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Herbaceous Peony Polyphenols Extend the Vase Life of Cut Flowers

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Abstract: Herbaceous peony is a potential material for cut flowers, but its short vase life seriously affects the development of cut herbaceous peony flowers industry. In this study, herbaceous peony polyphenols were applied to extend the vase life of cut flowers, and the results indicated that 8% mass concentration of herbaceous peony polyphenols increased the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities; increased the soluble protein content of the cut flowers; and effectively reduced the malondialdehyde (MDA) content. Meanwhile, herbaceous peony polyphenols increased the water balance value of cut flowers. In addition, the observation of microstructures indicated that herbaceous peony polyphenols reduced the blockage mainly caused by *Aspergillus* spp. at the stem ends and inhibited the growth of *Aspergillus* spp. Additionally, aquaporin genes (*AQPs*), including three plasma membrane intrinsic protein genes (*PlPIP1;2*, *PlPIP2;1*, and *PlPIP2;2*) and one intrinsic protein gene (*PlNIP*), were isolated. *PlPIP1;2*, *PlPIP2;1*, and *PlPIP2;2*, which were induced by polyphenol treatment, had common effects on maintaining the water balance of cut flowers. Therefore, herbaceous peony polyphenols can significantly extend the vase life of cut flowers; these results provide for the application of the theoretical reference of herbaceous peony polyphenols in extending the vase life of cut flowers.

Keywords: herbaceous peony polyphenols; vase life; stem; morphological indexes

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1. Introduction

As the 'prime minister of flowers', herbaceous peony has been favored by the cut flower market for its rich and auspicious symbolism, and it has experienced explosive development in China [1]. However, because of its short vase life, the industrial development of cut herbaceous peony flowers has been severely restricted. Therefore, extending the vase life of cut herbaceous peony flowers plays an important role in improving the ornamental value and economic value of cut flowers [2].

Many studies on extending the vase life of cut flowers have been reported. These studies mainly focused on chemical inhibitors [3,4]. Sun et al. [5] found that cut peony pretreated with 8-hydroxyquinoline (8-HQ) can extend the vase life. In addition, there are some studies on growth regulators [6–8]. Ichimura et al. [7] found that pulse treatments with STS (silver thiosulfate complex) at 0.1 mM in combination with GA (gibberellin) at 0.5 or 1 mM were optimum for extending the vase life of Narcissus tazetta var. chinensis. Moreover, there are also some studies focused on metal ions [9–11], ammonia sources [12–14], and signaling molecules [15,16]. Mashhadian et al. [17] found that salicylic acid (SA) and citric acid (CA) extended the vase life of chrysanthemum by increasing the water content and fresh weight. Tang et al. [18] found that 300 ppm nano-silver solution was effective in extending the vase life of herbaceous peony. Studies on the vase life of herbaceous peony also focus on the above pathways currently. However, the development of natural extracts for the preservation effect of cut flowers has been investigated less.

Polyphenols, as a natural plant extract, are widely found in the bark, roots, leaves, and fruits of natural plants [19,20]. Polyphenols have extremely strong biological activities,

such as antioxidant, antibacterial, and anti-inflammatory properties. Additionally, they are widely used in food, medical, and daily product research [21–24]. Ma et al. [25] found a positive correlation between polyphenol concentration and their antioxidant capacity in blanched and enzymatically digested carrot juice. Moreover, Xu et al. [26] found that the antioxidant activity of polyphenols was positively correlated with their antibacterial properties, and grape seed polyphenols significantly reduced the cell viability of Staphylococcus aureus. In addition, Zhang et al. [27] found that 20 mg L^{-1} tea polyphenols prolonged the vase life of cut flowers. At present, the extraction of natural plant polyphenols is widely studied, but the extraction studies on herbaceous peony are mainly focused on the roots, while the presence of polyphenols in other parts such as decaying petals, and whether they can be extracted for other applications, has been studied less.

In this study, we extracted polyphenols from the decaying petals of herbaceous peony and used the extracted polyphenol solution for cut flower vase solution. In order to explore the preservation effect of polyphenols on the vase life of herbaceous peony, morphological indexes, physiological indexes, and protective enzyme activities were measured during the vase life. Moreover, the mechanism of herbaceous peony polyphenols to extend the vase life of cut flowers was investigated. These results provide a sufficient basis for the application of herbaceous peony polyphenols in cut flowers.

2. Materials and Methods

2.1. Plant Materials

The herbaceous peony 'Hongyan Zhenghui' was harvest from the germplasm repository of Horticulture and Landscape Architecture College, Yangzhou University, Jiangsu Province, P. R. China (32°30′ N, 119°25′ E) on April 24th. The cut flowers were all selected at the color-change stage and then re-cut in water to about 30 cm. These flowers were divided into two groups, one using 8% mass concentration of herbaceous peony polyphenols as the vase solution and another group using distilled water as the control.

2.2. Preparation of Herbaceous Peony Polyphenol Extract

In total, 5 g herbaceous peony petals were weighed into a tube. Then, according to the material–liquid ratio of 1:50, an ethanol concentration of 40%, a sonication temperature of 40 °C, sonication power of 200 W, and a sonication time of 10 min were the conditions used to extract polyphenols by ultrasonic cleaner (KQ-200VDB, Kunshan Ultrasonic Instrument Factory, Kunshan, China) [28]. Macroporous resin (HPD100, Macklin, Shanghai, China) was used to purify the extract, and then it was distilled under reduced pressure by Rotary Evaporator (N-1300, Shanghai Ailang Instrument Co., Ltd., Shanghai, China); finally, 8% mass concentration of herbaceous peony polyphenols was obtained.

