Plant topping effects on the yield and active ingredient content of rhizome of Polygonatum cyrtonema, and analysis of functional composition, Antioxidant Activity its Waste

Wenwen Tang (tangwenwen6362@163.com)
College of Agronomy,Gansu Agricultural University
Yuan Chen
College of Agronomy,Gansu Agricultural University
Fengxia Guo
Gansu Agricultural University
Qian Li
College of Agronomy,Gansu Agricultural University

Article

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Abstract

Polygonatum cyrtonema is a perennial plant, and it has long been used in traditional Chinese medicine for food and medicine. The medicinal part of *Polygonatum cyrtonema* is the underground rhizome; however, the aerial part has not been studied so far for its medicinal activity. To promote the growth of underground rhizomes, the topping of aboveground plants of *Polygonatum cyrtonema* has been conducted. To understand the effect of the topping of aerial parts on the yield and medicinal components of rhizomes, the present study was conducted. Also, the chemical constituents, antioxidant, and *in vitro* hypoglycemic activities of the aerial stem, leave, and flower parts of *Polygonatum cyrtonema* were analyzed. The results showed that compared to the control (CK) treatment, the topping of the aerial part increased rhizome weight gain coefficient (3.43) significantly than the CK treatment (2.63). Moreover, the topping of the aerial part induces the biosynthesis of bioactive compounds. As such, the polysaccharide content in the rhizome (8.80%) and the total saponin content (37.60 mg/g) was significantly ($P < 0.01$) higher than CK treatment. The contents of total phenols and total flavonoids in PCL and PCF were significantly ($P < 0.01$) higher than those in rhizomes; however, the polysaccharide content (10.47%) in PCR (whole rhizome) was higher than that in PCS (3.65%), PCL (5.99%), and PCF (4.76%) content. The protein and amino acid contents in PCS, PCL, and PCF were higher than those in rhizomes. The protein and amino acid contents in PCS, PCL, and PCF were higher than those in rhizomes. PCS, PCL, and PCF showed strong antioxidant activities (DPPH, ·OH, ABTS, and FRAP), which were better than traditional medicinal parts (the rhizome).

To sum up, the topping measures can improve the rhizome yield of *Polygonatum cyrtonema* and the medicinal components of the rhizomes, which can be applied to improve production. The stems, leaves, and flowers had a much stronger antioxidant activity and higher the total polyphenols, flavonoids, proteins, and amino acid content. Therefore, stems, leaves, and flowers of *Polygonatum* can be fully developed according to different needs. they are typically used in animal feed, food storage and cosmetics.

1. Introduction

Polygonati rhizoma (Huangjing in chinese) is a Traditional Chinese Medicine (TCM) that has been known for its food and medicinal properties[1]. First described in "Shen Nong's Materia Medica", with the effects of invigorating qi, nourishing yin, invigorating spleen, and kidney[2]. It has a sweet taste and is traditionally known to be eaten as a food. It is a rich source of non-starch polysaccharides and fructooligosaccharides and has no starch content. It is one of the most cost-effective dietary products for diabetics and elderly people. It is expected to become an emerging high-quality crop with great potential[3]. Modern medical research shows that *Polygonati rhizoma* has various health beneficial effects such as enhancing immune function, regulating blood lipids, lowering blood sugar, and preventing cancer[4]. Moreover, it has been developed as an anti-aging health food and sports nutrition[5,6]. Since 2015, the *Polygonatum cyrtonema* planting area has been gradually increased in Zhejiang, Yunnan, Guizhou, Hunan, Jiangxi, Anhui, Guangxi, Sichuan, Hubei, and other Chinese provinces.

At present, the development and utilization of *Polygonatum cyrtonema* is limited to only underground rhizomes, whereas many aboveground parts, including stems, leaves, and flowers, are left behind. Also, to

promote the growth of its underground rhizomes, the aboveground parts were removed, which generates a lot of waste during the flowering period of *Polygonatum cyrtonema*. Furthermore, the above-ground parts of *Polygonatum cyrtonema* undergo drying and then burn in an open field, which pollutes the environment of the production area and its surroundings. So far, research on *Polygonatum cyrtonema* has focused on its traditional medicinal part (rhizome)[7–10], and only a few scholars have studied the aerial parts of *Polygonatum cyrtonema*[11–12]. These studies clearly postulated that the above-ground parts of *Polygonatum cyrtonema* could be utilized as food and medicine. Thus, there is an urgent need to study the functional components and physiological activities of the aerial parts (stems, leaves, and flowers) of *Polygonatum cyrtonema* to provide data for the utilization of these resources.

