JOURNAL OF
EMERGING INVESTIGATORS

Article

Enhanced flavonoids and antibacterial function in *Houttuynia cordata* with different organic fertilizers

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SUMMARY

***Houttuynia cordata*, or fish mint, is an important plant in traditional Chinese food culture. Traditional Chinese herbal medicine books also record *H. cordata* being used to heal wounds and treat diseases, including dysentery and pulmonary abscesses. However, little is known about factors affecting the nutritional or bioactive contents of *H. cordata*. Therefore, we sought to investigate how three common household wastes (pomegranate peel, eggshell, and citrus peel) used as organic fertilizers might affect the nutrient composition and antibacterial effect of *H. cordata*. We hypothesized that adding organic fertilizers to *H. cordata* would: (1) increase nutritional contents, including plant flavonoids and lysine, and (2) enhance antibacterial function, as measured by inhibition of *E. coli* growth. We further hypothesized: (3) orange peels would increase nutritional contents and enhance antibacterial function more than the other fertilizer treatments since previous research found the pH of orange peel-amended soils was closest to fish mint's preferred conditions. We found the overall impact on *H. cordata* nutritional contents and *E. coli* inhibitory effect varied by organic fertilizer. Although orange peel powder did increase the plant flavonoid concentration most, pomegranate peel powder enhanced the *E. coli* inhibitory effect most, and lysine content was not significantly impacted by any treatment. These findings raise the possibility of developing more sustainable *H. cordata* cultivation, particularly by utilizing relatively inexpensive, widely available, and environmentally friendly organic fertilizers to improve the nutritional and medical value of this important crop.**

INTRODUCTION

Houttuynia cordata Thunb., also known as fish mint due to its distinctive odor and flavor, is a member of the family Saururaceae (1). Native to the mountainous regions of East, South, and Southeast Asia, *H. cordata* primarily grows in moist and shaded environments (2). *H. cordata* is an important food and medicinal plant in East Asian cultures, especially in Southwest China (3–5). The therapeutic uses of *H. cordata* include enhancing the immune system, with antiviral, antibacterial, anti-inflammatory, toxin-relieving, and antioxidative functions (6). Due to fish mint's healing effects and nutrient contents, people throughout Asia use *H. cordata* as herbal medicine (2, 3, 7–10). In Japan and Korea, fish mint is used in cosmetics to prevent wrinkles and aging (3, 7). In India, fish mint is used to treat heart disorders, malaria, sores, and excess body heat (2). Traditional Chinese medicine uses fish mint to treat certain tumors, pneumonia, diabetes, and other diseases (1, 8, 9). In Southwest China's Sichuan Province, fish mint has both culinary and medicinal uses, such as for seasoning sauces and aiding digestion by the Yi people (1, 10).

Several chemical substances contained in *H. cordata* include flavonoids, vitamin C, amino acids, benzenoids, essential oils, and steroids (11). As flavonoids are able to modify important cellular enzyme activities, with anti-oxidative, anti-inflammatory, and anti-mutagenic qualities, they are widely used in pharmaceuticals and nutraceuticals (12). For example, being easily oxidized, flavonoids may help reduce cardiovascular disease in humans by reducing the oxidation of low-density lipoprotein (13–15). Additionally, lysine, one of the 20 amino acids contained in *H. cordata*, is important for human metabolic activities, protein synthesis, and the immune system (3, 16). Furthermore, the antibacterial effects of *H. cordata* are partly due to its essential oil components, of which decanoyl acetaldehyde (also known as Houttuynin) contributes to its distinctive odor (6, 17, 18). Other compounds found within the essential oils of *H. cordata*, including oxidized and non-oxidized monoterpenes and sesquiterpenes, oxidized diterpenes, and phenylpropene derivates, have antibacterial properties, inhibiting both Gram-positive (e.g., *Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria (19, 20).

Organic fertilizers have been documented to improve the agricultural production of *H. cordata*, with potential to also improve fish mint's overall growth and nutrition (21–23). More widely in agriculture, organic fertilizers are used to enhance plant growth and sustainable soil practices (24, 25). Farmers around the world use readily available daily household waste (e.g., fruit peels and inedible parts of vegetables) as raw materials for organic fertilizers. Studies have found organic fertilizers can improve the health benefits and nutritional levels of certain crops by increasing antioxidant activities, total flavonoid and phenolic contents, and essential amino acid levels (26, 27). Organic fertilizers also help regulate soil pH, support soil micro-organism communities, and provide important elements essential for plant growth such as carbon, nitrogen, and hydrogen (28, 29).