2.3. Measurement of Morphological Indexes

The morphological indexes of cut flowers were recorded every day, along with the time for which flowers with 80% wilted petals were considered to have reached the end of their vase life. The flower diameters were measured every day by the micrometer scale (Taizhou Xinshangliang Measuring Tools Co., Ltd., Taizhou, China). The fresh weight of flowers was weighted by electronic balance (T-500, Gandg Testing Instrument Factory, Tibetan Autonomous Prefecture of Golog, China).

2.4. Measurement of Physiological Indexes and Protective Enzyme Activity

The petals were accurately weighed and homogenized in an ice bath at a ratio of tissue mass (g): distilled water volume (mL) of 1:10, 8000× *g*, centrifuged at 4 °C for 10 min, and the supernatant was taken to be measured. Thereafter, the soluble protein and MDA were evaluated according to the reagent kit (Suzhou Kemin Biotechnology Co., Ltd., Suzhou, China). The water balance value is the difference between the water uptake and water loss of the two adjacent days. The petals were accurately weighed and homogenized in an ice bath at a ratio of tissue mass (g): a distilled water volume (mL) of 1:10, 8000× *g*,

centrifuged at 4 °C for 10 min, and the supernatant was taken to be measured. Thereafter, CAT, POD, and SOD content were evaluated according to the reagent kit (Suzhou Kemin Biotechnology Co., Ltd., Suzhou, China).

2.5. Microbe Identification

The stem ends were cut and immersed in 0.5% sodium hypochlorite solution for 2 min and then washed with sterilized distilled water for 2–3 times. Then, the dry tissue blocks were scattered in PDA medium and incubated for three days at 25 °C in dark conditions, and the colonies that grew out of them were purified. In addition, conidial morphology was observed using electron microscopy (CX31, Olympus Corporation, Tokyo, Japan). DNA was extracted using the fungal genomic DNA extraction kit (Beijing Solabao Technology Co., Ltd., Beijing, China), referring to the instructions in the kit. The PCR products were examined by agarose gel electrophoresis and compared with DL2000 Marker for size. Moreover, the PCR products were sent to Tsingke Biotechnology Co., Ltd. (Beijing, China) for sequencing.

2.6. Antibacterial Efficacy of Herbaceous Peony Polyphenols Observation

PDA medium was configured with herbaceous peony polyphenols added in one part and untreated in the other part; the final concentration of herbaceous peony polyphenols in the medium was 8%. All PDA accessed the same strains separately and measured and recorded their growth on days 0, 2, 4, 6 and 8, respectively.

2.7. Gene Isolation and Expression Analysis

RNA was extracted using the Plant RNA Extraction Kit (Shanghai TakaRa Bioengineering Co., Ltd., Shanghai, China). The cDNA was synthesized from 1 μg RNA using PrimeScript® RT reagent Kit With gDNA Eraser (TaKaRa, Japan). qRT-PCR was performed using the SYBR® Premix Ex Taq™ and contained 12.5 μL 2 × SYBR Premix Ex Taq™, 2 μL cDNA solution, 2 μL mix solution of target gene primers, and 8.5 μL ddH2O in a final volume of 25 μL. Gene-specific primers sequence for qRT-PCR detection are in the Table 1.

Table 1. Gene-specific primers sequence for qRT-PCR detection.

Gene	Forward Prime	Reverse Prime
Actin	ACTGCTGAACGGGAAATT	ATGGCTGGAACAGGACTT
PIP1-2	TTGGGGCTGAGATTATTG	GGAATGGTAGCCAAATGA
PIP2-1	CCTGTCTTGGCTCCACTT	CCCATGCTTTCTCATTATT
PIP2-2	AGACTTCTGGAATGCCTTGA	TATAAATCCGGCGGTGAC
NIP	ATATTCCGTTGGTCACATCT	CTAGGGTTGAACCAAGAAGT

2.8. Sequence and Statistical Analysis

All data were the mean of three replicates, and the experimental data were processed and analyzed using Microsoft Excel 2010 and SPSS 17.0 software.

3. Results

3.1. Effect of Polyphenols on Morphological Indices of Cut Herbaceous Peony Flowers

Herbaceous peony polyphenols significantly extended the vase life of cut flowers (Figure 1a). The whole process from bud stage to 80% wilted petals of herbaceous peony was about 8 days. Additionally, the herbaceous peony polyphenols extended the vase time by about 2 days. Moreover, polyphenol treatment significantly increased the flower diameter (Figure 1b). The flower diameter in polyphenol treatment showed a decreasing trend after 6 days of vase life, which was delayed by 2 days compared with the control. Additionally, the fresh weight of flowers in different treatments remained almost the same in the early stage, but the polyphenol treatment was higher than the control in the later stage (Figure 1b).

