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Actividad antibacteriana de flavonoides y alcaloides basada en cromatografía líquida de alto rendimiento

Antibacterial Activity of Flavonoids and Alkaloids Based on High Performance Liquid Chromatography

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Resumen
Berberidaceae El bambú de Nantian, el agracejo de hoja verde y el agracejo de hoja roja son importantes plantas de enverdecimiento de jardín, con excelente ecología y ornamentales únicos. Estudios recientes han demostrado que los componentes químicos de estas tres especies de berberidas tienen algún valor de desarrollo medicinal. El estudio de los componentes químicos y las funciones del bambú de Nantian, Berberis mandshurica y Berberis mandshurica tiene cierta función de referencia para el desarrollo científico y la utilización integral de los recursos vegetales de Berberid. Una gran cantidad de estudios han demostrado que los flavonoides y alcaloides en Berberidaceae a menudo tienen una fuerte actividad antibacteriana. Tres especies de Berberidaceae son plantas de jardinería comunes, fáciles de cultivar a gran escala, ricas en recursos y de bajo costo. En vista de esto, este estudio analizó el contenido de alcaloides y flavonoides de P. chinense, Berberis chinensis y Berberis pubescens en diferentes estaciones, y exploró las posibles razones de los cambios estacionales en alcaloides y flavonoides. La actividad bacteriostática de los alcaloides y flavonoides y su ley de cambio estacional, con miras a promover el desarrollo y la utilización de alcaloides y flavonoides en las berberidas.

Palabras clave: Berberidaceae; Flavonoides; Alcaloides; Actividad antibacterial

Abstract
Berberidaceae Nantian bamboo, green leaf barberry and red leaf barberry are important garden greening plants, with excellent ecology and unique ornamental. Recent studies have shown that the chemical constituents of these three species of Berberids have some medicinal development value. Studying the chemical constituents and functions of Nantian bamboo, Berberis mandshurica and Berberis mandshurica has certain reference function for scientific development and comprehensive utilization of Berberid plant resources. There are 17 genera in Berberidaceae. A large number of studies have shown that flavonoids and alkaloids in Berberidaceae often have strong antibacterial activity. Three species of Berberidaceae are common landscaping plants, easy to grow on a large scale, rich in resources, and low in cost. In view of this, this study analyzed the contents of alkaloids and flavonoids of P. chinense, Berberis chinensis, and Berberis pubescens in different seasons, and explored the possible reasons for the seasonal changes in alkaloids and flavonoids. The bacteriostatic activity of alkaloids and flavonoids and its seasonal change law, with a view to promoting the development and utilization of alkaloids and flavonoids in Berberids.

Key words: Berberidaceae; Flavonoids; Alkaloids; Antibacterial activity

1. Introduction

Berberidaceae, Nandina domestica, Berberis thunbergii, and B. thunbergii var. Atropurpurea are important landscaping plants with excellent ecology and unique ornamental properties [1]. Recent studies have shown that the chemical constituents of these three species of Berberids have some medicinal development value. The in vitro free radical scavenging test found that the polysaccharides of Nantian bamboo, Berberis mandshurica and Berberis mandshurica had certain antioxidant activity; the glycerin extract and ethanol extract of Berberis mandshurica could inhibit the growth of Streptococcus pyogenes, etc .; The red pigment of Berberis fortunei can inhibit the growth of Bacillus subtilis, Escherichi coli, and Staphylococus aureus; Nantianzhu essential oil can inhibit Pseudomonas aeruginosa and gold Growth of Staphylococcus aureus [2]. Studying the chemical constituents and functions of Nantian bamboo, Berberis mandshurica and Berberis mandshurica has certain reference function for scientific development and comprehensive utilization of Berberid plant resources [3].

Berberids have 17 genera. A large number of studies have shown that flavonoids and alkaloids of Berberids often have strong antibacterial activity. Berberidaceae Berberis are rutin, quercetin and other flavonoids with B. anstata, which can inhibit the growth of a variety of pathogenic microorganisms; Berberis is a heterogeneous

Berberis (B. anstata). heteropoda) B.iliensis) B.iberica B.kaschga / ia and Berberis amurensis Pathogenic bacteria have a significant inhibitory effect; the alkaloids of Epimedium (E. koreanum) of the genus Epimedium can inhibit the reproductive growth of E. coli in logarithmic stages, severely damage the surface of the bacteria, and make the bacteria between Clusters and adhesions with each other; Jatrorrhizine of M. aquifolium (Mahonia) can inhibit Epidermophyton floccosum and Propionibacterium acnes) 'S growth. However, there are few reports on the bacteriostatic effects of the alkaloids and flavonoids of Nantian bamboo, Berberis mandshurica and Berberis mandshurica [4].