Citrus peels, poultry eggshells, and pomegranate peels are relatively abundant and potentially ideal stock as organic fertilizers for *H. cordata* production in Sichuan. Citrus and poultry egg production are of high volume in the province (exceeding 563 kilotons and 175 kilotons annually, respectively) with steady growth (30). China ranks third globally for pomegranate production, with 11.12% of global output exceeding 1,200 kilotons annually (31, 32). Sichuan

https://doi.org/10.59720/25-156

ranks as the single largest production region in China (500 kilotons annually), being one of the eight main pomegranate producing regions in the world (33). Studies have shown that these three food wastes are rich in nutrients ideal for fertilizing food and medicinal crops (34–41). By dry weight, pomegranate peel contains 86% carbohydrate, 0.2% essential oil, 3.26% protein, and about 34 mg/g phenolic acids including chlorogenic, gallic, and caffeic (34–37). Orange peel has 16.9% soluble sugar, 42.5% pectin, 6.5% protein (by dry weight), and other small amounts of organic molecules including citric acid, malic acid, and vitamin C (38, 39). Poultry eggshells, rich in calcium (Ca^{2+}), are usually used as a Ca2+ supplement for plants and animals (40, 41). Moreover, Ca2+ as fertilizer can improve the productivity traits and essential oil contents of certain medicinal and edible plants (42, 43).

The most significant challenge associated with the massive volumes of food waste products is how to minimize their environmental impact (44). Each year, eggshells account for one of the most common wastes around the world, generated from both food processing and residential consumption (45). Consequently, many sustainable uses have been developed for eggshells, including as an ingredient in making concrete, conversion into a bioavailable source of Ca2+ for food fortification, and use as a pH neutralizer for organic fertilizers (44, 46, 47). In fact, all three of these potential organic fertilizers can influence soil pH, as soils become more neutral at about pH 7.1 with eggshells, or more acidic with pH 5.8 from orange and 4.8 pH from pomegranate peels (48, 49). Orange and pomegranate peels are sometimes also processed as functional foods for boosting the immune system and reducing risks of other diseases (50, 51).

Studies have not only shown how different organic fertilizers can affect the health benefits and nutritional levels of crops, but have also shown how organic fertilizers can affect the physiology and growth of medicinal and edible plants (26, 27, 52, 53). Previous studies investigating the nutritional levels of *H. cordata* have demonstrated that cultivated *H. cordata* has higher nutritive values than wild-collected *H. cordata* and that organic fertilizers or certain doses of nitrogen fertilizer positively impact the production of this plant (21–23, 54). However, empirical studies on *H. cordata* are scarce, not only regarding how the application of organic fertilizers can affect fish mint's growth and productivity, but also how the nutrient contents of *H. cordata* are affected by different organic fertilizers.

Therefore, this study sought to address this research gap by assessing how different commonly available organic fertilizers might affect the nutrition of *H. cordata* at the molecular level. We focused on three primary aspects of molecular level nutrition: 1) plant flavonoid concentrations, 2) lysine concentrations, and 3) antibacterial effects. In addition, considering their availability and abundance in Sichuan, as well as their documented effectiveness elsewhere, we chose to assess the suitability of using orange peels, eggshells, and pomegranate peels as organic fertilizers for *H. cordata* production (27, 30, 31, 33, 38, 40, 42). We hypothesized that: (1) the addition of organic fertilizers would increase the overall nutritional contents of *H. cordata*, including plant flavonoid and lysine concentrations; (2) the addition of organic fertilizers would enhance the antibacterial function of *H. cordata*; and (3) since orange peel-amended soils tend to have soil pH of 5.8, which is closest to the preferred soil pH (5.9) of *H. cordata*, orange peels would increase nutritional concentrations most and have stronger enhancement in antibacterial function than the other two fertilizer treatments (55). The results of this study will inform farmers and policy makers about ways to encourage more sustainable *H. cordata* cultivation, particularly by utilizing relatively inexpensive, widely available, and environmentally friendly organic fertilizers to improve the nutritional and medical value of this important crop.

