

# *Acer Buergerianum* a New Resource for the Extraction of Corilagin: Determination of Corilagin in Different Cultivars of *A. Buergerianum* UPLC

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## Article history

Received: 30-09-2021

Revised: 24-01-2022

Accepted: 26-01-2022

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**Abstract:** Corilagin is a promising anticancer drug candidate. *Acer nikoense* and *A. ammonium* are both known to contain corilagin. To explore whether corilagin occurs in congeneric species and to fully develop the medicinal value of maple, the corilagin content in different varieties of maple was determined by Ultrahigh-Performance Liquid Chromatography (UPLC). *Acer buergerianum* leaves contained corilagin and the corilagin content in leaves of different varieties at the deciduous stage varied from 1.2371 to 6.8887 mg/g, with an average of 4.3791 mg/g. Among the cultivars, 'Xingwang' and 'Qianlihong' showed relatively high corilagin contents and the content in 'Qilujin' was the lowest. The range of corilagin contents at the new leaf stage was 8.7452~11.2441 mg/g. Many parts of *A. buergerianum* contained corilagin, with the highest content in the leaves; based on this considerable corilagin concentration, this plant can be developed into raw material for corilagin extraction. There are large differences among varieties. To increase the output value and efficiency, it is necessary to further screen high-content varieties for industrial production; harvesting can be performed in spring and autumn, with autumn as the main season and spring as a supplemental season.

**Keywords:** Corilagin, *Acer Buergerianum*, Ultra Performance Liquid Chromatography, Leaf, Petiole

## Introduction

Corilagin,  $\beta$ -1-O-galloyl-3,6-(R)-hexahydroxydiphenyl-d-glucose, a water-soluble tannin, has a variety of pharmacological activities, including antitumor, antioxidant, antiatherosclerotic, antifibrinolytic, blood pressure-lowering, antiviral, antibacterial and anti-inflammatory activities (Yeo *et al.*, 2015, Li *et al.*, 2018a; Huang *et al.*, 2013; Wu *et al.*, 2021), especially antitumor activity. Studies have shown that this compound has a significant inhibitory effect on the cell growth of cancers such as liver cancer, cholangiocarcinoma, ovarian cancer, cervical cancer, and gastric cancer (Ming *et al.*, 2013; Gu *et al.*, 2016; Attar *et al.*, 2017). In addition, corilagin has low toxicity to normal human cells and

tissues. Therefore, this tannin has become a promising anticancer drug candidate. In addition, it has been reported in the latest research that corilagin can be used as a small molecule to interfere with the interaction between the new coronavirus (SARS-CoV-2) and host cells (Hanson *et al.*, 2020). At present, corilagin is mainly extracted from plants. Schmidt and Lademann (1951) were the first to isolate corilagin from a plant, *Caesalpinia coriaria*, and identified it as tannin. To date, corilagin has been identified in 15 families and 50 species of plants (Li *et al.*, 2018b), mainly in the families Euphorbiaceae (19 species), Geraniaceae (10 species), and Combretaceae (7 species), including, *Phyllanthus Emblica* (Liu *et al.*, 2020), *Phyllanthus urinaria* (Fan, 2016), *Mallotus japonicus* (Tabata *et al.*, 2010),

*Erodium stephanianum*, *Erodium cicutarium* (Fecka and Cisowski, 2005), *Geranium carolinianum*, *Geranium sibiricum*, *Geranium thunbergii*, *Geranium wilfordii* (Bastian *et al.*, 2018), *Terminalia catappa* (Kinoshita *et al.*, 2007), *Terminalia bellirica* and *Terminalia chebula* (Avula *et al.*, 2013). Longan and rambutan in the *Sapindus* genus (Rakariyatham *et al.*, 2020) and *Acer nikoense* and *Acer amoenum* in the *Acer* genus have also been identified as containing corilagin (Okabe *et al.*, 2001; Honma *et al.*, 2010). Since the only known synthetic pathway of gallic acid, an important precursor for the synthesis of corilagin, is the shikimic acid pathway (Werner *et al.*, 1997), the entire biosynthesis pathway of corilagin is not clear and artificial synthesis cannot be carried out at present. Therefore, it is of great significance to continue to expand the exploration of raw materials of corilagin. In particular, the abovementioned families and genera have been reported to contain corilagin.