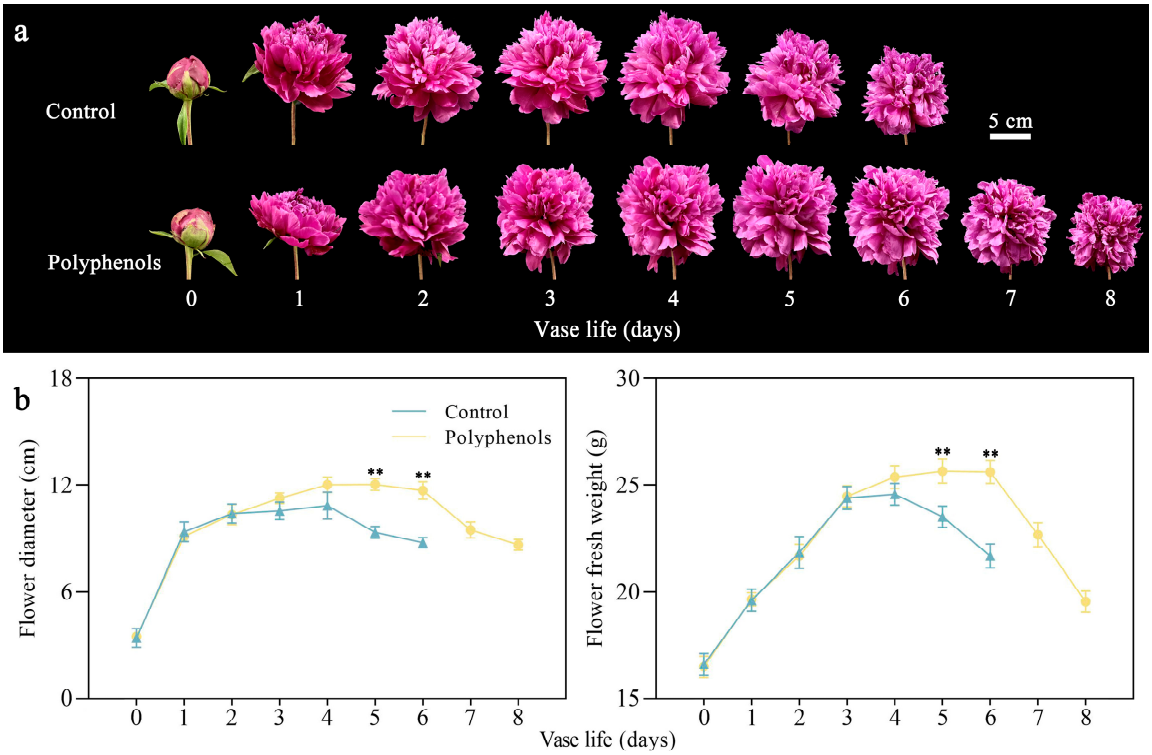

Figure 1. Effect of polyphenols on morphological indices of cut herbaceous peony flowers. (**a**) Effect of polyphenols on the vase performance of cut herbaceous peony flowers during the vase life. (**b**) Effect of polyphenols on the flower diameter and fresh weight of cut herbaceous peony flowers during the vase life. ** indicates highly significant difference of data, $p < 0.01$.

3.2. Effect of Polyphenols on Physiological Indices of Cut Herbaceous Peony Flowers

The water balance value between polyphenol treatment and the control was not different in the first 3 days, but the polyphenol treatment was higher than the control in the late stage of vase life (Figure 2a). Moreover, herbaceous peony polyphenols significantly increased the soluble protein content and kept the soluble protein content at a high level (Figure 2b). Additionally, the MDA content of flowers in polyphenol treatment showed a slower increasing trend than the control after 5 days of vase life (Figure 2b).

3.3. Effect of Polyphenols on Protective Enzyme Activities of Cut Herbaceous Peony Flowers

Herbaceous peony polyphenols significantly increased the POD activity and decreased the SOD activity of flowers compared to the control (Figure 3). Moreover, herbaceous peony polyphenols significantly limited the reduction in CAT activity, so that the CAT activity in cut flowers was always maintained at a high level and reached its maximum value after 4 days of vase life (Figure 3).

3.4. Microstructures of Stem Ends

The stem ends of herbaceous peony were sliced and placed under a microscope for observation (Figure 4). At first, they all had a green appearance and no microbes were observed under the microscope (Figure 4a,b). After 6 days, the stem ends of the flowers in herbaceous peony polyphenols had a light-green color and almost no microbes were observed (Figure 4c). In contrast, the stem ends of herbaceous peony in the control were black and were almost covered with microbes (Figure 4d).