RESULTS

To test the effects of organic fertilizers on *H. cordata*, we compared flavonoid and lysine concentrations and antibacterial activity across four fertilizer treatments: orange peel, eggshell, and pomegranate peel powders and control (no fertilizer).

Plant Flavonoids & Lysine

To test the effect of organic fertilizer treatment on *H. cordata* flavonoid content, we measured flavonoid concentrations in fresh leaves through a plant flavonoid enzyme linked immunosorbent assay. *H. cordata* grown with eggshells, pomegranate, or orange peels had significantly more flavonoids compared to the control group ($F(3, 9.19) = 6.80$, $p = 0.010$, Welch's one-way ANOVA) (**Figure 1**). Furthermore, according to Games-Howell post hoc comparison, only the orange peel group had significantly higher flavonoid concentrations compared to the control group ($p = 0.003$; **Figure 1**).

We also tested the impact of organic fertilizer treatment on *H. cordata* lysine concentrations. Across all fertilizer types, there was no statistically significant difference in lysine concentration compared to the control (**Figure 2)**.

Antibacterial Functions

We then tested the impact of organic fertilizer treatment on the antibacterial activity of *H. cordata* by observing the growth of *E. coli* following treatment with leaf extracts from *H. cordata* grown in the pomegranate peel, eggshell, orange peel, or control conditions. Since the number of bacteria in a culture dish is inversely proportional to its antibacterial effect, we determined the inhibitory effect of different organic fertilizers on *E. coli* by comparing the coverage of bacteria colonies in each petri dish (**Figure 3A**) (56). Calculation of surface area was assisted by using Procreate (v5.3) and ImageJ (v1.54m) software (**Figure 3B**) (57, 58). In the control group, the coverage percentage was 5.56%. For the treatment groups, bacterial coverage was 2.89% in the pomegranate peel group; 13.35% in the orange peel group, and 5.13% in the eggshells group. For the petri dish without added *H. cordata* extract, *E. coli* colonies covered 34.11% of the culture area (**Figures 3, 4**).

DISCUSSION

Our findings revealed that adding different organic fertilizers can impact flavonoid concentrations and the antibacterial function of *H. cordata*, partially supporting our first two hypotheses that fertilizer amendments would influence the plant's nutritional composition and antibacterial activity (**Figures 1, 4**). Specifically, the addition of certain organic fertilizers enhanced the overall nutritional level of *H. cordata*, with plant flavonoid concentrations increasing significantly when orange peel powder was added (**Figure 1**).

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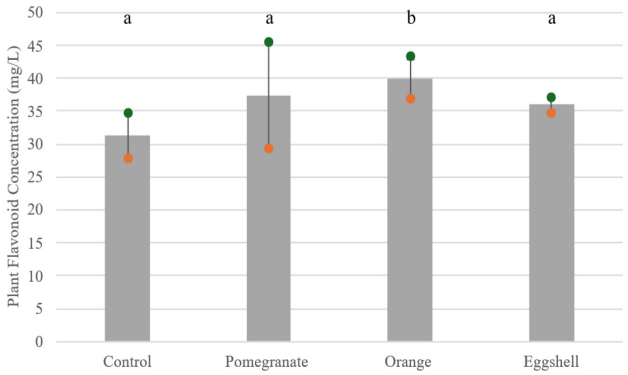


Figure 1. Mean plant flavonoid concentrations (mg/L) of *H. cordata* leaves from plants grown in four different organic fertilizer treatment groups. Treatment groups using H. cordata grown without organic fertilizer (control); with pomegranate peel powder organic fertilizer (pomegranate); with orange peel powder organic fertilizer (orange); with eggshell powder organic fertilizer (eggshell). Bars represent mean ± SD (n = 6). Green dots represent the maximum value and orange dots the minimum value observed in each treatment group. The lowercase letters indicate statistical groupings based on the post hoc comparisons (Welch's ANOVA followed by Games-Howell post hoc tests, $p < 0.01$). Groups that share the same letter are not significantly different from one another, while groups with different letters are significantly different.

However, the content of lysine was not significantly affected by the addition of any organic fertilizer, so our first hypothesis was only partially supported (**Figure 2**). Since antibacterial function increased after adding extracts of *H. cordata* leaf grown with pomegranate peel powder, our second hypothesis was supported (**Figure 4**).