*Acer buergerianum* is a deciduous tree of the Sapindaceae family (*Acer*); it is native to China and distributed in the middle and lower reaches of the Yangtze River. It is often used as an autumn leaf tree in garden applications and as sidewalk trees, garden trees, and hedges (Xu *et al.*, 2017). In recent years, its medicinal and food value has been explored. Studies have shown that its seeds can be pressed into oil. The seed oil content can reach 30.56%, with a nervonic acid content of approximately 6% and it contains 21.85% protein and 36.07% vitamin E. The mature leaves are very rich in volatile components. In particular, many of these chemical components have aromatic or pharmacological effects and have application potential in the fields of flavors and fragrances or medical treatment (Li *et al.*, 2018c), but they have not been fully developed for use. The leaves of *A. nikoense* and *A. amoenum* are known to contain corilagin (Okabe *et al.*, 2001; Honma *et al.*, 2010). Whether *A. buergerianum* contains corilagin has not been reported. With the increasing demand for corilagin, it is necessary to identify high corilagin-content plants. Thus, it is of great significance to explore whether there are high corilagin-content *Acer* species. Furthermore, in recent years, as the slow sweep of fallen leaves' has become popular, urban management has begun to pay attention to the landscape of fallen leaves, which adds natural beauty to the city in autumn. However, fallen leaves should not only be used for viewing but also be developed concerning their economic value to turn fallen leaves into "treasures". As an important garden landscape tree species, *A. buergerianum* has concentrated autumn leaves. It is thus meaningful to make full use of fallen leaves to create added value in the garden. Accordingly, this study uses *A. buergerianum* as a trial harvest plant and analyzes the presence and contents of corilagin in different varieties, harvest periods, and tree parts. The aim is to explore the potential of *A. buergerianum* as a

raw material for corilagin extraction and to provide a scientific basis for the development of the medicinal value of *A. buergerianum*.

## Materials and Methods

### Plant Materials

Sampling materials: Three *A. buergerianum* cultivars were used in this study: 'Xingwang', 'Qianlihong' and 'Qilujin'. The sampling times in the defoliation period were as follows: October 27 and November 6, 15, and 21, 2019. Three plants of each variety were selected and healthy, disease-free mature leaves were sampled and transported to the laboratory for drying at 50°C, followed by milling. In the new leaf stage, shoots and tender leaves were collected twice, specifically, on April 15 and 20, 2020, and the samples from the two dates were mixed, dried, and ground. Collection from different parts of *A. buergerianum* tetraploid 'Xingwang': Samples of leaves, petioles, shoots, bark, pericarp, and seeds of 'Xingwang' were collected on August 25, 2020. Three plants were selected at random and all the materials were healthy and undamaged. The materials were brought to the laboratory, dried, and ground.

### Experimental Methods

#### Solution Preparation

Reference substance solution. A total of 5.00 mg of corilagin standard product (purity  $\geq 98\%$ ) was accurately weighed, dissolved in 50% methanol (chromatographically pure) in a 50 mL volumetric flask, and diluted to the mark and shaken well. In this way, a reference solution containing 0.10 mg/mL corilagin was prepared.

Test solution. In an extraction method modified by Li *et al.* (2018a), approximately 1.00 g of sample powder was accurately weighed and placed in a 100 mL conical flask. Then, 50 mL of 50% ethanol aqueous solution (analytical purity) with a volume fraction of 50% was added, and ultrasonic extraction with an ultrasound instrument (kQ-250DE; power, 500 W; frequency, 40 kHz) was performed for 30 min. Next, the supernatant was collected and evaporated with a rotary evaporator (RE-2000A). The solid powder was dissolved in 50 mL of 50% methanol and the sample solution was passed through a 0.22  $\mu\text{m}$  filter membrane for testing.

#### Chromatographic Conditions

A two-dimensional Ultra-Performance Liquid Chromatography (UPLC) system (model: Arc) was used to determine the sample, with an XBridge C18 column (4.6  $\times$  150 mm, 3.5  $\mu\text{m}$ ) as the chromatographic column and 0.4% formic acid aqueous solution and methanol as the mobile phase for gradient elution (0.4% formic acid: 0-2 min, 80-75%;

2-15 min, 75-55%; 15-20 min, 55-10%; 20-30 min, 10; 30-32 min, 10~80%). The flow rate was 0.3 mL/min, the column temperature was 25°C, the detection wavelength was 270 nm and the injection volume was 2  $\mu$ L.