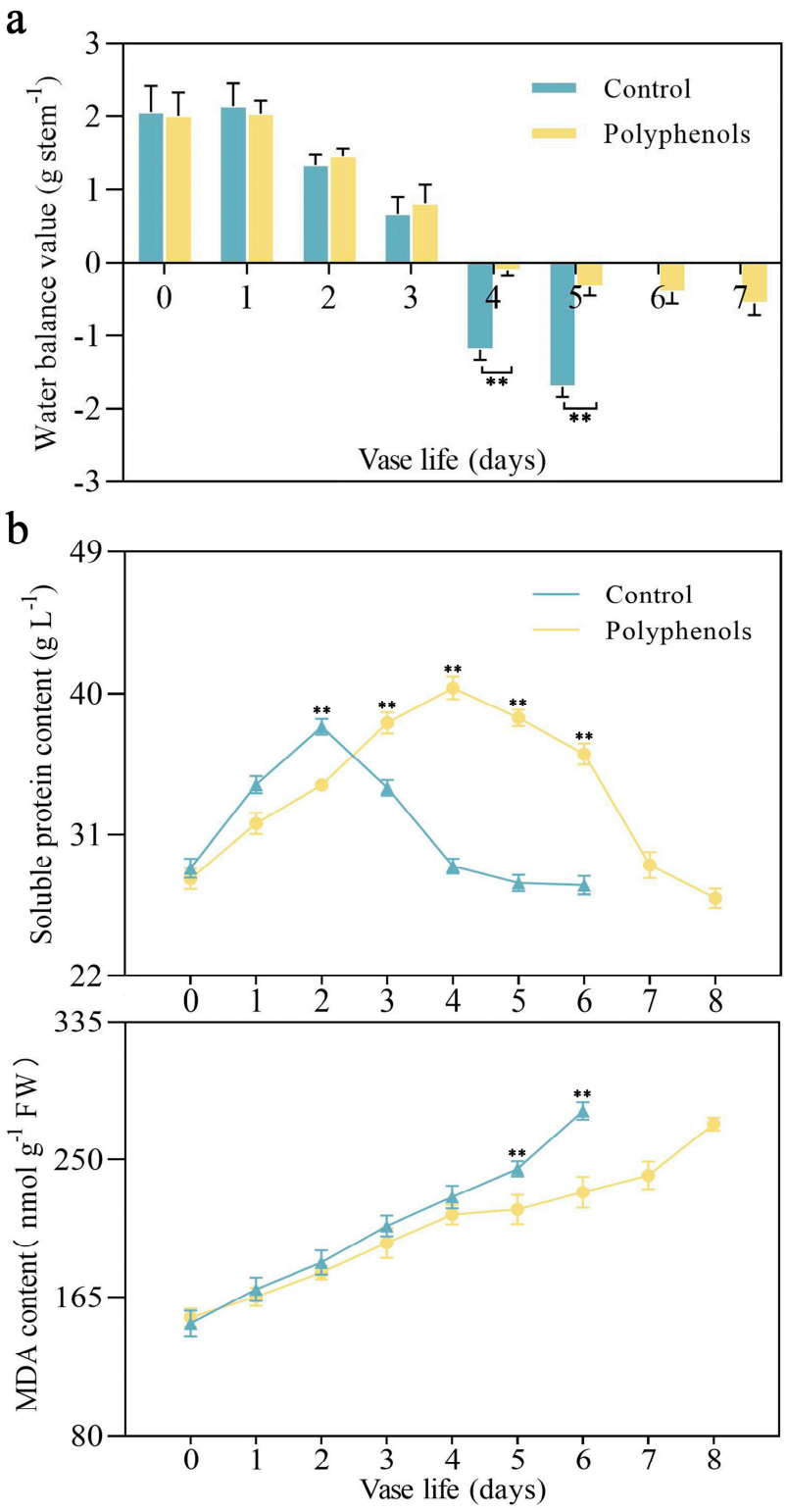

Figure 2. Effect of polyphenols on physiological indexes of cut herbaceous peony flowers. (**a**) Effect of polyphenols on the water balance value of cut herbaceous peony flowers during the vase life. (**b**) Effect of polyphenols on soluble protein and MDA content of cut herbaceous peony flowers during the vase life. ** indicates highly significant difference of data, $p < 0.01$.