We expected that orange peel-amended soils with a pH of 5.8 would be the optimal treatment group for *H. cordata* growth, being closest to the ideal soil pH (5.9) of *H. cordata* (48, 55). Consequently, for our third hypothesis, we also expected *H. cordata* grown in orange peel-amended soils would have the highest nutritional contents and antibacterial effect. But this was only partially true. Although flavonoid concentrations of the orange peel group were highest, there was no significant impact on lysine content, and antibacterial function improved in the pomegranate-peel group but worsened in the orange-peel group, both contrary to our expectations (**Figure 4**). Thus, the fact that *only* the flavonoid content was significantly higher in the orange peel group may be due to the content of the citrus peels themselves. Since citrus peels (including orange peels) contain high concentrations of plant flavonoids, *H. cordata* grown in orange peel-amended soil may produce more flavonoids (59). Flavonoids in soils are also known to promote plant absorption of elements like nitrogen, phosphorus, and iron (60, 61).

Another factor that might contribute to the difference in flavonoid production is the varying microbial characteristics of each *organic* fertilizer. Tangerine and pomegranate peels have been shown to contain similarly large amounts of yeast, with yeast levels on pomegranate peels being slightly higher (31, 62). However, mold levels on pomegranate peels are significantly higher than tangerine peels, which may negatively impact plant growth in pomegranate peel-amended soils (31). Overall, the microbial profile of citrus (tangerine) peels may have more positive effects on plant growth, with yeasts bringing benefits for plant growth and competitive prevention of diseases (63, 64). Yeast soil amendments have been shown to increase nitrogen and phosphorus contents, thereby further increasing flavonoid production in plants since the nutritional levels and contents of these elements in soils can increase flavonoid production (63, 65). In addition, since orange peels and pomegranate peels both contain large amounts of carbohydrates, moderate proteins, and beneficial yeasts, the possibility of enhancing *H. cordata* nutritional yield by using a combination of these two different organic fertilizers may be higher than using them separately. Future studies should, therefore, investigate the impact of various proportions of these organic fertilizers used in combination.

The insignificant effect of organic fertilizers on lysine content in *H. cordata* may have been due to the relatively low concentrations of free lysine amino acids in *H. cordata* (**Figure 2**). In a study investigating the flavonoid, phenolic acid, nucleoside, and amino acid contents of different parts of *H. cordata*, lysine was listed as one of twenty amino acids found in the plant, which is why we tested it in our study (66). Nevertheless, a different study did not detect lysine as a free amino acid (67). A subsequent review also did not list lysine as one of the free amino acid components of *H. cordata* (68). Thus, future research should examine the amino acids that are more abundant in *H. cordata*, such as leucine, glycine, and serine, particularly since leucine is also one of the essential amino acids of the human body (66, 69).

It is important to note that multiple vegetative parts of *H. cordata* are consumed as food and medicine in China, including the above-ground portions (stems and leaves) and below-ground portions (roots and rhizomes) (9). But studies of their nutrient and bioactive compounds have variously assessed these parts individually, grouped together (above- or below-ground parts), or together as the whole plant, sometimes using the terms "root" and "rhizome" interchangeably (9, 70, 71). Leaves and roots tend to have higher nutrient contents than stems (70, 71). Nevertheless, since leaves are easier to process due to their softness and thinness, yielding a

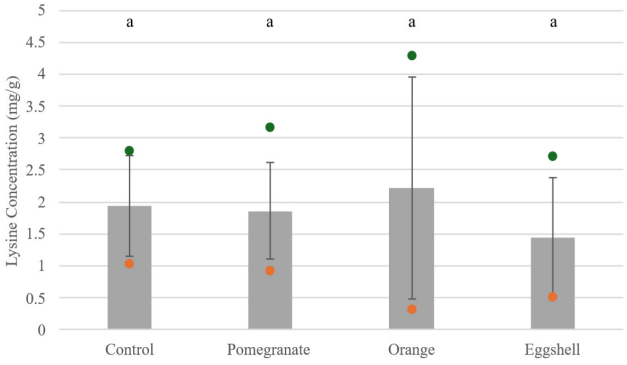


Figure 2. Mean lysine concentrations of *H. cordata* leaves from plants grown in four different organic fertilizer treatment groups. Treatment groups using *H. cordata* grown without organic fertilizer (control); with pomegranate peel powder organic fertilizer (pomegranate); with orange peel powder organic fertilizer (orange); with eggshell powder organic fertilizer (eggshell). Bars represent mean ± SD (n = 4). Green dots represent the maximum value, and orange dots the minimum value observed in each treatment group. One-way ANOVA showed no significant differences among fertilizer treatments ($p > 0.05$), so all groups share the same letter.

