusion

The present study investigated the antioxidant and hypolipidemic activities of bioactive constituents extracted from Hibiscus hamabo. The crude ethanol extract of H. hamabo was sequentially fractionated using a systematic solvent extraction procedure, yielding five fractions of different polarities: petroleum ether, chloroform, ethyl acetate, n-butanol, and water fractions. The in vitro antioxidant assays demonstrated that all fractions exhibited varying degrees of scavenging activity against both DPPH radicals and hydroxyl radicals (·OH). Within the tested concentration range, the ethyl acetate fraction displayed the strongest antioxidant activity. The overall order of radical-scavenging capacity among the fractions was: Ethyl Acetate Fraction >

Chloroform Fraction > n-Butanol Fraction > Water Fraction > Petroleum Ether Fraction. Among the tested fractions, the ethyl acetate extract exhibited the most pronounced free radical-scavenging activity, achieving a DPPH radical-scavenging rate exceeding 85% of that observed for vitamin C.

Furthermore, the in vitro hypolipidemic evaluation conducted under simulated gastrointestinal digestion conditions revealed that the ethyl acetate fraction possessed strong binding capacities toward sodium glycocholate, sodium taurocholate, and sodium cholate. Its highest bile salt-binding activity exceeded 65% of that achieved by an equivalent dose of the positive control, cholestyramine.

In summary, among the solvent fractions obtained from H. hamabo, the ethyl acetate fraction exhibited both outstanding antioxidant activity and promising hypolipidemic potential in vitro, indicating considerable value as a source of biologically active compounds. Future studies will employ in vivo animal models to further elucidate its lipid-lowering mechanisms. These investigations are expected to facilitate the high-value utilization and industrial development of H. hamabo, a characteristic salt-tolerant plant resource of the Zhoushan region.

## Funding project

Zhoushan Basic Research Project 2024 (Grant No. 2024C31042)

## References

[1] Chi, H., & Chen, J. (2025). Research progress on the cultivation and application value of Hibiscus hamabo. *Modern Agricultural Science and Technology*, (17), 123–127.

[2] Shen, Y. J., Cheng, S. Y., Wu, X. J., Wang, S., Wang, J. Y., Huang, Y. R., & Cao, X. Y. (2026). Three natural plant flavonoids extraction and their antioxidant properties. *Food Engineering*, (1), 43–46, 70.

[3] Li, S. Y., Xu, Y. P., Zheng, J. X., Zheng, Y., Yu, J. H., Li, Y. Q., & Guo, W. D. (2026). Evaluation of main polyphenol composition and antioxidant capacity in leaves of 34 Vaccinium species. *Scientia Agricultura Sinica, 59*(4), 874–886.

[4] Zhao, Z. H., Dai, J. Y., Liu, Y. N., Fu, H., Ying, Y., Huang, Y. Y., ... Wu, H. X. (2025). Antioxidant activity of extracts from 16 plant species in Inner Mongolia grassland. *Chinese Agricultural Science Bulletin, 41*(32), 116–123.

[5] Wang, Y. L., Feng, Y., Li, Z. F., Chen, D. F., Feng, Y. L., & Zhong, G. Y. (2025). Study on chemical constituents and in vitro anti-inflammatory and antioxidant activities of different sources of Tibetan medicine Yegexing plants. *Journal of Jiangxi University of Chinese Medicine, 37*(5), 56–64. https://doi.org/10.20140/j.2095-7785.2025.05.16

[6] An, X. M., Liang, Z. Y., Wu, L. F., Yuan, Y. T., Xia, L. Z., Wu, G. J., ... Guo, Y. X. (2025). Comparative analysis of in vitro antioxidant capacity of four natural plant extracts. *Chinese Agricultural Science Bulletin, 41*(29), 113–121.

[7] Wu, Q. T., Meng, X., Wang, M. L., & Xing, H. Y. (2025). Analysis of in vitro antioxidant and hypolipidemic activities of three plant oils. *Food and Machinery, 41*(10), 121–125. https://doi.org/10.13652/j.spjx.1003.5788.2024.80634