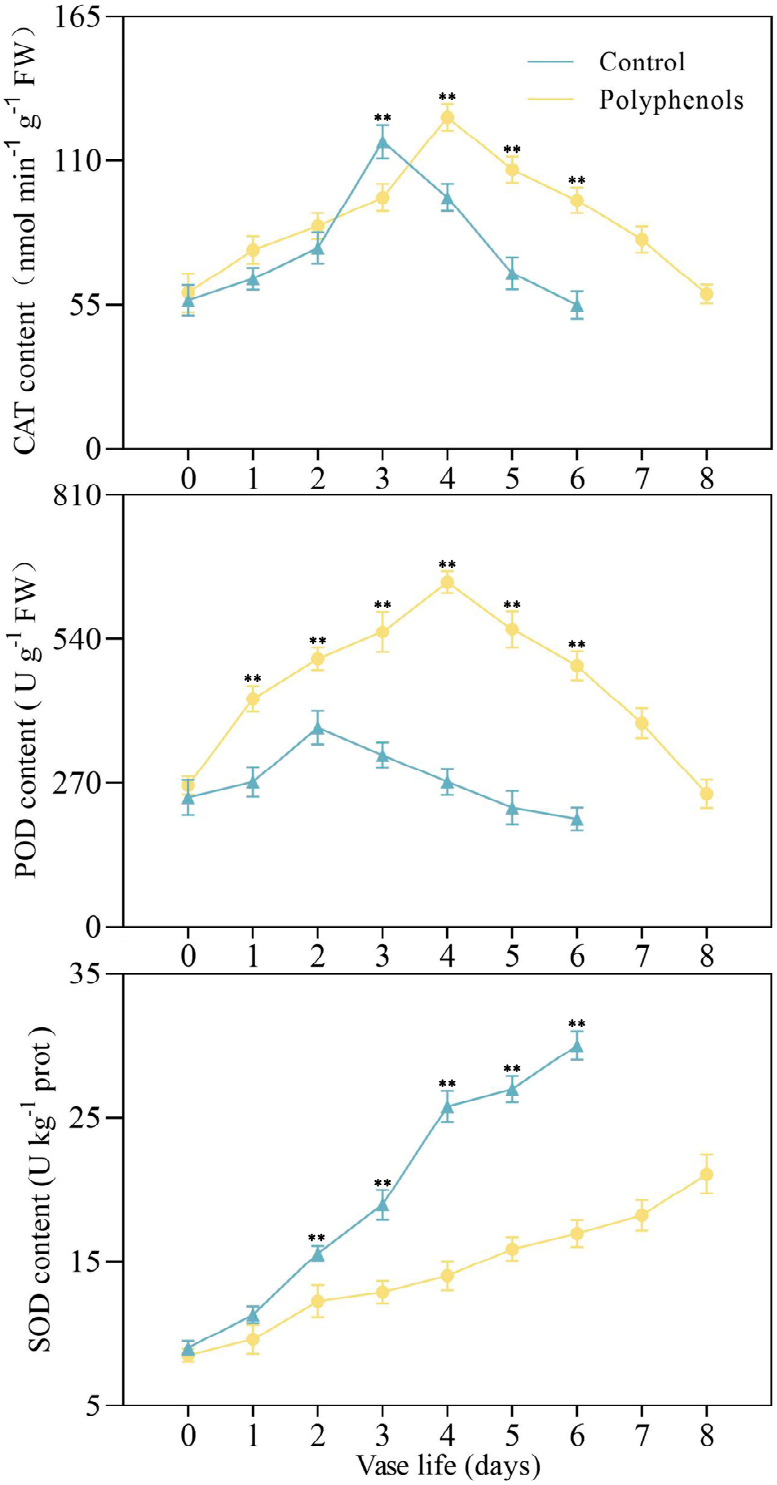

Figure 3. Effect of polyphenols on protective enzyme activity of cut herbaceous peony flowers. ** indicates highly significant difference of data, $p < 0.01$.

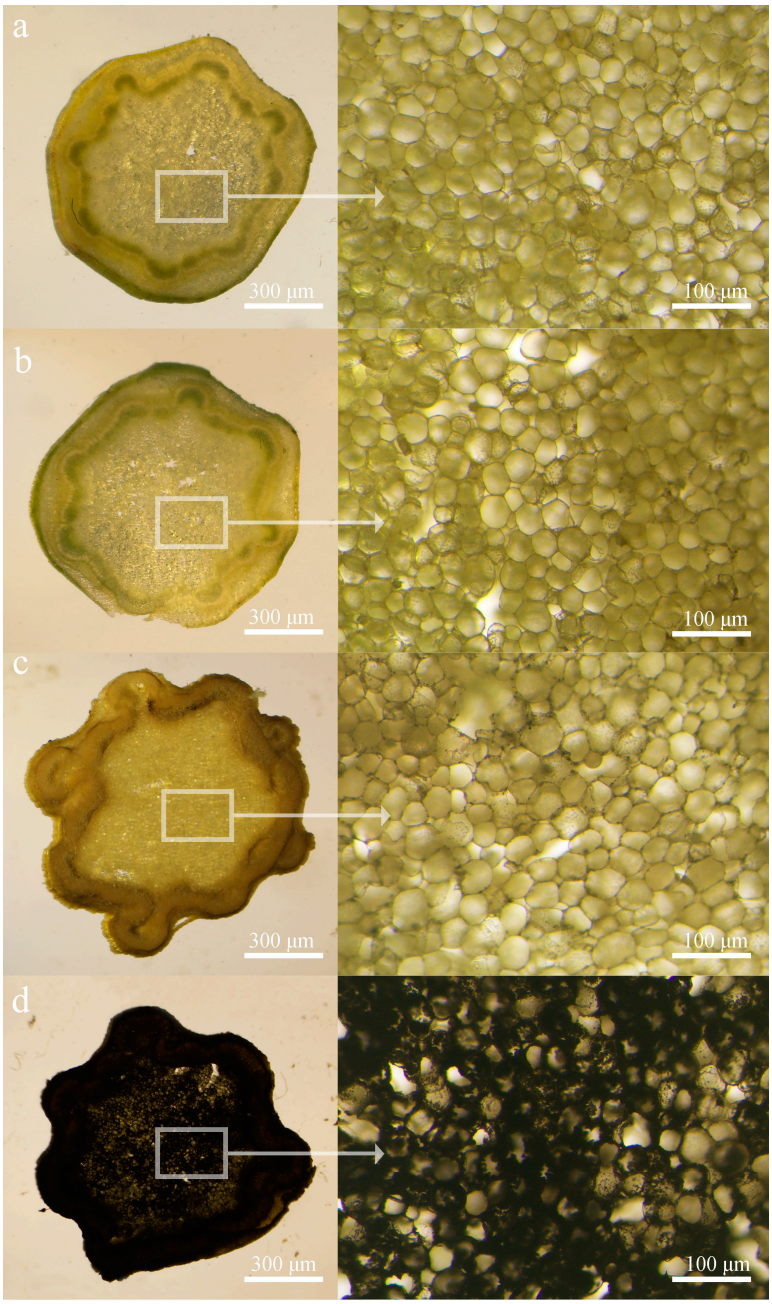

Figure 4. Microstructures of stem ends. (**a**) Polyphenol treatment for 0 day; (**b**) control for 0 day; (**c**) polyphenol treatment for 6 days; and (**d**) control for 6 days.