Polygonatum cyrtonema Hua is one of the original species of *Polygonatum* included in the 2020 edition of the "Chinese Pharmacopoeia". In the present study, *Polygonatum cyrtonema* Hua was used as the research object, to determine the effect of topping on the growth of *Polygonum cyrtonema* rhizomes, this study also compared the rhizome yields and the contents of polysaccharides, saponins, flavonoids, and other bioactive components with and without topping.In addition, the functional components and antioxidant activities of the wastes plant topping(stems, leaves, flowers) were evaluated to provide functional data for the potential development and future utilization of the aerial parts of *Polygonatum cyrtonema*.

2. Materials And Methods

2.1 Polygonatum planting experimental area

The field experiments were carried out in the planting base of Zhengda in Songtao County, Guizhou Province, China. The test area is located at 108°35'-109°23' east longitude, 27°49'-28°30' north latitude, with an average altitude of about 750 mm asl and an annual average temperature of 16.5°C. The frost-free period is of 293 days, the annual precipitation is 1378.3 mm, the average annual rainy period is 183 days, and the average sunshine time is 1228 h. The soil is sandy, and the soil fertility is medium. The Phellodendron saplings were planted at 5m×2m spacing with a tree height of 1. 5 – 2. 5m, which has the shading effect in the field.

2.2 Plant sample

The roots of *Polygonatum cyrtonema* Hua of weight between 18–25 g were collected. The stems, leaves, and flowers of Polygonatum were collected in early May 2021, while the rhizomes of Polygonatum were collected in December 2021. The botanical authentication was carried out by Professor Yuyong Liang at Tongren Polytechnic College. A voucher specimen (TRZYHM_1763) was deposited at in the herbal medicine collection of Tongren Polytechnic College. The rhizomes, stems, leaves, and flowers of *Polygonatum cyrtonema* were washed to remove impurities, dried at 105°C and 60°C subsequently for half an hour, vacuum-packed, and stored at -80°C until analysis. All materials were identified as *Polygonatum cyrtonema* Hua by Prof. Chen Yuan from Gansu Agricultural University and Prof. Liang Yuyong from Tongren Vocational and Technical College, China.

2.3 Instruments and Reagents

Instruments including UV-1801 UV-Vis Spectrophotometer (Beijing Ruili Analytical Instrument Co., Ltd.); SB5200DTD Ultrasonic Cleaner (Ningbo Xinzhi Biotechnology Co., Ltd.); CP214 Electronic Balance (Ohaus Instrument Co., Ltd.); and Ultrapure Water Meter (South Korea) Human Company) were used in this study. Diosgenin (batch number: 111539–202102, purity: 98.7%), D-anhydroglucose reference substance (batch number 110833–201908, mass fraction: 99.8%) were all purchased from China Food and Drug Administration Research Institute. Rutin (batch number: B20771, purity: ≥98%), gallic acid (batch number: B20851, purity: ≥98%) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Folin phenol reagent, and amino acid mixed reference substances were purchased from Sigma reagent company (Sigma-Aldrich). Methanol, ethanol, anhydrous glucose, dimethyl sulfoxide, anthrone, sulfuric acid, sodium nitrite, aluminum nitrate, sodium hydroxide, n-butanol, vanillin, and glacial acetic acid were all analytical grades and purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd.

2.4 Topping test method

At the beginning of May 2021, the topping test was carried out during the flowering period of the second year of planting *Polygonatum cyrtonema* (the growth of *Polygonatum cyrtonema* has not been completely and stably recovered in the first year of planting) and CT treatment (removal of 1/3 of the height of the above-ground plant) and CK treatment was set (no removal the top, keep all the plants). Each treatment was repeated 3 times, and a total of 6 plots were made with 9 m^2 (3m × 3m) area.

At the end of November 2021, the production was measured per meter square. The "S" type sampling method was adopted in the plot, and 15 plants were sampled in each plot. The fresh weight of the plant, according to the fresh weight of the roots of the sampled plants, calculate the rhizome weight gain coefficient, measure the yield per unit area, cut off the rhizomes of the current year, fixing them at 105°C for half an hour, and then dry them at 60°C, and determine the rhizomes of *Polygonatum cyrtonema* in the current year under different treatments. Rhizome weight gain coefficient was determined as:

Rhizome weight gain coefficient = (fresh weight of rhizome per plant - fresh weight of seed root) / fresh weight of seed root

2.5 Analysis of functional components

The polysaccharide content was determined as per the 2020 edition of "Chinese Pharmacopoeia"[2], using the anthrone-sulfuric acid chromogenic method. The polysaccharide content was determined at 582 nm wavelength using anhydrous glucose as reference ($y = 5.1307x + 0.0064$; $R^2 = 0.9993$). To calculate the polysaccharide content in the sample, the sample absorbance value was brought into the standard curve.