To study the bacteriostatic effects of alkaloids and flavonoids in Nantian bamboo, Berberis mandshurica and Berberis mandshurica, and to analyze the effects of seasons on flavonoids, alkaloid content and antibacterial activity, may have the following application prospects: Flavonoids, alkaloids, bacteriostatics or preservatives, serving the pharmaceutical, food or feed industry; 2) helping to make full use of waste leaf resources obtained by thinning and pruning, increasing the added value of technology; 3) helping to expand 3 small The application range of plovers extends the industrial chain. Compared with traditional Chinese herbal medicine, the three species of Berberidaceae are common landscaping plants, easy to grow on a large scale, rich in resources, and low in cost. In view of this, this study analyzed the contents of alkaloids and flavonoids of P. chinense, Berberis chinensis, and Berberis pubescens in different seasons, and explored the possible reasons for the seasonal changes in alkaloids and flavonoids. The bacteriostatic activity of alkaloids and flavonoids and its seasonal change law, with a view to promoting the development and utilization of alkaloids and flavonoids in Berberids [5].

2. Materials and Methods

2.1 Test Materials and Reagents

The leaves of Nantian Bamboo, Berberis mandshurica and Berberis mandshurica in different seasons were collected from Xi'an, Shaanxi Province in 2015. Season division refers to the method of Miao Qilong and Wang Yong. After the leaves are picked, they are rinsed with distilled water, then placed in a ventilated place to dry in the shade, crushed, sieved (180 ± 7.6) gm, sealed and refrigerated in a refrigerator, and set aside. E. coli (strain code: ATCC25922) was purchased from China Industrial Microbial Strain Collection and Management Center.

2,3,5-triphenyltet-razolium chloride (TTC) (purity ≥ 99%), ampicillinsodiumsalt (USP grade), quercetin (Purity 3 95%), berberine (purity ≥ 95%) were purchased from Shanghai Yuanye Company; beef extract peptone medium (pH 7.2 ~ 7.4), agar was purchased from Beijing Aoxing Company; aluminum trichloride Bismuth nitrate and ethanol are domestic reagents (analytical grade) [6].

2.2 Instruments, Materials and Reagents

Agilent 1100 high performance liquid chromatograph with quaternary gradient pump, in-line degasser, autosampler, column oven, and UV detector.

Berberidaceae plants are the dried roots of the Ranunculaceae plant, Coptis chineisis Franch, and Nantian bamboo is the dried, near-ripe fruit of the Rutaceae plant, Evodia rutaecarpa (Juss) Benth, all provided and identified by a Chinese medicine practitioner. Berberine hydrochloride, palmatine hydrochloride, and jatrorrhizine hydrochloride were purchased from the China National Institute for the Control of Pharmaceutical and Biological Products; berberidae, hypericin, and isorhamnetin-3-0-galactoside the control was made by our laboratory [7].

2.3 Chromatographic Conditions

Chromatographic column: Diamonsil G8 column (250mm × 4.6mm.id, 5 m, Dima) Mobile phase: Phase A is H2OSDSH3PO4 (1000mL: 5g: 1mL), Phase B is CH3CN-H2OSDSH3PO4 (600mL: 400mL: 5g: 1mL) ; Use linear gradient elution procedure 25% B 50% B 80% B (hold 30min) 90% B flow rate 0.8mL / mn column temperature 40oC; injection volume 10 L; detection wavelength 360nm.

2.4 Preparation of Sample Solution

2.4.1 Preparation of Berberid alkaloid samples

The Berberidaceae plants were extracted by refluxing with 50% ethanol, and the filtrate was concentrated under reduced pressure to obtain an extract. The extract was dissolved with an appropriate amount of water and adsorbed by D-101 macroporous adsorption resin column chromatography, washed with water and then eluted with 30% ethanol [8]. The alkaloid-containing components were combined and concentrated to dryness. The qualitative identification of the alkaloids of Berberidaceae plants during the extraction and purification process was carried out using silica gel thin layer chromatography. Purification by macroporous adsorption resin column chromatography can increase the purity of total alkaloids of the plant Berberid from 37.7% to 83.7% of the extract. The refined Berberid plant alkaloid concentrate was made into a solution of 3.20 g / L with 10% ethanol.