3.5. Microbe Identification

A strain was isolated by culturing the stem ends of herbaceous peony in PDA (Figure 5). The colonies of the isolate strain spread over the plate for about 10 days. They were white at first, then turned yellow, and finally changed to gray-green. Under the microscope, its mycelial cells expanded into a spherical shape. The conidial peduncle was vertical with a rough surface and no septum, and its conidia was born on the tip of the pedicel. It was presumed to be *Aspergillus* spp.

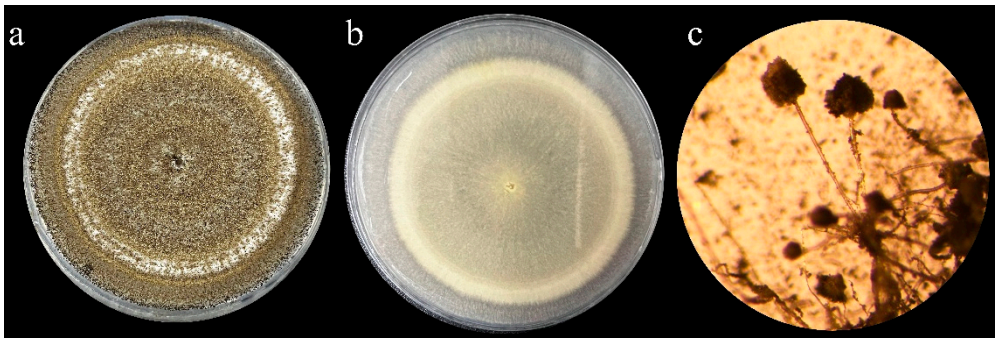

Figure 5. Colonial and conidial morphology of the isolate strain. (**a**) The front side of the colony; (**b**) the back side of the colony; and (**c**) conidial morphology.

The DNA of the strain was identified by PCR using universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC). The product was detected by agarose gel electrophoresis, and a single, bright band of approximately 550 bp was obtained (Figure 6a). The sequence results were highly homologous to *Aspergillus* spp. (Figure 6b), which showed that the strain was *Aspergillus* spp.

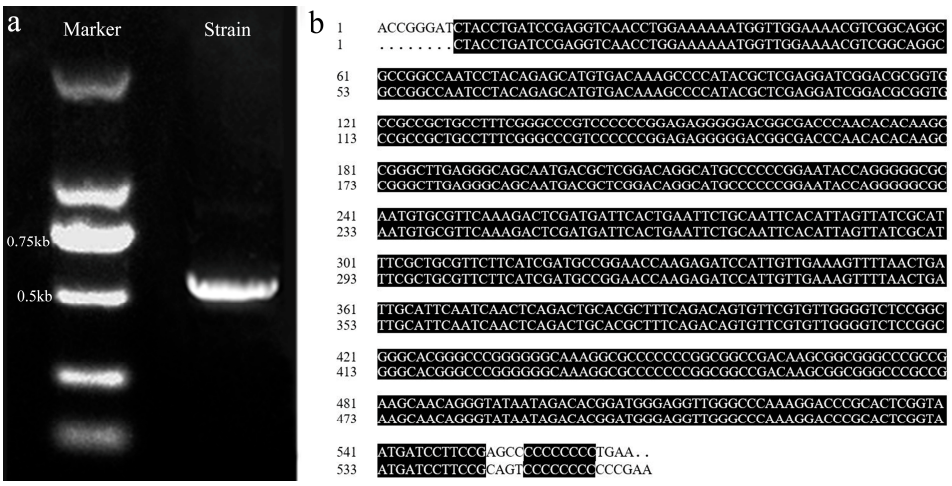

Figure 6. Molecular biology identification of isolated strain. (**a**) Agarose gel electrophoresis of the isolated strain; (**b**) the result of the isolated strain in blast. Comparison by blast; the upper line represents the speculative sequences, and the lower line represents the resulting sequences.

3.6. Antibacterial Efficacy of Herbaceous Peony Polyphenols

The inhibition of the strain by the PDA with the addition of herbaceous peony polyphenols was significant (Figure 7a). *Aspergillus* spp. grew well and spread over the plates after approximately 10 days in the control, while they grew slowly in the PDA with polyphenols. By measuring the diameter of the strain, the diameters in the PDA with polyphenols were significantly lower than in the control (Figure 7b).

3.7. Expression Analysis of AQPs

The expression levels of *AQPs* were inconsistent during the vase life, with higher levels of *PlPIP1;2*, *PlPIP2;2*, and *PlNIP* and a lower level of *PlPIP2;1* (Figure 8). As far as individual genes were concerned, *PlPIP2;1* accumulated in increasing amounts during the vase life. *PlPIP1;2* and *PlPIP2;2* showed a general trend of increasing and then decreasing expression, but the change in *PlNIP* was less pronounced than in the other three genes.