Total saponins content was determined according to the vanillin-glacial acetic acid-perchloric acid colorimetric method [13], with minor modifications. Briefly, 1.0 g of each sample powder was mixed with 30 mL of 80% ethanol and extracted using an ultrasonic bath at 25 ℃ for 30 min (3 times). The filtrates were combined, placed on a water bath, and evaporated to dryness. The residue was dissolved in 15 mL of 1% sodium hydroxide solution and extracted with 10 mL of water-saturated n-butanol (3 times), and the n-butanol solution was combined. The n-butanol solution was evaporated to dryness on a water bath, and the residue was dissolved in 5 mL of methanol. Accurately in 0.5 mL of test solution, 0.2 mL of 5% vanillin-glacial acetic

acid solution and 0.8 mL of perchloric acid were added. The mixture was shaken well and heated at 60°C (water bath) for 15 min, immediately placed in an ice bath, and added 5.0 mL of glacial acetic acid. The mixture was shaken well to develop color, and the absorbance was measured at 532 nm. Methanol as blank control was used to draw standard curve with diosgenin as reference ($y = 6.2371x - 0.0055$; $R^2 = 0.9997$).

The total polyphenol content was determined according to Folin's phenol colorimetric method[14]. Briefly, 0.1 g of each sample powder was added to 10 mL of 70% methanol solution and ultrasonically extracted at 25°C for 30 min (3 times). The extract was filtered and combined, and the volume was adjusted to 50 mL. A standard curve was drawn with gallic acid as reference ($y = 3.7514x + 0.006$; $R^2 = 0.9991$), and the total polyphenol content in the sample was calculated.

The total flavonoids content was determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric method[15]. Briefly, 0.1 g of each sample powder was weighed, and 5 mL of 70% methanol solution was added. The mixture was ultrasonically extracted 3 times at 25°C for 30 min each. The extract was filtered, combined, and diluted to a 25 mL volumetric flask. A standard curve was drawn with rutin as the reference ($y = 10.39x + 0.0014$; $R^2 = 0.9998$), and the total flavonoid content in the sample was calculated.

The Kjeldahl method was used to determine the protein content[16]. Briefly, 0.1 g of each sample was weighed into a digestion tube, and to the 1 piece of catalyst tablet, 8 mL of concentrated sulfuric acid was added. The mixture was placed in a digestion furnace at 420°C for 2 h. After the digestion was completed, a blue-green transparent solution was formed, removed, and cool down to room temperature. The Kjeldahl nitrogen analyzer was used to detect and record the percentage of protein.

2.6 Antioxidant activity analysis

2.6.1 Extract preparation

To 1.0 g of *Polygonatum cyrtonema* rhizome, stem, leaf, and flower sample powder, respectively, add 50 mL of 50% ethanol. The mixture was ultrasonicated for 10 min and then heated under reflux for 1 h, filtered, and transferred to a 50 mL volumetric flask. The volume was makeup using 50% ethanol.

2.6.2 Determination of DPPH free radical scavenging ability

DPPH was prepared as DPPH methanol solution with a concentration of 0.2 mM. Each sample was diluted into solutions of different concentrations, and 2 mL of each diluted solution was mixed with 2.0 mL of DPPH methanol solution. The absorbance was measured at 517 nm [17], and vitamin C (Vc) was considered as the positive control. The scavenging rate of DPPH free radicals for each sample was calculated using the equation (Eq. 1):

$$Y(\%) = 100*[A_0-(As-A_{S0})]/A_0 \quad \text{(Eq. 1)}$$

where Y (%) is the scavenging rate of DPPH free radicals, A_0 is the absorbance value of the blank group, As is the absorbance value of different samples, and A_{S0} is the absorbance value of the sample itself. According to

the clearance rate of DPPH for each sample at different concentrations, IC_{50} was calculated using the probability regression analysis of SPSS.