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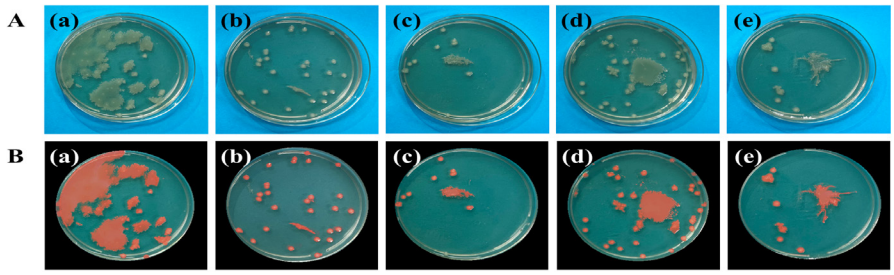


Figure 3. Five *E. coli* petri dishes after the addition of different *H. cordata* treatments (A) and after processing by Procreate v5.3 software (B). (A) Five *E. coli* petri dishes were grown for 24hr after adding 200µL of each treatment: (a) purified water, (b) control (leaf extract of *H. cordata* grown without amendment), (c) pomegranate peel-amended *H. cordata* leaf extract, (d) orange peel-amended *H. cordata* leaf extract, and (e) eggshell-amended *H. cordata* leaf extract. (B) ImageJ-processed pictures of the *E. coli* petri dishes from the same five treatment groups. The round petri dish parts were selected and retained, while the *E. coli* growth areas were enhanced with red color to aid size calculation.

more homogenous, low-viscosity supernatant after cutting, grinding, and centrifuging, we chose to examine the nutrient and antibacterial effect of the leaves only for this study.

Our results provide important insight into the antibacterial function of *H. cordata* leaf extracts. Purified leaf extracts effectively reduced *E. coli* growth, of which *H. cordata* grown with pomegranate peel powder had the most antibacterial effect overall. In a previous study, *H. cordata* leaf extract had a negligible effect restraining Gram-negative bacteria, including *E. coli* (72). However, since the antibacterial effects result from several different components of *H. cordata*, further research should compare different pathways that *H. cordata* may produce antibacterial substances. Moreover, future studies should compare the antibacterial effect of extracts from different parts of *H. cordata* (e.g., leaves, versus roots, stems, or rhizomes), as extracts from each part grown under similar conditions may differ in overall antibacterial effect.

This study demonstrated a practical application for using three kinds of household wastes as organic fertilizers (orange peels, eggshells, and pomegranate peels) in the commercial production of *H. cordata*. Use of these wastes as organic fertilizers have important implications on sustainable industry and development of a more circular economy, since all three are currently widely available and inexpensive sources for organic fertilizers in Sichuan (30–33). Use of these three organic fertilizers in *H. cordata* cultivation could partially reduce reliance on chemical fertilizers. Excessive use of chemical fertilizers in China causes serious environmental problems, including increasing amounts of phosphorous and nitrogen washed off from fields into rivers and groundwater, as well as intensified evaporation of nitrogen gas from agricultural fields (73, 74). Organic fertilizers have been found to improve *H. cordata* quality, encouraging farmers to reduce their use of chemical fertilizers, in keeping with the United Nations Sustainable Development Goals (73). In addition, our study highlights practical and potentially cost-effective ways to increase the nutritional and medicinal value of *H. cordata*.

Moreover, based on our results, we now believe the effect that each organic fertilizer had on the *H. cordata* nutrition and antibacterial function may also be influenced by their varying impact on soil pH. Since we did not measure changes to soil pH following the addition of each organic fertilizer, future research should carefully plan for this, particularly in how each organic fertilizer independently, and in combination, may differently affect soil pH as well as plant nutrition and antibacterial function. Furthermore, our analysis looked at each treatment separately, but soil properties like pH can have confounding impacts on nutrient levels. Because these variables can interact, future studies should use multivariate statistics (such as principal component analysis or multiple regression) to analyze several factors at the same time. This would help identify which variables truly drive the differences we found. In particular, since eggshell-amended soils become basic, and soils with pomegranate peels are slightly more acidic than the ideal soil pH for *H. cordata*, these two organic fertilizers might be best used in combination together to better regulate the soil pH (48, 49, 55).