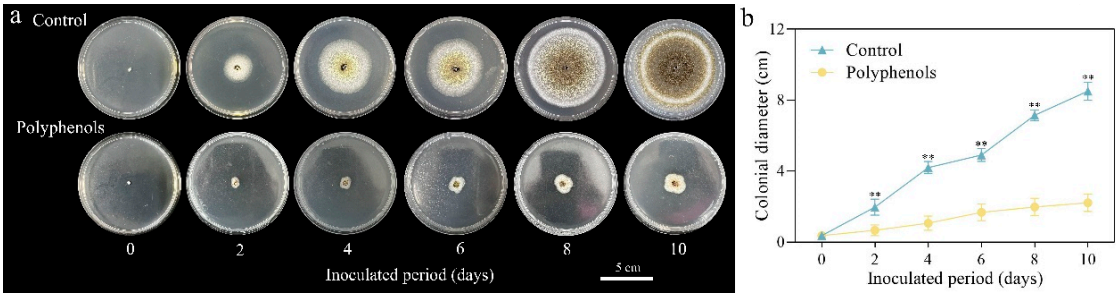

Figure 7. Antibacterial efficacy of herbaceous peony polyphenols on *Aspergillus* spp. (**a**) The performance of strain; (**b**) colonial diameter of strain. ** indicates highly significant difference of data, $p < 0.01$.

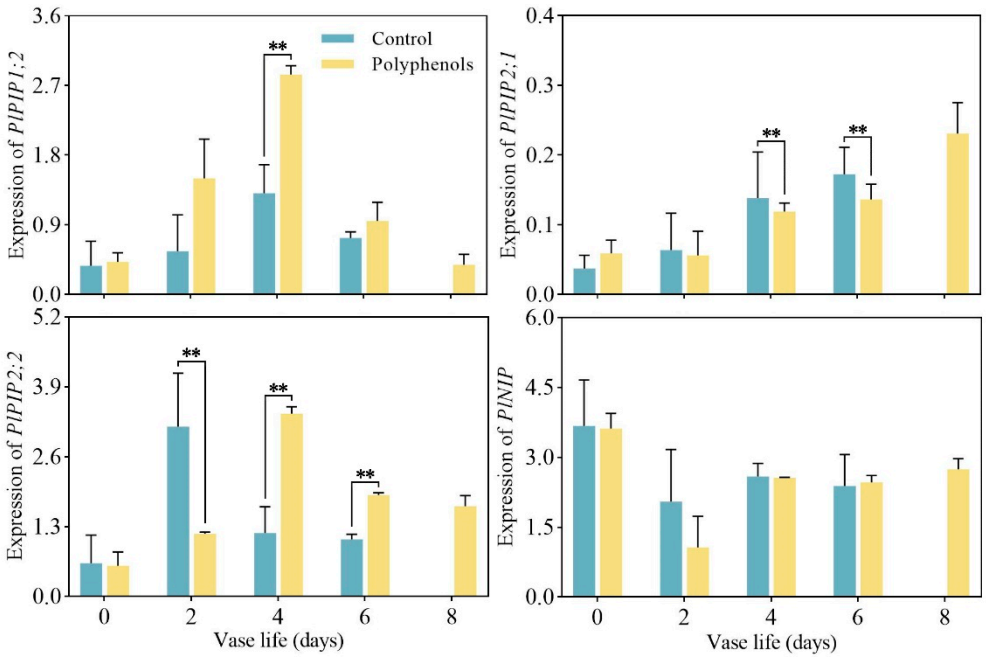

Figure 8. Expression analysis of *AQPs* during the vase life. ** indicates highly significant difference of data, $p < 0.01$.

4. Discussion

In this study, 8% mass concentration of herbaceous peony polyphenols was used as the experimental group and distilled water was used as the control. Moreover, the polyphenol treatment significantly increased the flower diameter of cut flowers, it remained at a higher level when the flower diameter in control became smaller. Compared with control, the decay time of polyphenol treatment flowers was generally delayed by 2 days, and the changes in fresh weight and water balance values of cut flowers were flatter. Tang et al. [18] used nano-silver on the same species of herbaceous peony, and the vase life of herbaceous peony was extended by 2 days compared to this experiment. The reason for this gap is the different period of herbaceous peony chosen for these two studies; the bud-stage flowers were chosen for Tang's study, and these flowers fully opened only after 4 days in the vase, while the herbaceous peony chosen for this study was the color-change stage, which fully opened in 1–2 days in the vase. However, the trends of the relevant morphological indicators, physiological indicators, and protective enzyme activities of herbaceous peony in both studies were basically the same. These results indicated that polyphenol treatment could extend the vase life of cut herbaceous peony flowers.

In addition to morphological changes, there were also some changes in physiological indices. Soluble protein is an important osmoregulatory substance and nutrient in plants,

and it is an important indicator for evaluating cut flowers [29]. The soluble protein content of polyphenol treatment flowers accumulated steadily in the early stage of the vase and decreased in the late of the vase due to proteolysis, which was consistent with the reports in *Gladiolus grandiflora* [30]. In this study, polyphenol treatment not only increased the maximum value of soluble protein in flowers but also slowed down its reduction rate, so that the flowers were always maintained at a high level during the vase life. MDA is the final product of membrane lipid peroxidation free radicals, and the level of lipid oxidation in plants can be reflected by detecting the level of plant MDA [31]. Fan et al. [32] found that humic acid treatment decreased the MDA content in cut chrysanthemum flowers. Moreover, Zheng et al. [33] found that that Ce(NO3)3 significantly decreased the MDA content in the cut *Dianthus caryophyllus* flower. In this study, the MDA levels of herbaceous peony with polyphenol treatment were significantly lower than the control in the late of vase life, which was consistent with the previous studies [33]. Meantime, SOD, CAT, and POD activities are the key enzymes in the antioxidant system of cut flowers [34–36]. Polyphenol treatment significantly increased the activities of SOD, CAT, and POD. However, it could not completely remove reactive oxygen species from the cut flowers. In the late stage of the vase, the enzyme activity decreased and reactive oxygen species accumulated continuously, leading to senescence of cut flowers. These trends are consistent with the results of previous experiments [37–39].