2.6.3 Determination of the free radical scavenging ability of ABTS

To 5 mL of 7 mM ABTS solution, 5 mL of 2.45 mM potassium persulfate solution was mixed and shaken well. The solution was kept in the dark for 12 h at room temperature. Before use, the ABTS solution was diluted with ethanol with an absorbance of 0.70 ± 0.02 at 734 nm. To prepare the working solution, 2 mL of ABTS + was added to 50 μL of each sample solution at different concentrations and left for 6 min in the dark at room temperature. The absorbance was measured at 734 nm [18], with Vc as a positive control. The scavenging rate of the sample to ABTS free radicals was calculated following the equation (Eq. 2):

$T(\%) = 100*[(A_0-As)/A_0]$ (Eq. 2)

where T (%) is the ABTS free radical scavenging rate, A_0 is the absorbance value of the blank group, and As is the absorbance value of different samples. According to the clearance rate of ABTS of each sample at different concentrations, IC50 was calculated using the probability regression analysis of SPSS.

2.6.4·OH radical scavenging rate

To 1.5 mL of each sample solution with different concentrations, 1.0 mL of 2.5 mmol/L salicylic acid solution, 1.0 mL of 5 mmol/L $FeSO_4$ solution, and 2.0 mL of distilled water were added and mixed well. To this solution, 1.0 mL of 5 mmol/L H_2O_2 was added. The mixture was placed at a constant temperature water bath at 37°C for 30 min, and the absorbance was measured at a wavelength of 510 nm, with V_C as a positive control. The scavenging rate of·OH radicals was calculated according to the equation (Eq. 3)[19]:

$·OH(\%) = 100*[A_0-(A_2-A_1)]/A_0$ (Eq. 3)

where·OH (%) is the scavenging rate of ·OH radicals, A_0 is the absorbance of the blank control; A_2 is the absorbance of the sample solution with H_2O_2; A_1 is the absorbance of the sample solution without H_2O_2.

2.6.5 Determination of FRAP reducing power

Referring to the method of Wang et al. [20], 0.5 mL of each sample solution after proper dilution into a 10 mL colorimetric tube was mixed with 5 mL of FRAP working solution (prepared for current use) in a water bath at 37°C. After 30 min, the absorbance of the reaction solution was measured at 593 nm. The standard curve was drawn with different concentrations of V_C solutions ($y = 0.0024x + 0.1269$ $R^2 = 0.9992$), and the total reducing power of each sample was represented by the equivalent content of V_C (unit: μg/mL VC eq/gsample).

2.7 Data Analysis

Significant differences between mean values were determined using one-way ANOVA at different significance levels. In addition, one-way ANOVA and determinations of the IC50 values for DPPH and ABTS radical

scavenging activity were performed using SPSS software V. 19.0. All experiments were conducted in triplicate, and IC50 values were determined by probit regression analysis.

3. Results And Discussion

3.1 Effects of plant topping on rhizome yield and functional components of *Polygonatum cyrtonema*

The rhizome yield and polysaccharide, total saponins, total flavonoids, and total polyphenols contents of topping and non-topping of *Polygonatum* samples are shown in Table 1. From the data in Table 1, topping *Polygonatum cyrtonema* has a significant impact on the fresh weight of the rhizome per plant and the yield per unit area. Furthermore, it was found that the rhizome weight gain coefficient can more accurately reflect the effect of topping on the rhizome weight gain of *Polygonatum cyrtonema*. These data showed that the topping of *Polygonatum cyrtonema* during the flowering period can significantly increase the yield of underground rhizomes. Picking off some flowers reduces the pulling force of flowers and fruits on carbon assimilates, while the pulling force of tubers on carbon assimilates increases sharply, which promotes the continuous flow of carbon assimilates to tubers [21,22]. On the other hand, it may be possible to reduce the nutrient consumption of the aerial part of *Polygonatum cyrtonema* by topping so that the underground rhizome can distribute more nutrients and promote its expansion. The wound-inducing effect after the topping causes the strong compensatory growth of *Polygonatum cyrtonema*[23,24], thereby driving the accumulation of underground rhizome biomass.