### Linear Relationship Investigation

The reference solution was diluted with 50% methanol, a concentration gradient of 0.005, 0.01, 0.025, 0.05, 0.10, and 0.20 mg/mL solutions was prepared and sample injection and determination were performed under the chromatographic conditions described above. With the concentration of corilagin as the abscissa and the chromatographic peak area as the ordinate, a standard curve was drawn and regression analysis was performed.

### Methodological Review

**Precision test:** From the reference solution, 6 consecutive sample injections under the chromatographic conditions of 'Chromatographic conditions' were performed, the peak area was recorded and the Relative Standard Deviation (RSD) was calculated.

**Repeatability test:** Six samples of the 'Qianlihong' test solution were analyzed under chromatographic conditions described in 'Chromatographic conditions'. The peak area was recorded and the RSD was calculated.

**Stability test:** Every 2 h, 2  $\mu$ L of the reference solution was injected. The peak area was recorded 5 times

continuously according to the chromatographic conditions described in 'Chromatographic conditions' and the RSD was calculated.

**Sample recovery test:** The powder of the 'Qianlihong' sample was accurately weighed and 1.410 mg of corilagin standard was added to a total of 5 parts; the samples were prepared according to the methods of 'Solution preparation' and measured under the chromatographic conditions described in 'Chromatographic conditions'. The chromatogram was recorded and the sample recovery rate and RSD were calculated.

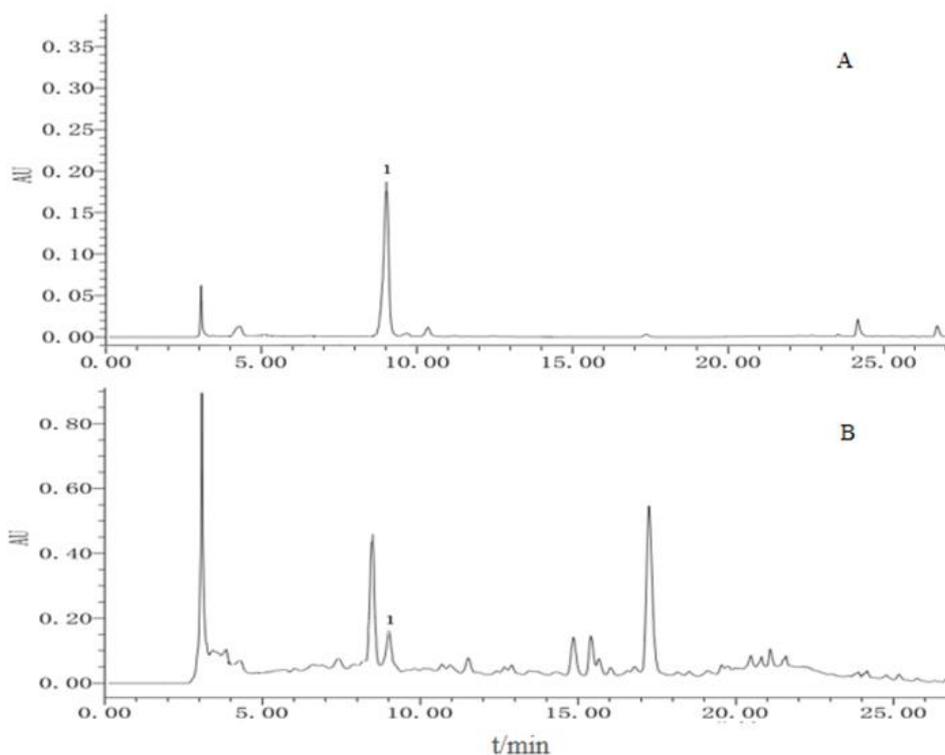
### Sample Determination

Three replicates of each sample powder, each approximately 1.0 g, were precisely weighed to prepare a sample solution and measured according to the chromatographic conditions described in 'Chromatographic conditions'.