The inhibition of microbial growth is an important aspect of cut-flower preservation [40]. Bacteria, fungi, and viruses multiply so that they block the ducts during the vase life of cut flowers, disrupting the nutrient uptake and water balance of cut flowers, thus accelerating the senescence of cut flowers [41,42]. Williamson et al. [43] found that STS treatment significantly increased the vase life in *Boronia heterophylla*. In this study, the water balance values of cut flowers with polyphenol treatment increased compared to the control, and the stem ends in the control became darker. Further observation by microscopy showed that the stem ends of polyphenol treatment remained yellow-green, and no microorganisms were observed, while the stem ends of the control were covered with black microorganisms. This phenomenon indicates that polyphenols have the function of inhibiting the growth of microorganisms. In addition, the mechanisms to extend the vase life of polyphenols and nano-silver were also basically the same, both of which were mainly through the inhibition of microbial growth at the stem ends and through the regulation of the water balance of cut flowers [18]. On this basis, the pure strain was obtained by picking, isolating, and purifying the bacteria. The DNA of the strain was extracted, and the sequences of the strain were amplified and sequenced using universal primers ITS1 and ITS4. The fungi were identified as *Aspergillus* spp. Moreover, the inhibition effect of polyphenols on *Aspergillus* spp. was subjected to a vitro inhibition test, which proved that polyphenols have strong antibacterial activity. This vitro inhibition method has also been applied in other studies. Li et al. [44] found that NS solution at a concentration of 5 mg L^{-1} showed significant inhibition of *Pseudomonas fluorescens* and *Aeromonas aeruginosa* by using this method.

Aquaporins (*AQPs*) have a role in mediating water transport across membranes and play a key role in the water-regulation pathway [45]. Based on the specificity of their peptide sequences and distribution differences, the aquaporins in higher plants are mainly classified into four categories: *PIPs* (plasma-membrane intrinsic proteins), *TIPs* (vesicle-membrane intrinsic proteins), *NIPs* (a class of membrane intrinsic proteins), and *SIPs* (basic-membrane intrinsic proteins) [46]. Additionally, *PIPs* and *TIPs* have high protein activity and water transport permeability [47]. Kong et al. [48] studied *AQPs* during flowering in Dianthus and found that *AQPs* slowly accumulate during flowering and slowly decrease in later. Ye et al. [49] found that *HuPIP1;2* and *HuPIP2;1* showed increasing patterns and decreased thereafter. Referring to the study of Tang et al. [18], this study analyzed the expression of *PlPIP1;2*, *PlPIP2;1*, *PlPIP2;2*, and *PlNIP* during the vase life of cut herbaceous peony flowers. The results showed that the expression levels of *PlPIP1;2* and *PlPIP2;2* increased during the flowering of cut flowers and then decreased, which was consistent with the study of Ye et al.

PlPIP2;1 showed an overall upward trend throughout the vase life, which suggests that the gene may be involved in petal development and cellular senescence [50,51]. However, the expression of *PlNIP* did not change significantly compared to the other three genes. Compared with the control, *PlPIP1;2* and *PlPIP2;2* showed an up-regulation trend; they may help absorb the water of cell and maintain cell stability [51], while *PlPIP2;1* may help reduce water loss to maintain cell stability [52].

5. Conclusions

In summary, polyphenol treatment increased the content of soluble protein, as well as the activities of antioxidant enzymes SOD, POD, and CAT in herbaceous peony. Additionally, the excess oxygen radicals could be scavenged in time within a certain time frame, which delayed the senescence of cut flowers. Moreover, the polyphenol treatment had a significant inhibitory effect on *Aspergillus* spp. at the stem ends of cut flowers, which reduced the blockage of ducts at the stem ends, thus extending the vase life of herbaceous peony. Meanwhile, the *AQPs* of polyphenol treatment played an important role in maintaining the water balance of cut flowers. Therefore, polyphenol treatment could effectively maintain the fresh weight and flower diameter of cut flowers and extend the vase life of cut herbaceous peony. The results of this study can be applied to the preparation of the fresh-keeping agent and the natural bacteriostatic agent of cut flowers. At the same time, further study should be conducted to see if the natural polyphenols have the same effect on other cut flowers or species.

Author Contributions: Conceptualization, D.Z.; methodology, data curation, and software, P.L. and W.Z.; investigation, writing—original draft preparation, P.L.; writing—review and editing, P.L., W.Z. and D.Z.; and project administration, supervision, D.Z. and J.T. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable for studies not involving humans or animals.

Data Availability Statement: Not applicable.

Conflicts of Interest: All authors declare no conflict of interest.

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