The output of Chinese herbal medicines is related to its economic value, and the content of the medicinal components is a decisive factor for the quality of Chinese herbal medicines. *Polygonatum cyrtonema* contains polysaccharides, saponins, flavonoids, and other chemical components. The underground rhizomes of *Polygonatum cyrtonema* are similar to bamboo whips, Aboveground parts grow on the current year rhizomes(Fig. 1). Therefore, the effect of topping on the quality of underground rhizomes was determined, and the functional components of the rhizomes of the current year correspond to the plants with and without topping. Polysaccharide is one of the main active components of *Polygonatum cyrtonema* [25], and it is commonly used to identify the quality of the medicinal material. With and without topping, there is no significant difference in polysaccharide content, and both meet the requirement of "2020 Pharmacopoeia" i.e., polysaccharides should not be less than 7.0%. Like polysaccharides, saponins are also the active ingredients and present in high content in *Polygonatum cyrtonema*[26,27], and showed anti-inflammatory, hypoglycemic, intestinal flora regulation, antidepressant, and other pharmacological activities[28,29]. From the data in Table 1, the saponin content of the rhizome of topping was 37.60 mg/g, which was significantly higher ($P < 0.01$) than that of the rhizome without topping (32.53 mg/g). Topping can significantly increase the content of total saponins, which could promote the synthesis of saponins and terpenoid skeletons after removing the tops of *Polygonum cyrtonema* [30].

Interestingly, the content of total polyphenols and total flavonoids in Polygonatum rhizomes of topping (7.51 mg/g, 1.08 mg/g) were lower than those without topping (7.94 mg/g, 1.42 mg/g). Light exposure should increase the content and activity of flavonoid synthases such as phenylalanine ammonia-lyase, chalcone

isomerase, flavanone-3-hydroxylase, and flavonol synthase [31]. Light played a major role in increasing the flavonoids content in plants. After topping, the aboveground plants receive less light, thus decreasing the content of flavonoids in *Polygonatum cyrtonema*. Most flavonoids contain phenolic hydroxyl group, an important part of polyphenols. The decrease in the flavonoids content after topping is also one of the reasons for the decrease in the polyphenols content. These metabolites may be synthesized in the stems and then transferred to the roots or directly synthesized in the roots. The rhizomes' physiological and secondary metabolic mechanisms in response to light are still unclear, and further research is needed. Although the content of flavonoids and polyphenols in Rhizoma Polygonatis rhizomes decreased after decapitation; however, saponins, the main active ingredient usually used to evaluate its quality, increased significantly in *Polygonatum cyrtonema*. As a result, the content and yield of polysaccharides increased significantly. However, further research needs to be conducted to determine the regulatory mechanism of the distribution of nutrients in the aboveground and underground parts of *Polygonatum cyrtonema* to accumulate to the roots.

3.2 Functional components of stems, leaves, and flowers of Polygonatum

The contents of polysaccharides, total saponins, total phenols, total flavonoids, and proteins in each sample are shown in Table 2. The data in Table 2 showed that the rhizomes (PCR, the whole rhizomes), stems (PCS), leaves (PCL), and flowers (PCF) of *Polygonatum cyrtonema* contain functional components such as polysaccharides, total saponins, total phenols, total flavonoids, and proteins; however, their concentrations were significantly different. For instance, the polysaccharide content in PCR was the highest (10.47%), followed by leaves (5.99%) and flowers (4.76%), while the polysaccharide content in the stem was the lowest (3.65%). The content of total saponins in flowers (36.68mg/g) is very close to the rhizomes (39.09mg/g) content, and there is no significant difference between the two contents. The content of total saponins in stems and leaves is lower and represents only 1/7 of that in flowers (~ 1/5). The contents of total phenols and total flavonoids in different samples were consistent, all of which were in the order of PCF > PCL > PCS > PCR, which was inconsistent with the study of Zhao et al.[32]. The contents of total phenols and flavonoids in flowers, leaves, and stems were significantly ($P < 0.01$) higher than those in rhizomes, especially the polyphenols content in flowers was much higher than that in rhizomes. The results were consistent with Zhang et al.[12], which could be related to the higher content of anthocyanins in flowers. The protein content in the rhizome was the lowest (3.08%), which was only half of the protein content in the stem. The protein content in the leaf and flower was higher, 13.81% and 11.64%, respectively, and about 3.7–4.5 times in the rhizome. These data showed that the non-medicinal parts such as stems, leaves, and flowers of Polygonati contain more bioactive compounds than the rhizomes. The content of total phenols, total flavonoids, and protein in the leaves is high, and it has a good potential to be developed into functional food and skincare products.

The data in Table 3 showed the total amino acids, their content, and individual content in rhizomes (PCR), stems (PCS), leaves (PCL), and flowers (PCF). *Polygonatum cyrtonema* stems, leaves, and flowers are rich in amino acids, which are much higher than rhizomes. The total amino acid content in leaves is the highest (23.25%), about 3.16 times that in the rhizomes. The contents of valine, leucine, isoleucine, phenylalanine, and lysine in the stems, leaves, and flowers are all higher, especially in the leaves; the contents of these essential

amino acids are above 1%. These amino acids provide better health care functions such as regulating blood sugar and improving immunity. The contents of umami amino acids such as aspartic acid and glutamic acid are higher in the leaves. These results indicated that stems (PCS), leaves (PCL), and flowers (PCF) contained more abundant amino acids than rhizomes.