2.4.2 Preparation of Nantian Bamboo Flavonoid Sample

Nantian bamboo was extracted by refluxing with methanol, and the filtrate was concentrated under reduced pressure to obtain an extract. The extract was dissolved with an appropriate amount of water, and extracted with petroleum ether, chloroform, and ethyl acetate in this order. The ethyl acetate extract was adsorbed on a polyamide column and eluted successively with water, 10% ethanol, 30% ethanol, 50% ethanol, 70% ethanol, and 95% ethanol [9]. The flavonoid-containing components were combined and passed through a polyamide column. Chromatographic adsorption, eluting with 30% ethanol, to obtain a mixture of Hyperoside and isorhamnetin-3-O-galactoside. The qualitative identification of Flavonoids in Nantianzhu during the extraction and purification was carried out using polyamide thin layer chromatography. Nantian bamboo flavonoid mixture was made into a solution of 1.13g / L with 10% ethanol [10].

2.4.3 Preparation of test products

Berberid plant alkaloids and Nantian bamboo flavonoid solutions were matched at a volume ratio of 12: 0.9: 3.8: 4.6: 6.4: 8.3: 9.0: 12 (the total volume was the same). The compatible samples were sealed in sample bottles at 70oC. Heat for 4h. Filter through a 0.45 m microporous membrane before HPLC analysis. The solvent used in Berberidaceae plant alkaloids and Nantian bamboo flavonoids is 10% ethanol (lower solubility in pure water), which is limited by its boiling point. 70oC is used as the reaction temperature of alkaloids and flavonoids. Each compatible sample was a clear solution before and after heating without turbidity.

2.5 Preparation, Identification and Quantitative Analysis of Flavonoids

Weigh 1.0g of leaf powder, add 40mL of 60% (V: V) ethanol, soak for 30min, and heat and reflux at 85°C for 3h. The residue was removed by filtration, and the filtrate was concentrated to a thick paste. After lyophilization, a flavonoid extract was obtained. Purification and qualitative and quantitative analysis were performed with reference to literature methods. Each experiment was repeated 3 times [11].

2.6 Preparation, Identification and Quantitative Analysis of Alkaloids

Isolate and purify alkaloids according to Hao Miao's method. Identification of alkaloids using bismuth potassium iodide color method. Quantitative analysis of alkaloids by UV spectrophotometry. Each experiment was repeated 3 times.

Figure 1. Berberidaceae Plants

2.7 Antibacterial Activity Analysis

2.7.1 Strain activation and bacterial suspension preparation

The test bacteria were inoculated on slant medium and activated at 37 ° C for 24h. Then inoculated in 100 mL of liquid culture medium and shake-cultured at 37oC for 18 h (oscillation speed 110 r · min-1) to obtain a suspension of activated bacteria, which was diluted to 106-107 cfu · mL-1 with liquid medium for later use.

2.7.3 Determination of minimum inhibitory concentration (MIC)

The classic double dilution method was used to investigate the minimum inhibitory concentration of different samples on E. coli [12]. Take 9 pre-sterilized test tubes, numbered A, B, C, D, E, F, G, H, I in this order. Add 2 mL of liquid culture medium to test tube A, and then add 2 mL of sample solution (100 mg · mL-1). After mixing, draw 2 mL of the mixed solution, add test tube B, and then add an equal volume of liquid culture medium to test tube B. After mixing, the sample solution is continuously diluted until the G test tube.

Only liquid medium (without sample solution) was added to the H test tube as a negative control. As a positive control, liquid medium and ampicillin sodium were added to the I test tube. Add 400μL of bacterial suspension to A ~ I test tubes, and shake culture at 37°C for 18h (oscillation speed 110r ± 0min-1). TTC staining was used to evaluate the growth of E. coli. Each experiment was repeated 3 times [13].

2.8 Data Processing

Test data was entered using Microsoft Excel software, tables were created using Microsoft Word software, and one-way analysis of variance (One-WayANOVA) was performed using SPSS 12.0 software. $P < 0.05$ and $P \geq 0.05$ indicated significant differences and no significant differences, respectively.

3. Results and Analysis

3.1 Preparation of Flavonoids and Alkaloids

The flavonoids and alkaloids of Berberis formosana, Berberis fortunei and Nantian bamboo were prepared. The three types of Berberidaceous plants flavonoids were positive for aluminum trichloride reaction and hydrochloric acid-magnesium powder reaction [14]. The alkaloid bismuth potassium iodide color reaction was also positive, which met the standard.