Since this study focused on the relationship of organic fertilizers on the nutrient contents in *H. cordata*, details about the underlying mechanisms behind this relationship should also be explored. For example, chemical elements, such as zinc, potassium, nitrogen, and iron, have been shown to affect the growth, yield, and chemical substances contained in plants (75). Similarly, this could be a reason why some chemical substances in *H. cordata* have been shown to be influenced by certain groups of organic fertilizers. Other possible reasons for the effects of organic fertilizers on chemical substance concentrations could be some internal (e.g., plant hormone signaling and flavonoid biosynthesis) and external pathways (e.g., interactions between plant

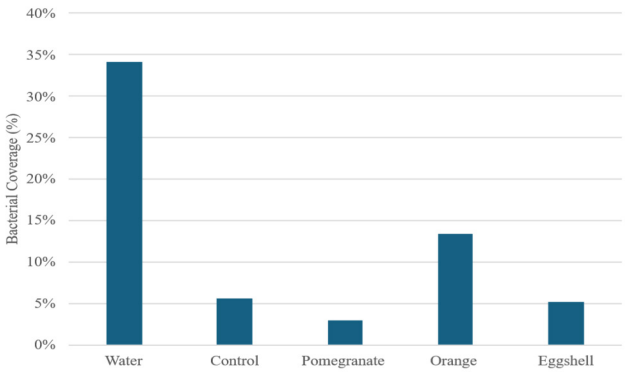


Figure 4. Bacterial coverage (%) of *E. coli* plates following five *H. cordata* leaf extract treatments. The treatment groups were purified water, (control) leaf extract of *H. cordata* grown without amendment, pomegranate peel-amended *H. cordata* leaf extract, orange peel-amended *H. cordata* leaf extract, and eggshell-amended *H. cordata* leaf extract.

https://doi.org/10.59720/25-156

roots and the soil microbial communities and soil enzyme activities). It is known that organic fertilizers can increase the soil microbial population, which then boosts plant growth (25). For example, *Azotobacter*, as a kind of nitrogen-fixing bacteria promoting plant growth, could play a role as a plant hormone synthesizer, contributing to plant growth and nitrogen and phosphate uptake by making these molecules soluble (76). It is also important to consider how the organic fertilizers used in this study might promote particular strains of microorganisms with beneficial impacts on plant growth. Beyond understanding the potential ways organic fertilizers can directly alter nutrient levels and production of plants, it is also essential to identify how they influence other specific plant growth-promoting factors.

The addition of three different organic fertilizers was found to significantly enhance some of the nutritional contents of *H. cordata*. However, the nutrient enhancement of *H. cordata* varied between the three organic fertilizers. Plant flavonoid concentrations increased the most when orange peel powder was used, while pomegranate peel powder was most effective at enhancing the antibacterial effect on *E. coli*. The contents of lysine did not show significant changes from any of the organic fertilizers. Our findings serve as a valuable reference for farmers to provide a possible source of fertilizers, which are cheap, easily attainable, and environmentally friendly. Moreover, the findings generated in this study can be applied to improve the nutritional value of *H. cordata* or increase the content of certain nutrients.

MATERIALS AND METHODS

Experimental Materials

Orange peels, eggshells, and pomegranate peels were collected from daily household waste (Chengdu, China). After collecting, the wastes were washed and sundried for about 48-72 hours, then made into powdered forms using a high-speed blender (**Figure 5**) (28). Several healthy *H. cordata* rhizomes were chosen and purchased from Chengdu Binlan Gardening Co., LTD (Chengya Highway, Shuangliu District, Chengdu, China). They were cut into 5 cm long sections for planting in potting media at a depth of 3 cm, evenly spaced four-by-four into four styrofoam containers (with outer dimensions measuring 41 x 31 x 20 cm and inner dimensions measuring 36 x 26 x 15 cm). The potting media consisted of nutrient peat soil with over 90% organic matter purchased from Xiu Zheng Hua Le Duo® (No. 36, Zhengxiu Road, Tonghua, Jilin Province). Each container held one organic fertilizer treatment group: orange peel, eggshells, and pomegranate peel or control, with sixteen rhizome segments of *H. cordata* per group. The boxes were placed near bright windows of an upper-story room with direct afternoon sunlight.