3.3 Antioxidant activity

Antioxidants can be divided into synthetic antioxidants and natural antioxidants. With the research on the toxicology of synthetic antioxidants and the enhancement of people's health awareness, the development of natural antioxidants came to light. Most natural antioxidants are obtained from plants [33]. Intake of natural antioxidants Many studies have shown that the natural antioxidant components contained in plants can effectively reduce the incidence of aging-related diseases such as cardiovascular disease, diabetes, cancer, etc. by scavenging free radicals[34,35].

DPPH is a purple, very stable free radical, which is used to measure the ability of various antioxidants to scavenge free radicals. The scavenging effect on DPPH free radicals is shown in Fig. 2-A. Both leaves and flowers have high DPPH free radical scavenging ability and the scavenging rate increases with increasing concentration. Among them, flowers have the strongest scavenging ability to DPPH free radicals, and at 1000 μg/mL, the scavenging rate reaches 88.9%, respectively. This could be related to the high polyphenols and flavonoids concentration in the flowers. In the case of stems and leaves samples at 3000 μg/mL, the scavenging abilities of DPPH free radicals were 79.2% and 85.1%, respectively, which were higher than those of rhizomes (61.2%). This may be related to the fact that stems and leaves contain more flavonoids and polyphenols than rhizomes and can directly capture free radicals.

As shown in Fig. 2-B, the stems, leaves, and flowers of *Polygonatum cyrtonema* also showed comparatively stronger ·OH radicals scavenging ability than rhizomes. For example, at a concentration of 4000 μg/mL, the·OH radicals scavenging abilities of the stems, leaves, and flowers are very close, reaching more than 85%, while the scavenging power of rhizomes is only 43.6%. However, at lower concentrations (250–1000 μg/mL), the leaves showed stronger ·OH radical scavenging ability than stems and flowers. Moreover, the scavenging ability of flowers changed greatly with increasing concentration, indicating that the flower has a strong ability to scavenge ·OH radicals.

The scavenging effect on ABTS free radicals is shown in Fig. 2-C. Similar to the results of DPPH and ·OH free radical scavenging experiments, flowers and leaves showed higher scavenging ability to ABTS free radicals at a concentration of 1500 μg/mL. At this concentration, the ABTS clearance rates of leaf and flower extracts reached 88.4% and 89.3%, respectively, while the ABTS clearance rates of stems at this concentration are weaker (60.3%), like those of underground rhizomes.

The IC_{50} values of the stem, leaf, and flower extracts for scavenging DPPH, ·OH, and ABTS free radicals, and the FRAP total reducing power analysis results are shown in Table 4. The smaller the IC_{50}, the stronger the free radical scavenging ability. It can be seen from Table 4 that for the IC_{50} of DPPH and ABTS, the scavenging effect of stems, leaves, and flowers is stronger than that of rhizomes ($p < 0.01$), but compared with stems (719.35, 862.39 μg/mL), leaves (301.86, 342.87 μg/mL) and flower (251.44, 264.61 μg/mL) showed stronger

DPPH and ABTS free radical scavenging effects ($p < 0.01$). However, unlike DPPH, the IC_{50} of leaf against·OH (1069.93 μg/mL) was the smallest, indicating that leaves had a stronger ability to scavenge ·OH, followed by flowers and stems.

The reducing power is also an important indicator of measuring antioxidant activity. As shown in Table 4, the total reduction of FRAP scavenging power was similar to that of DPPH and ABTS, with higher reducing power for flowers (1343.65 μg/mL VC eq/gsample), leaves (1232.43 μg/mL VC eq/gsample), and stems (552.69 μg/mL VC eq/gsample), but both were significantly stronger ($p < 0.01$) than the reducing power of underground rhizomes.

The above results of DPPH, ·OH, ABTS, and FRAP showed that the stems, leaves, and flowers of *Polygonatum cyrtonema* have strong free radical scavenging ability and antioxidant activity. The inhibitory activity of the rhizomes was significantly lower than other samples ($P < 0.01$) ,especially ,the aboveground parts (leaves and flowers) showed high antioxidant effects related to the higher content of flavonoids and polyphenols. In addition, there are differences in the free radical scavenging ability of DPPH, ·OH, ABTS, and the antioxidant capacity of FRAP among the stems, leaves, and flowers of *Polygonum cyrtonema*, which could be related to the different mechanisms of different antioxidant methods. The antioxidant activities of stems, leaves, and flowers in the oxidation system were lower than Vc, which may be because the content of active antioxidant substances in the crude extracts of stems, leaves, and flowers are not high enough.