3.2 Dynamics of Flavonoids and Alkaloids in Different Seasons

3.2.1 Changes in flavonoid content

The average value of flavonoid content in the four seasons of green leaf bark was significantly higher than that of red leaf bark ($P < 0.05$), and there was no significant difference with Nantian bamboo ($P \geq 0.05$). The relationship between green leaf berberis and red leaf berberis was closer, but the difference in flavonoid content between the two was larger. The content of flavonoids in Berberis pubescens and Nantian bamboo gradually increased with the season from spring to winter, while the levels of flavonoids in Berberis phyllostachys showed an upward trend and then a decrease (Table 1) [15]. The level of flavonoids in red leaf barberry was highest in winter (3.908%), which was significantly higher than in other seasons ($P < 0.05$). The content of flavonoids in Nantian bamboo was also highest in winter (6.138%), which was significantly higher than that in spring and summer ($F < 0.05$), but there was no significant difference from autumn ($P \geq 0.05$). The level of flavonoids in barley was highest in summer (6.277%), which was significantly higher than in other seasons ($P < 0.05$).

Table 1: Flavonoid Content in 3 Species of Berberids

Species	Spring	Summer	Autumn	Winter	Average value
Barberry	2.615±0.015Db	3.292±0.123Cc	3.677±0.169Bb	3.908±0.015Ac	3.373±0.490b
Barberry	4.662±0.200Ca	6.277±0.000Aa	5.550±0.120Ba	4.882±0.140Cb	5.343±0.631a
Nantianzhu	4.815±0.000Ca	5.062±0.090Bb	5.569±0.123Aa	6.138±0.338Aa	5.396±0.507a

Different capital letters in the same group indicate significant differences between different seasons of the same plant ($P < 0.05$), and different lower case letters in the same column indicate significant differences between different species in the same season ($P < 0.05$). Table 2 is the same.

3.2.2 Changes in alkaloid content

The average value of alkaloid content in green leaf barley in four seasons was significantly higher than that in red leaf bark ($F < 0.05$), but there was no significant difference from Nantianzhu ($P > 0.05$). The content of alkaloids in the red leaf barberry was highest in autumn (5.517%), followed by winter and summer, and lowest in spring [16]. The alkaloid content of Berberis acuminata was expressed as summer> autumn> spring> winter. The content of alkaloids in Nantian bamboo is summer> winter> autumn> spring. Overall, the alkaloid levels of the three Berberid families were generally higher in summer (Table 2) [17].

Table 2: Alkaloid Content in 3 Species of Berberids

Species	Spring	Summer	Autumn	Winter	Average value
Barberry	3.759±0.043Dc	4.496±0.350Cc	5.517±0.203Aa	5.000±0.080Bb	3.373±0.490b
Barberry	5.369±0.117Ca	6.574±0.043Ab	5.578±0.068Ba	4.718±0.080Dc	5.560±0.666a
Nantian Bamboo N	4.281±0.270Cb	7.435±0.141Aa	5.548±0.430Ba	7.304±0.100Aa	6.142±0.308a

3.3 Seasonal Changes in Bacteriostatic Activity of Flavonoids and Alkaloids

3.3.1 Changes in the bacteriostatic activity of flavonoids

At a certain concentration, barley green leaf bark, red leaf barberry, and Nantian bamboo flavonoids all showed an inhibitory effect on E. coli (Table 3), but all were weaker than ampicillin sodium (10 g). The MIC values of flavonoids in different seasons of the same plant are different. The MIC value of flavonoids in red leaf berberis was smaller in spring and winter, followed by autumn and larger in summer. The order of MIC value of

flavonoids in green leaf berberis was spring <winter <autumn <summer. The MIC values of flavonoids from Nantian bamboo were smaller in spring, winter and autumn, and larger in summer. Generally speaking, the bacteriostatic effects of flavonoids of the three Berberid families were relatively strong in spring and winter, and relatively weak in summer and autumn [18]. In winter, the content of flavonoids in red leaf berberis is relatively high (Table 1), and the antibacterial activity is strong, which is worth further research and development.