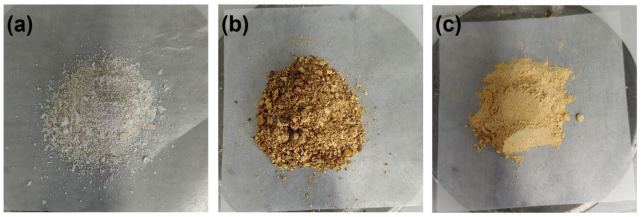


Figure 5. Three types of powdered organic fertilizers (5.00g each) used for treating *H. cordata*. After drying, eggshells, pomegranate peels, and orange peels were made into powder forms using a high-speed blender: (a) Eggshell powder, (b) pomegranate peel powder, and (c) orange peel powder.

Before planting, for each treatment group, the organic fertilizer powder was mixed with water (15g/500mL) using a magnetic stirrer then added to the soil media of the corresponding box. The fertilized soil media was fermented for three weeks before the *H. cordata* rhizome segments were planted. After planting, 5g of the corresponding organic fertilizer was mixed with 500mL water and added to the corresponding box of each treatment group once per week. This process continued for two months (April-May). For the control group, each time water was added to treatment groups, the same volume of water was added, but without organic fertilizer. After two months, all *H. cordata* leaves from each box were harvested for further analysis.

Determination of Nutritional Chemical Substances

After harvest, the leaves of each treatment group were analyzed for key nutritional chemical properties including lysine and plant flavonoid concentrations, as well as antibacterial function. Leaves of each group were divided into three sets to go through three experiments separately.

The lysine content of the leaves from each treatment group and control was measured using a lysine test box UPLC-MS-5100 produced by Shanghai Liquid Quality Testing Technology Co., LTD (77). After collecting the leaves, a portion of each sample was packed into paper bags, put into a drying oven for 24hr, and then ground into powder. Lysine was extracted and reacted from the powdered leaf samples according to the lysine testing kit manufacturer's instructions. The resulting assay and blank solutions were then mixed thoroughly, and 1,000μL from each tube was transferred to a 1mL glass cuvette. Absorbance (*A*) was measured at 530nm wavelength, and *ΔA* was calculated as *A* (assay tube) - *A* (blank tube). Given the regression equation under standard conditions, $y = 0.0062x - 0.0212$, in which x was the lysine content in an assay tube (μg/mL) and y was the absorbance value, the lysine content according to dry weight of a sample was calculated by the following equation:

$$C = [(\Delta A + 0.0212) \div 0.0062 \times V1] \div (W \times V1 \div V2) \times 1.1515$$

The simplified equation was:

$$C = 185.73 \times (\Delta A + 0.0212) \div W$$

Where *V1* referred to the sample size in the assay tube, 0.2mL; *V2* referred to the liquid volume in the assay tube, 1.0mL; *C* referred to the lysine content in the sample leaves (μg/g dry weight); *W* referred to the sample mass (g), with a correction coefficient of 1.1515. The calculation equations and regression equation were given by the lysine testing kit instructions.

Plant Flavonoids

To measure flavonoid concentration, we used a Plant Flavonoid Enzyme Linked Immunosorbent Assay (ELISA) Kit from Beijing Zhiyi Technology Co., Ltd. (18-1, 2nd Floor, No. M18, Xinxi Middle Road, Shangdi Information Industry Base, Haidian District, Beijing). For each treatment group and

https://doi.org/10.59720/25-156

control, 2.5g of fresh leaf parts of *H. cordata* were collected from each group. All fresh leaf parts were collected from the first leaves under the new buds. After the collection, 2.5g of leaf parts from each group were ground with 12.5mL phosphoric acid buffer in four separate mortars. The grinding process continued until no obvious leaf tissues remained visible. After grinding, the extracted *H. cordata* liquids from the four different groups were injected into four different empty centrifuge tubes then the four tubes were centrifuged under 1,000g for 20 minutes. After that, supernatant from these four samples was injected into a centrifuge to prevent fixing with precipitate in the old centrifuge tubes.