4. Conclusions

This study firstly integrated factors such as underground rhizome yield, rhizome weight gain coefficient, and the content of bioactive components, and postulated that topping effectively increased the cultivation yield of *Polygonum cyrtonema*. The functional components, such as polysaccharides, flavonoids, polyphenols, saponins, proteins, and amino acids, showed their presence in stems, leaves, and flowers of *Polygonatum cyrtonema* is comparable to that of the traditionally used part (the rhizome). Especially, the stems, flowers and leaves exhibited much higher antioxidant activities as well as higher protein, amino acid, total flavonoid and total polyphenol contents.These findings provide an important database for the development and application of above-ground stems, leaves, and flowers for medicinal and food purposes.

So far, the stems, leaves, and flowers of *Polygonum cyrtonema* are considered wastes. The present study results showed that *Polygonum cyrtonema* resources could be used as a source of bioactive components for health needs. For instance, flowers can be used as raw materials for extracting natural antioxidants (high content of polyphenols and flavonoids) in beauty and skincare products. Also, it can be used to make health scented tea, functional food additives, pharmaceutical products, etc. The protein and amino acids in leaves are significantly higher than common vegetables, which contain more umami amino acids and have better taste, and can be eaten as vegetables. Old leaves and stems can be used as food preservatives, feed additives, etc. Compared with the underground rhizomes of *Polygonum cyrtonema*, the aboveground stems, leaves, and flowers have the characteristics of faster growth, strong regeneration ability, and large biological yield. Moreover, they have broader development potential and application value. To develop these parts of *Polygonum cyrtonema* into a more widely used health product, further research is needed on the pharmacological activities and long-term toxicological experiments.

Declarations

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Additional Information

All data generated or analysed during this study are included in this published article [and its supplementary information files(Related files)].Supplementary information accompanies this paper at http://www.nature.com/srep

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Tables

Table1

Effects of Plant topping on Rhizome yield and functional components of *Polygonatum cyrtonema*

treatments	The fresh rhizome weight gain coefficient	the yield per unit area (kg/m2)	polysaccharide(%)	total saponins mg/g	total polyphenols mg/g	total flavonoids mg/g
CT	3.43^{Aa}	1.17^{Aa}	8.80 ± 0.39^{Aa}	37.60 ± 2.91^{Aa}	7.51 ± 0.48^{Ab}	1.08 ± 0.11^{Bb}
CK	2.63^{Bb}	0.93^{Bb}	8.64 ± 0.30^{Aa}	32.53 ± 2.67^{Bb}	7.94 ± 037^{Aa}	1.42 ± 0.15^{Aa}

The data are presented as the means ± SD. Within each column, the different superscripted small and capital letters indicate significant and highly significant differences at $p < 0.05$ and $p < 0.01$, respectively, based on ANOVA-Duncan multiple comparison results.

Table 2

Polysaccharide (POL),total saponins(TS), total polyphenol (TP) , total flavonoid (TF),and protein contents

Samples	POL(%)	TS mg/g	TP mg/g	TF mg/g	Protein %
PCS	3.65 ± 0.28^{Dd}	4.99 ± 0.20^{Bb}	21.52 ± 0.43^{Cc}	2.62 ± 0.15^{Cc}	9.87 ± 0.79^{Cc}
PC L	5.99 ± 0.22^{Bb}	7.11 ± 0.17^{Bb}	37.57 ± 1.12^{Bb}	3.09 ± 0.12^{Bb}	13.81 ± 0.91^{Aa}
PCF	4.76 ± 0.17^{Cc}	36.68 ± 2.53^{Aa}	53.83 ± 2.33^{Aa}	4.48 ± 0.13^{Aa}	11.64 ± 0.70^{Bb}
PCR	10.47 ± 0.47^{Aa}	39.09 ± 1.37^{Aa}	9.63 ± 0.56^{Cc}	1.64 ± 0.09^{Dd}	3.08 ± 0.33^{Dd}

The data are presented as the means ± SD. PCR, *Polygonatum cyrtonema* Hua rhizome;

PCS,*Polygonatum cyrtonema* Hua stem;PCL,*Polygonatum cyrtonema* Hua leaves;

PCF, *Polygonatum cyrtonema* Hua flower. Within each column, the different superscripted small and capital letters indicate significant and highly significant differences at $p < 0.05$ and $p < 0.01$, respectively, based on ANOVA-Duncan multiple comparison results.