Changes in the bacteriostatic activity of alkaloids Dynamics Berberis erythematosus, Berberis erythematosus and Nantian bamboo alkaloids all have inhibitory effects on E. coli [19]. The MIC value of the alkaloids of the red leaf barberry was the smallest in spring, followed by autumn and winter, and the largest in summer (Table 3). The MIC value of the alkaloids of Berberis mandshurica was as follows: winter <spring <summer <autumn. The MIC value of Nantianzhu alkaloids is winter <spring <autumn <summer. Generally speaking, the antibacterial effect of alkaloids of the three Berberid plants in winter and spring was relatively strong, while it was relatively weak in summer and autumn. In spring, the MIC value of the alkaloids of red leaf berberis was the lowest (5mg ± 0mL-1) [20]. In summary, the alkaloids and flavonoids of Berberis amurensis have good inhibitory effects on E. coli.

Table 3: Minimum Inhibitory Concentrations of Flavonoids and Alkaloids from Three Berberid Families (n = 3)

Season	Barberry		Barberry		Nantianzhu	
	Flavonoids / (mg ± mL^{-1})	Alkaloid / (mg ± mL^{-1})	Flavonoids / (mg ± mL^{-1})	Alkaloid / (mg ± mL^{-1})	Flavonoids / (mg ± mL^{-1})	Alkaloid / (mg ± mL^{-1})
Spring	7	5	20	15	15	12
Summer	10	8	75	20	18	14
Autumn	8	7	40	38	15	13
Winter	7	7	25	13	15	10
Average value	800	675	4000	2150	1575	1225

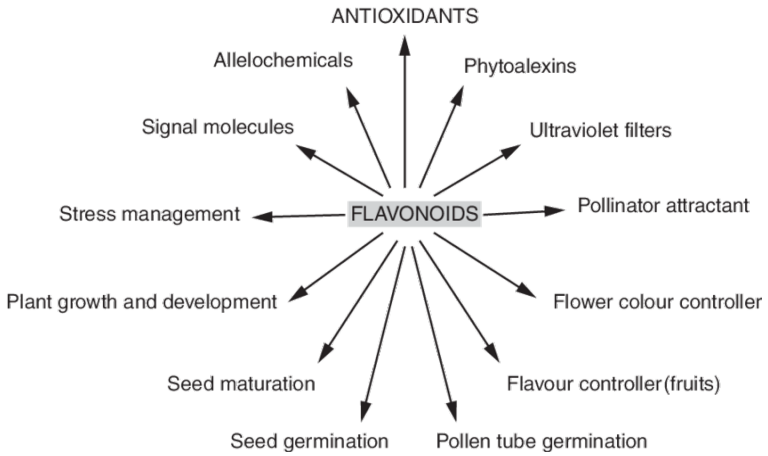

Figure 2. Plant Flavonoid

4. Discussion

4.1 Causes of Variations in Flavonoids and Alkaloids

The content of flavonoids in the four seasons of red leaf bark was significantly different from that of green leaf bark (Table 1). Red leaf barberry is a variant of green leaf barberry. Its leaf color (brick red) may be related to the high content of anthocyanidin-3,5-diglucoside. Plant anthocyanins are generally synthesized with flavan-3,4-diol as a substrate and catalyzed by anthocyanidin synthase [21]. The levels of anthocyanins such as methyl anthocyanin-3,5-bisglucoside in red leaf bark are very different from those in green leaf bark, suggesting that there may be a large difference in the flavonoid metabolism pathway between the two. Different flavonoid metabolism pathways may be the main reason for the significant difference in flavonoid content between the two.

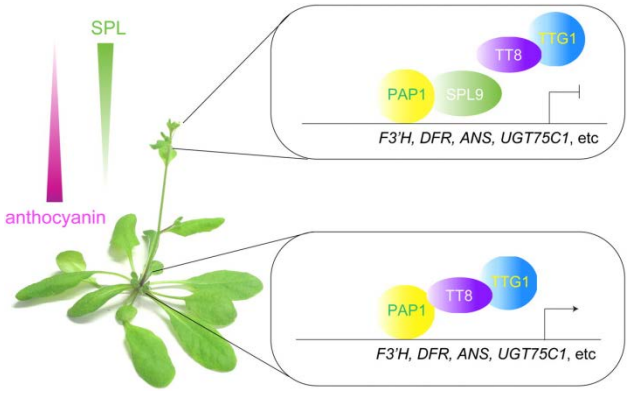

Figure 3. Plant Anthocyanin

Carmona et al. Found that low temperature is helpful for the synthesis and accumulation of anthocyanins in Cit-mssinensis. The lower temperature in winter is helpful for the biosynthesis of anthocyanins, which may be red leaf blight and Nantian bamboo The reason for the highest flavonoid content in winter. The biosynthesis of plant flavonoids is often affected by environmental factors such as ultraviolet rays. Guidi and other studies believe that plant mesophyll flavonoids can protect chloroplasts from photooxidative damage caused by ultraviolet rays, so ultraviolet rays can Promote the biosynthesis of flavonoids. In Shaanxi Province, the average light duration in summer is longer, and the intensity of ultraviolet radiation may be higher. In order to cope with UV stress, plants often synthesize more flavonoids, which may be because the content of flavonoids in green leaf berberis is higher in summer. s reason.

