The plant original identification of Inner Mongolia *Arnebia Radix* based on morphology and DNA barcoding

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Research

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Abstract

Background: It's a long history for the use of herbal medicines to derive remedies in Chinese, and the popularity of herbal medicines has risen worldwide. It is necessary to assure the safety, efficacy and quality of herbal medicines by the corrected identification. Inner Mongolia *Arnebia Radix* is an important and historied used herbal medicine in Inner Mongolia.

Methods: The methods of morphological characteristics and DNA barcoding was used to identify the plant origin of Inner Mongolia *Arnebia Radix*.

Results: The identification results of morphological characteristics and DNA barcoding showed that the original plant of Inner Mongolia *Arnebia Radix* was *Arnebia szechenyi*, which was distinguished with *Arnebia guttata*, the official original plant of Inner Mongolia *Arnebia Radix*.

Conclusions: We inferred that that the original plant of Inner Mongolia *Arnebia Radix* was *Arnebia szechenyi*.

Background

Members of the Boraginaceae s.str. (= subfamily Boraginoideae) distribute worldwide. There is a wide range of variation in them, particularly in their floral and fruit characters. The family is a very heterogeneous group that has been variously divided into groups whose number and limits are not very clearly defined. Researches on the phylogeny of Boraginaceae s.str. have been carried out based on the molecular systematics[1–4]. But the phylogeny of Boraginales has been controversial since the early days of molecular systematics in the early 1990s[5]. Langstrom and Chase[3] established a new tribe Echiochileae (Riedl) Langstrom and Chase composed of Echiochilon Desf., Antiphytom DC. Ex Meisn., Ogastemma Brummitt., and Sericostoma Stocks ex Wight based on the sequences of *atpB* gene. Weigend et al.[4] revealed the polyphyly of the tribe Trigonotideae and accepted four tribes Boragineae s. l., Lithospermeae s. lat., Cynoglosseae s. l., and Echiochileae on the basis of *trnL-F* data. In the most classifications, Eritrichieae has been recognized as a distinct tribe[6–11]. Combined utilizing morphological characters and DNA sequence data to reconstruct phylogenetic relationships within Boraginaceae and investigating the morphological character evolution patterns in the family set the stage for future work creating an updated taxonomic system of Boraginaceae[12–20].

Arnebia Radix is an important Chinese herbal medicine, there are 122 Chinese patent medicines and 195 Chinese medicine prescriptions that used *Arnebia Radix*[21], and the demand for *Arnebia Radix* is incresing. In the 2015 *Pharmacopoeia of the People's Republic of China*[22], the official source plants of *Arnebia Radix* are *Arnebia euchroma* (Sinkiang *Arnebia Radix*) and *Arnebia guttata* (Inner Mongolia *Arnebia Radix*). However, the inspection of *Arnebia Radix* in the national markets in 2015 showed that none of Inner Mongolia *Arnebia Radix* was saled in the national marketsand the unofficial source plant of *Arnebia Radix* accounted for more than half in the national medicines markets. The field investigation in Sinkiang and Inner Mongolia showed that the natural resource of Sinkiang *Arnebia Radix* was scarce and

the annual production was difficult to meet the market demand, in the meantime, Inner Mongolia *Arnebia Radix* was discovered natural resource in Inner Mongolia. Inner Mongolia *Arnebia Radix* is the official source plant of *Arnebia Radix* in the 2015 *Pharmacopoeia of the People's Republic of China*[22], and it is still a traditional ethnic medicine used by Mongolian medical staff. The official source plants of Inner Mongolia *Arnebia Radix* is *Arnebia guttata* in the 2015 *Pharmacopoeia of the People's Republic of China*[22], *Arnebia guttata* mainly distribute in Tibet, Sinkiang, western Gansu and Ningxia, secondly distribute in Inner Mongolia to northern Hebei according to *Flora of China*[23]. It was strange for Sinkiang *Arnebia Radix* was in shortage of resources but there was still none of Inner Mongolia *Arnebia Radix* in the medicine market. The content determination results negated the hypothesis that Inner Mongolia *Arnebia Radix* was deteriorating and difficult to confirm with the authenticated official standards. Thus, the real reason for the absence of Inner Mongolia *Arnebia Radix* in the medicine markets is unclear. Reviewed previous standards, the official source plants of Inner Mongolia *Arnebia Radix* was *Arnebia guttata* in the 1990 to 2015 *Pharmacopoeia of the People's Republic of China*[22, 24–28], *Chinese medicine dictionary*[29] and *Chinese Materia Medica*[30].