Table3

Amino acid contents of PCR, PCS,PCL, and PCF

Amino acid	Amino acid content (%)			
	PCS	PC L	PCF	PCR
Asp	1.93	2.42	1.13	1.57
Thr	0.94	1.08	0.43	0.25
Ser	0.96	1.11	0.5	0.37
Glu	3.45	4.52	2.72	1.36
Gly	0.98	1.10	0.67	0.29
Ala	1.14	1.30	1.00	0.23
Val	1.29	1.54	0.83	0.31
Met	0.28	0.32	0.06	0.05
Ile	0.85	1.02	0.56	0.14
Leu	1.82	2.02	0.98	0.56
Tyr	1.04	1.19	1.02	0.77
Phe	1.12	1.25	0.69	0.23
Lys	1.03	1.45	0.78	0.28
His	0.46	0.60	0.26	0.14
Arg	1.12	1.32	0.87	0.67
Pro	0.86	1.01	0.58	0.13
Total content	19.27	23.25	13.08	7.35

Table 4

IC_{50} values of DPPH ·OH and ABTS radicals inhibition and test results of FRAP assays of extracts of rhizome,stem , leaf and flower

Samples	DPPH IC_{50} values (μg/mL)	·OH IC_{50} values (μg/mL)	ABTS IC_{50} values (μg/mL)	FRAP assays result (μg/mL VC eq/gsample)
PCS	$719.35±6.06^{Bb}$	$1348.63±38.89^{Aa}$	$862.39±26.04^{Bb}$	$552.69±12.99^{Cc}$
PCL	$301.86±9.48^{Cc}$	$1069.93±54.89^{Cc}$	$342.87±9.21^{Cc}$	$1232.43±17.52^{Bb}$
PCF	$251.44±2.19^{Dd}$	$1280.97±16.60^{Bb}$	$264.61±18.42^{Dd}$	$1343.65±49.65^{Aa}$
PCR	$2069.67±26.53^{Aa}$	ND	$1039.87±25.13^{Aa}$	$307.85±3.46^{Dd}$

Note:The data are presented as the means ± SD. ND, not detected;Within each column, the different superscripted small and capital letters in indicate significant and highly significant differences at $p < 0.05$ and $p < 0.01$, respectively, based on ANOVA-Duncan multiple comparison results.

Figures

Figure 1

Legend not included with this version

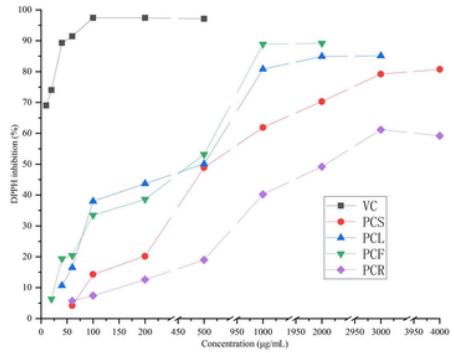

Figure 2-A

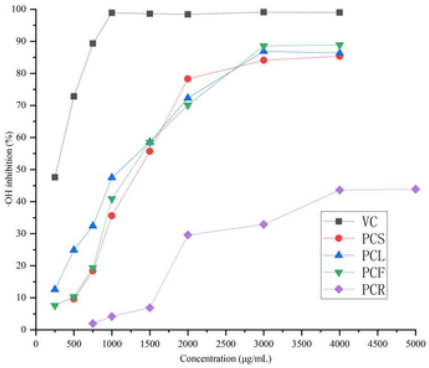

Figure 2-B

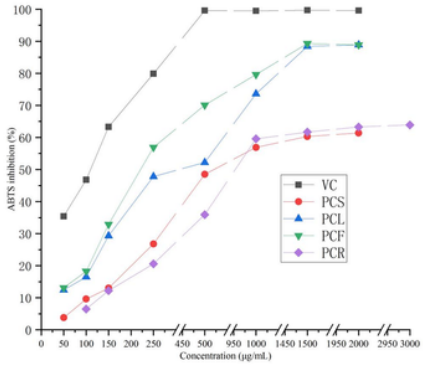

Figure 2-C

Figure 2

Inhibition of DPPH,ABTS,·OH radical for samples

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ABTS.opju
- DPPH.opju
- OH.opju