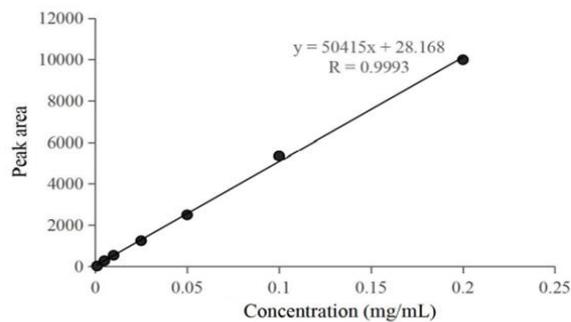
## Results

### Determination of *A. buergerianum* Leaf Corilagin

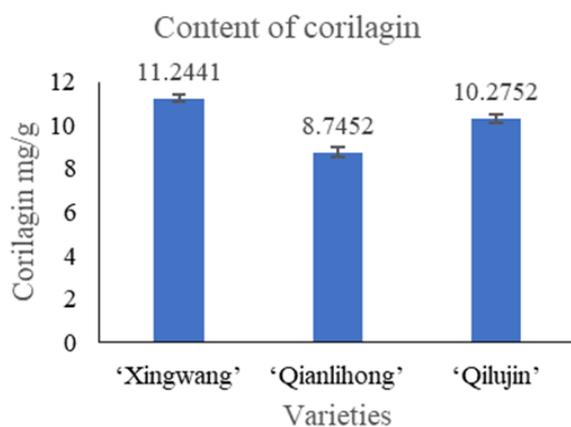
The UPLC method was used to detect corilagin in the leaves of *A. buergerianum*. Chromatograms are shown in Fig. 1. A comparison of the retention times of the sample and corilagin standard revealed that the leaves of *A. buergerianum* contained corilagin.



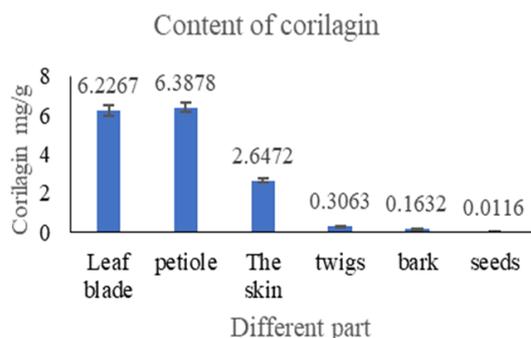
**Fig. 1:** UPLC diagram of the corilagin standard and the sample of *A. buergerianum* (1: Corilagin)



**Fig. 2:** The standard curve of the corilagin standard



**Fig. 3:** The content of corilagin in the leaves of different varieties of *A. buergerianum* at the new leaf stage



**Fig. 4:** Corilagin contents of different parts of the *A. buergerianum* cultivar 'Xingwang'

The linear relationship under the test conditions was investigated and the external standard method was used to quantitatively analyze the corilagin (Fig. 2). The regression equation of the standard curve is  $Y = 50415X + 28.168$ , the correlation coefficient  $R$  is 0.9993 and the linear range is 0.005-0.250 mg. In the linear range, the correlation coefficient is greater than 0.999, indicating that the corilagin standard curve has a good linear relationship.

The RSDs of the precision test, repeatability test, and stability test were 2.12, 0.92, and 0.42%, respectively,

indicating that the method has good repeatability, stability, and precision. The sample recovery rate reached 95.31% and the RSD was 1.45% (Table 1), which confirms that this method has good accuracy in detecting corilagin. The UPLC method established in this experiment can be used to determine the corilagin content in *A. buergerianum* leaves.

#### *Changes in Leaf Corilagin Content in Different A. Buergerianum Cultivars at the Deciduous Stage*

The leaf corilagin contents of different varieties of *A. buergerianum* at the deciduous stage are shown in Table 2. The corilagin content ranged from 1.2371 to 6.8887 mg/g, with an average of 4.3791 mg/g. In 'Xingwang', the highest corilagin content was observed on November 6, at 6.4435 mg/g and the average content over the deciduous period was 5.4707 mg/g; in 'Qianlihong' the highest content was found on October 27, at 6.8887 mg/g and the average content over the deciduous period was 5.6951 mg/g. The highest content in 'Qilujin', at 3.3204 mg/g, was found on October 27, with lower contents detected for later dates and the average content in the defoliation stage was 1.9714 mg/g. The results showed that the contents of corilagin in 'Xingwang' and 'Qianlihong' were significantly higher than that in 'Qilujin'. The CV ranged from 27.99~65.90%, indicating that the content of corilagin was quite variable during the deciduous period.

#### *Corilagin Content in the Leaves of Different Varieties of A. Buergerianum at the New Leaf Stage*

The leaf content of corilagin at the new leaf stage was significantly different among the three *A. buergerianum* cultivars, with 'Xingwang' having the highest value, 11.2441 mg/g, followed by 'Qiluhong', with 10.2752 mg/g and 'Qianlihong', with the lowest value of 8.7452 mg/g (Fig. 3). Compared with that in the deciduous stage (Table 2), the content of the new leaf stage was significantly higher. The new leaf stage of *A. buergerianum* is thus more suitable for harvesting for the extraction of corilagin. Of course, we must also pay attention to the differences among varieties and select high-content germplasms.