Traditional method for plant identification is mainly based on morphological characteristics, the plants of herbal medicines cover a broad range of taxa, which may be phylogenetically less related but morphologically very similar, and the similarity between species may lead to misidentification and inappropriate use. There are several alarming situations of misidentification in world. Supporting traditional plant identification is an urgent matter to secure human health[31, 32]. Given herbal medicines usually are poorly conserved or processed materials, the chance is very low to extract and amplify the long DNA fragments (> 200 bp) successfully[32–34]. The rapid development of DNA barcoding has been proved that it were extremely powerful tool to differentiate closely related species of herbal medicines[35–38]. Wang et al.[32] compared seven candidate DNA barcodes (*psbA-trnH*, *matK*, *rbcL*, *rpoC1*, *ycf5*, ITS2, and ITS) from medicinal plant species, and the result suggested that the second internal transcribed spacer (ITS2) of nuclear ribosomal DNA represents the most suitable region for DNA barcoding applications. Chen et al.[31] tested the discrimination ability of ITS2 in more than 6600 plant samples belonging to a broad range of taxa and found that the rate of successful identification with the ITS2 was 92.7% at the species level. The ITS2 region of nuclear ribosomal DNA possesses several valuable characteristics, such as the availability of conserved regions for designing universal primers, the ease of amplification, and sufficient variability to distinguish even closely related species[39], so it is a potentially standard DNA barcode to be used for the identification of herbal medicines. In this study, The methods of morphology and DNA barcoding were used to the plant original identification of Inner Mongolia *Arnebia Radix*.

Materials And Methods

Plant collection and morphological examination

Thirteen samples representing the two official origin of *Arnebia Radix* (*Arnebia euchroma*, *Arnebia guttata*) and Inner Mongolia *Arnebia Radix* were collected from different provinces in China, including

Inner Mongolia, Sinkiang, Guangxi province and Guangdong province (Table 1). Plant samples were collected during June–July, representing the summer season in China, samples of medicine material were collected from the medicine market. The authenticity of the samples was done taxonomically, and all species were identified by DNA barcoding.

Morphological traits were identified for one of the Inner Mongolia *Arnebia Radix* plant samples which was collected from Bayannaoer League, Inner Mongolia. The morphological traits were identified according to *Flora of China* (Volume 16, Boraginaceae)[40] and *Flora Reipublicae Popularis Sinicae* (Volume 64, Book 2, Boraginaceae)[41]. One plant sample of *Arnebia guttata* was comparative analysis on morphological characteristics which was collected from Altay prefecture, Sinkiang.

DNA extraction and PCR condition

Tiagen plant genomic DNA kit was used to extract genomic DNA. Extracted DNA was stored at -20°C until required. The ITS2 sequences of 13 samples were amplified by the forward primer 5'-ATGCGATACTTGGTGTGAAT-3' and reverse primer 5'-GACGCTTCTCCAGACTACAAT-3' [30,38,39]. The 20 µL PCR amplification system included 10 µL 2 × Taq master mix buffer (Tiagen, China), 15 ng DNA per reaction volume as well as 1 pmol of each primer. PCR program was performed with 94°C denaturation temperature for 4 min, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C, 1 min at 72°C, and a final extension for 10 min at 72°C. Molecular weights were estimated using a DL2000 DNA Ladder. After electrophoresis analysis, PCR products were sequenced bidirectionally by Invitrogen™ Life Technology.

DNA sequences data analysis

The forward and reverse sequences of ITS2 were spliced together with CodonCode Aligner 3.7.1 program (CodonCode Co., USA). Two conserved regions of the 5.8S and 28S gene for plants were used to delimit the ITS2 region. The trimmed sequences of 2 sequences downloaded from NCBI database (Table 2) and 13 sequences obtained from the 13 samples (Table 1) were analyzed. The intra- and inter-specific divergences were calculated based on different taxa. Sequences were aligned using Clustal W, and Kimura 2-parameter (K2P) distances were calculated using MEGA 6.06 program (molecular evolutionary genetics analysis).

Results

Morphological characteristics and identification

Inner Mongolia *Arnebia Radix* (Fig. 1), herbs pubescent. Leaves simple, alternate. Inflorescences scorpioid cymes, bracts present. Flowers bisexual, actinomorphic, Calyx usually 5-persistent, Corolla tubular, limb usually 5-parted. Taproot, containing purple matter. According to *Flora of China*[40] and *Flora Reipublicae Popularis Sinicae*[41], it was a preliminary judgment that Inner Mongolia *Arnebia Radix* belong to the family of Boraginaceae.

Inner Mongolia *Arnebia Radix*, Ovary 4-lobed, style gynobasic (between ovary lobes) (Fig. 2A). Ovules 4, potentially maturing into 4 nutlets (Fig. 2C). Corolla actinomorphic, lobes subequal, Corolla throat unappendaged (Fig. 3A, Fig. 3B). Stamens in single whorl, included, inserted at same level in corolla tube, connective of anthers not subulate, not spirally twisted (Fig. 3A, Fig. 3C, Fig. 3E). Stigma 2-divided (Fig. 3D). According to the identification key to genera of Boraginaceae [40,41], the morphological characteristics of Inner Mongolia *Arnebia Radix* match up with the description of *Arnebia* in *Flora of China* [40] and *Flora Reipublicae Popularis Sinicae* [41].