Six standard wells were set on the MicroElisa Strip plate by injecting 50μL standard solutions with different concentrations (the standard concentration wells S0 to S6 are 0, 1.25, 2.5, 5, 10, 20mg/L). After setting the six standard wells, a matched group well was set by injecting 50μL purified water into one well. To set four sample wells, 10μL supernatant from four samples and 40μL sample diluent were injected into each well. Before 60 minutes of incubation in a 37°C incubator, 100μL HRP-conjugate reagents were added to the five standard wells and the four sample wells. After incubation, all liquid on the stripboard was discarded and all sample and standard solutions on the stripboard were filled with wash solution. We let the stripboard stand for one minute, then discarded all solutions on the stripboard and repeated the above washing operations five times. After washing, 100μL chromogen solution was injected into the five standard wells and the four sample wells, then incubated 15min at 37°C in darkness. After 15min incubation, 50μL stop solution was added to each standard well and sample well. The absorbance (OD) of flavonoids at 450nm was read using a spectrophotometer.

Antibacterial Function

To test the effect of different organic fertilizers on the bacteriostasis of *H. cordata*, we used *E. coli* in the assay. The *E. coli* strains were from Shanghai Luwei Technology Co., Ltd. (78). We used the spread plate method to perform the experiments under sterile conditions.

First, to ensure the viability of the strains, we grew the colonies by transferring them before use. Colonies were inoculated into 5mL purified water in a beaker. Later, 20μL mixture was added to and evenly spread in each petri dish. We placed the petri dishes in an incubator at 37°C. After 24hr post-transfer, a single colony was inoculated in 5mL purified water, shaken, and then 10μL of the mixture was added and mixed in 1mL purified water. We transferred 100μL mixture to 400μL purified water, and after mixing evenly, 20μL of this dilute solution was applied to the petri dish each time with a spreading rod, and finally 200μL *H. cordata* supernatant after centrifugation was added to each dish. After the addition of reagents, similarly, the petri dishes were incubated at 37°C.

Following 24hr of incubation, we observed the colonization. In quantifying bacteriostasis of *H. cordata*, we captured a clear image of each petri dish against a blue background from the same directly overhead position and at the same height. Subsequently, the color bacterial colonies in each image was set to red and areas outside the petri dishes were removed using Procreate software (v5.3) (57). Then, the images were imported into the ImageJ software (v1.54m), the red portions were selected, and the pixel values of the bacterial colony parts and that of the entire petri dishes were calculated respectively (58). The proportion of the areas occupied by the bacterial colonies in each petri dish were calculated using the ratio of the pixel values of the bacterial colonies to those of the whole petri dish. Finally, bacteriostasis of *H. cordata* with different organic fertilizers on *E. coli* was compared by contrasting the proportion of bacterial colonies in each group under the addition of different organic fertilizers with that of the blank group with the addition of purified water only, which was specifically reflected in the fact that those with fewer colonies had a better bactericidal effect.

Statistical Methods

Statistical tests were completed using IBM SPSS Statistics (v27) (79). For tests assessing lysine and plant flavonoid contents, the effects of the three treatments and control on *H. cordata* plants were evaluated: orange peel organic fertilizer, pomegranate peel organic fertilizer, eggshell organic fertilizer, and no organic fertilizer (control). For assessing antibacterial function, the effects of four treatments to *E. coli* were measured: extraction from plants in the orange group, extraction from plants in the pomegranate group, extraction from plants in the eggshells group, extraction from plants in the control (with *H. cordata* plants grown without organic fertilizer), and purified water only. The flavonoid concentration data were normally distributed but had unequal variances, as indicated by a significant test of homogeneity of variances ($p < 0.05$). The lysine concentration data were normally distributed and had homogeneous variances, as indicated by a non-significant test of homogeneity of variances ($p > 0.05$). Welch's one-way ANOVA followed by Games-Howell post hoc tests were used to investigate the effects of different fertilizer treatments on the flavonoid concentrations, while a standard one-way ANOVA was used to analyze the effects of fertilizer type on lysine concentration in *H. cordata*. The mean value, standard deviation, and standard error of flavonoid concentrations were also calculated.

ACKNOWLEDGMENTS

We would like to thank Chengdu Shude High School for providing the facilities and support to conduct this study.

Received: April 15, 2025
Accepted: November 30, 2025
Published: November 15, 2026

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