#### *Corilagin Content in Different Parts of 'Xingwang'*

Taking the higher content of 'Xingwang' as a trial harvest, the corilagin content in different plant parts of this variety was measured. The results showed that the amount significantly differed among the different parts (Fig. 4). The petiole had the highest content, 6.3878 mg/g, which was similar to the leaf content (6.2267 mg/g), so it is recommended that the leaves be harvested with the petiole for corilagin extraction.

**Table 1:** Recovery test of corilagin sample from *A. buergerianum*

Number	Sample weight/ (g)	Corilagin content in the sample/ (mg)	Added amount/ (mg)	Measured amount/ (mg)	Recovery rate/ (%)	Average recovery rate/ (%)	RSD/ (%)
1	1.109	7.2694	1.410	8.6007	94.42	95.31	1.45
2	1.112	7.2823	1.410	8.6051	93.82		
3	1.105	7.2908	1.410	8.6705	97.85		
4	1.118	7.3792	1.410	8.7256	95.49		
5	1.107	7.2617	1.410	8.6010	94.98		

**Table 2:** Changes in the content of corilagin in *A. buergerianum* leaves at the deciduous stage (mg/g)

Cultivars	October 27	November 6	November 15	November 21	Average
'Xingwang'	5.8187	6.4435	4.1498	--	5.4707
'Qianlihong'	6.8887	5.6891	4.1842	6.0184	5.6951
'Qilujin'	3.3204	1.3899	1.9381	1.2371	1.9714
Average	5.3426	4.5075	3.4240	3.6278	4.3791
Coefficient of Variation	27.9900	49.3800	30.6900	65.9000	38.9300

Note: "--" means that the leaves had fallen at that time

The content in the peel was 2.6472 mg/g, which indicates that the peel can also be used for extracting corilagin. Generally, the peel is treated as waste during oil extraction. This experiment shows that the peel can be used to extract corilagin, which can save resources and fully exploit the medicinal value of the peel. The twigs, bark, and seed kernels had low corilagin contents. As these parts are important parts for plant growth or oil extraction, they should not be used as raw materials for corilagin extraction.

## Discussion

In this study, a suitable UPLC method was established to determine corilagin contents in *A. buergerianum* and it was demonstrated for the first time that the leaves, petioles, twigs, bark, and seeds of this plant contain corilagin. This method provides a basis for the identification and determination of corilagin in other species of the genus *Acer*. The method provides only a preliminary determination of the corilagin content in *A. buergerianum*. In actual production applications, extracted corilagin needs to be separated and purified. In addition, it is necessary to optimize the method to achieve a higher extraction rate. Rangsriwong *et al.* (2009) used subcritical water extraction to extract corilagin from *Terminalia chebula* Retz. The extraction technology of this method is green, the extraction rate is high, the cost is low and selective extraction can be achieved. Prasad *et al.* (2009) used high-pressure extraction technology to extract longan fruit corilagin under conditions of 30°C, 50% ethanol, 1:50 (w/v) solid-to-liquid ratio, and 500 MPa for 2.5 min and the extraction rate was increased by 22% compared with that using ultrasonic extraction. Therefore, different processes to extract corilagin from *A. buergerianum* as the raw material need to be explored.