Alkaloids have important physiological and ecological significance to the plant itself, have antifeedant or toxic effects on plant-eating animals and insects, and can also defend against microbial pathogens. In addition, environmental factors such as temperature and light often have a greater impact on alkaloid biosynthesis. Generally, high temperatures are beneficial for the synthesis of nitrogen-containing substances such as alkaloids. The average summer temperature in Shaanxi Province is relatively high, and the threats of insects and microbial pathogens to plants are relatively serious. Therefore, plants may synthesize more alkaloids, which may be the reason for the high alkaloid content in Berberis chinensis and Nantian bamboo .

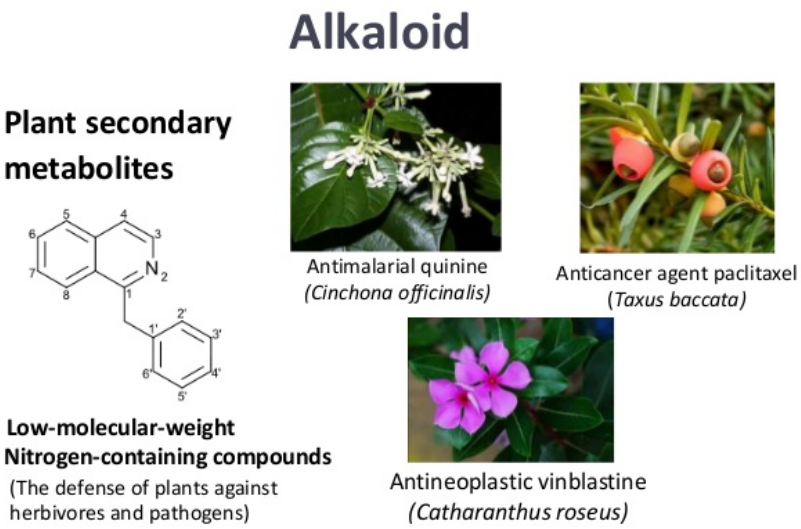

Figure 4. Plant Alkaloids

4.2 Mechanism Analysis of Bacteriostatic Activity of Flavonoids and Alkaloids

In this study, bacteriostatic tests showed that green leaf barberry, red leaf barberry, and Nantian bamboo flavonoids and alkaloids all have a certain inhibitory effect on E. coli. Studies have found that plant flavonoids can inhibit bacterial quorum-sensing signal receptors, toxins, and enzymes and other virulence factors, affect the formation of bacterial biofilms, and disrupt the adhesion of bacteria to host ligands. Alkaloids can disrupt the formation of Z-loops in bacterial divisions, inhibit nucleic acid synthesis, affect plasma membrane function, inhibit topoisomerase and dihydrofolate reductase activity, and thus exhibit specific antibacterial effects. The bacteriostatic mechanism of flavonoids and alkaloids from three species of Berberidaceae need further investigation.

5. Conclusion

This study analyzed the content of alkaloids and flavonoids in P. chinensis, Berberis chinensis, and Berberis pubescens in different seasons. The possible causes of seasonal changes in alkaloids and flavonoids were explored. The bacteriostatic activity of flavones and its seasonal change rule provided ideas for the development and utilization of flavonoids and alkaloids in Berberids. This study found that the contents of flavonoids, alkaloids, and bacteriostatic activities of Berberis formosana, Berberis fortunei, and Nandenbamboo differed from season to season. Seasonal changes in flavonoids and alkaloid content of three Berberids may be closely related to environmental factors such as light and temperature. The flavonoids and alkaloids of three species of Berberids showed a certain inhibitory effect on Escherichia coli. The inhibitory activities of flavonoids and alkaloids on E. coli in different seasons of the same plant are different. Among the three species of Berberidaceae, the inhibition effect of E. coli on E. coli was relatively strong, and in spring, the alkaloid activity of E. repens was the strongest. In the future, we can further explore the material basis and action mechanism of the bacteriostatic activity of red leaf berberis.

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