The corilagin content in the deciduous stage was quite different among the three *A. buergerianum* cultivars in this study and the contents of 'Xingwang' and 'Qianlihong' were significantly higher than that of 'Qilujin'. The content ranged between 1.2371 and 6.8887 mg/g across the three varieties, with an average of 4.3791 mg/g; this value is much higher than that reported for other species from the same genus, *A. nikoense* and *A. amoenum* (Okabe *et al.*, 2001; Honma *et al.*, 2010), as well as for *geranium sibiricum* (0.59 mg/g) of *Geranium* (Liu *et al.*, 2020) and *Dimocarpus longan* (1.1726 mg/g) of *Dimocarpus* (Li *et al.*, 2018b). However, the corilagin content of *A. buergerianum* is lower than that of *Phyllanthus Emblica* leaves (13.16-28.76 mg/g) (Liu *et al.*, 2020) and the young fruit of *Terminalia chebula* (10.36-12.70 mg/g) (Avula *et al.*, 2013). Rambutan (3.80 mg/g) (Rakariyatham *et al.*, 2020), *Phyllanthus urinaria* L. (2.288 mg/g) (Fan, 2016), *Geranium wilfordii* Maxim (2.0 mg/g), and *Geranium carolinianum* (4.10 mg/g) (Bastian *et al.*, 2018), *Erodium cicutarium* (6.5 mg/g) (Fecka and Cisowski, 2005) and *Terminalia catappa* (6 mg/g) (Kinoshita *et al.*, 2007) exhibit similar corilagin contents as *A. buergerianum*. The results of this study show that it is feasible to extract corilagin from the autumn leaves of *A. buergerianum*. In addition, although the leaf corilagin content of *A. buergerianum* is higher in the new leaf stage than in the deciduous stage, the new leaf stage is not suitable for a large number of leaf picks since the leaves are very important for the growth of the tree; thus, an appropriate number of young leaves can be harvested in early spring. In industrial corilagin production using *A. buergerianum* leaves as raw materials, the leaves can be harvested in spring and autumn, with autumn as the main season and spring as a supplement.

The determination of corilagin content in different parts of 'Xing wang' showed that the leaves and petioles had the highest content, above 6 mg/g,

followed by husks; the lowest contents were in kernels. In other plants that have been reported to contain corilagin, the contents are usually highest in leaves. For example, in longan, leaves contain the highest corilagin contents, followed by twigs, seeds, pericarp, bark, and pulp. Rambutan, Jatropha, Phyllanthus, and geranium also have very high leaf corilagin contents (Li *et al.*, 2018c; Fan, 2016; Manpong *et al.*, 2011; Rakariyatham *et al.*, 2020). Whole plants and fruits are also used as the main raw materials for corilagin extraction; examples include wild geranium (whole plant), celery leaf ox seedlings (whole plant), and Terminalia chebula (fruit) (Fecka and Cisowski, 2005; Avula *et al.*, 2013; Li *et al.*, 2018a). In general, leaves are the main raw material used to extract corilagin.

According to a recent report (Li *et al.*, 2018b), the plants containing corilagin are mainly concentrated in the families Euphorbiaceae (19 species), Geraniaceae (10 species), Combretaceae (7 species), and Sapindaceae (5 species). There are many plants in these families; there are more than 200 species of *Acer* in Sapindaceae alone. Therefore, we believe that these families and related plants have great potential as raw materials for the extraction of corilagin, which is the direction of our next work.

## Conclusion

*A. buergerianum* leaf has potential as a material for the extraction of corilagin because of its high corilagin content. Petiole and pericarp of this plant could also be developed as raw material for extracting corilagin. There were great differences among the different varieties in this study. The contents of 'Xingwang' and 'Qianlihong' were higher than those of 'Qilujin' in the deciduous stage, whereas the contents of 'Xing wang' and 'Qiluhong' were higher than those of 'Qianlihong' in the new leaf stage. To increase the output value and efficiency, it is necessary to further screen high-content varieties for industrial production. There was a great difference in corilagin content between the different seasons, with that in the deciduous stage being significantly lower than that in the new leaf stage (4.3791 and 10.0881 mg/g, respectively). The harvest period can be divided into spring and autumn, with autumn as the main season and spring as a supplement.

## Acknowledgment

This study was supported by the Central Fiscal Forestry Science and Technology Extension Demonstration Fund Project (NO: [2020]TG01) and Major Agricultural Projects of Shandong Province (NO: 2017LZN027 and 2020LZGC009). Shandong Province Central Guiding Fund Project (No. YDZX2021101).

## Practical Application

Many parts of *Acer buergerianum* contain corilagin and the leaves contain the highest content, with a considerable amount, which can be developed into raw material for extracting corilagin.

## Author's Contributions

**Qian Qiao, Chong Wu, Jiangyong Wang, Yajing Su, and Yu Yan:** Designed and performed the experiments, analyzed the data and prepared the paper.

**Tiantian Cheng, Lin Zhang, Yongchang Yu, Po Hong, Dongzi Zhu, and Jiawei Wang:** Participated to collect the materials related to the experiment.

**Qingzhong Liu and Zhen Feng:** Designed the experiments and revised the manuscript.

## Ethics

The author declares their responsibility for any ethical issues that may arise after the publication of this manuscript.

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