Inner Mongolia *Arnebia Radix*, corolla yellow (Fig. 3A, Fig. 3B), stamens inserted at same level in corolla tube (Fig. 3E), leaf blade less than 1.2 cm wide (Fig. 4). According to identification key to species of *Arnebia* [40,41], the morphological characteristics of Inner Mongolia *Arnebia Radix* match up with the description of *Arnebia guttata* or *Arnebia szechenyi* in *Flora of China* [40] and *Flora Reipublicae Popularis Sinicae* [41]. Summarized the morphological characteristics of *Arnebia guttata* and *Arnebia szechenyi* in Table 3 for the further identification of Inner Mongolia *Arnebia Radix*.

According to *Flora of China* [23,42], stems of *Arnebia guttata* usually 2-4, sometimes only 1, erect, and stems of *Arnebia szechenyi* sparsely branched, 20-30 cm tall. There was no obvious difference between *Arnebia guttata* and *Arnebia szechenyi* on the stem morphological characteristics.

In order to further determine Inner Mongolia *Arnebia Radix* is belong to *Arnebia guttata* or *Arnebia szechenyi*, more morphological characteristics comparison has been done between *Arnebia guttata* and Inner Mongolia *Arnebia Radix*. Leaves of *Arnebia guttata* are spatulate-linear to linear, densely hirsute, hairs discoid at base, apex obtuse (Fig. 5D, Fig. 5E). Leaves of Inner Mongolia *Arnebia Radix* are narrowly ovate to linear-oblong, short strigose and minutely hispid, hairs discoid at base, margin obtusely serrate, apex acute, bristly (Fig. 5A, Fig. 5B, Fig. 5C). Flowers of *Arnebia guttata*, Inflorescences cymes (Fig. 6A) bracts linear-lanceolate (Fig. 6E). Calyx lobes linear, long hispid (Fig. 6D). Corolla yellow, tubular-campanulate, pubescent outside; limb 7–12 mm wide, lobes broadly ovate or semiorbicular (Fig. 6B, Fig. 6C). Stamens inserted at the middle in corolla tube (Fig. 6C). Ovary 4-lobed (Fig. 6H). Style filiform, apex 2-lobed; stigma reniform (Fig. 6F). Flowers of Inner Mongolia *Arnebia Radix*, Inflorescences cymes (Fig. 1) bracts same shape as leaves (Fig. 7B, Fig. 5A). Calyx lobes linear, densely hirsute and hirtellous on both sides. (Fig. 7C, Fig. 7E). Corolla yellow, tubular-campanulate, pubescent outside, limb 5–7 mm wide (Fig. 3A, Fig. 7A, Fig. 7D). Stamens inserted at the middle in corolla tube (Fig. 3A). Ovary 4-lobed. Style filiform, apex 2-lobed (Fig. 2A, Fig. 3D). The leaves and flowers morphological characteristics of Inner Mongolia *Arnebia Radix* match up with the description of *Arnebia szechenyi* in *Flora of China* [42].

Nutlets of Inner Mongolia *Arnebia Radix*, triangular-ovate (Fig. 8A, Fig. 8B), tuberculate (Fig. 8C), short appressed pubescent (Fig. 8D). The nutlets morphological characteristics of Inner Mongolia *Arnebia Radix* match up with the description of *Arnebia szechenyi* in *Flora of China* [42].

Through a comparative study on the relative ubiquity between bracts and flowers in inflorescences between the specimen of *Arnebia guttata* (Fig. 9A) and Inner Mongolia *Arnebia Radix* (Fig. 9B), we identified the

inflorescences morphological characteristics of Inner Mongolia *Arnebia Radix* had some differences with *Arnebia guttata*.

The results of DNA sequences data analysis

The success rate of PCR amplification for ITS2 fragments was 100% in our experiments. The lengths of ITS2 sequences for ZC-1 to ZC-5 and ZC-6 to ZC-13 were 224 and 223 bp, respectively.

Phylogenetic tree of 15 sequences was divided into three clades which were identified as *Arnebia szechenyi*, *Arnebia euchroma*, and *Arnebia guttata*, respectively (Fig. 10). ZC-1, ZC-2, ZC-3, ZC-4 and ZC-5 clustered together with EF199863.1, an ITS2 sequence of *Arnebia szechenyi* which was downloaded from NCBI database that submitted by Hong Kong Baptist University. ZC-6 and ZC-7 clustered together with JX976805.1, an ITS2 sequence of *Arnebia guttata* which was downloaded from NCBI database that submitted by Xinjiang Institute of Ecology and Geography. The neighbor-joining (NJ) tree also showed that the three species of *Arnebia szechenyi*, *Arnebia euchroma* and *Arnebia guttata* formed three clades individually. This result strongly endorsed the opinion that Inner Mongolia *Arnebia Radix* should be contained in *Arnebia szechenyi*, which was consistent with the identification result of Morphological characteristics.

The results of intra-specific distance and inter-specific distance based on genetic divergence were shown in Table 3. The result of genetic variation test based on K2P-distance for ITS2, showed that the minimum inter-specific distance was 15 times higher than the maximum intra-specific distance ($\text{inter}_{\max} = 0.075$ vs. $\text{intra}_{\min} = 0.005$), indicating that genetic variation of ITS2 demonstrated separately, non-overlapping distributed between intra- and inter-specific samples. Our results showed that the distributions of intra- and inter-specific variation of ITS2 region exhibited distinct gaps. Therefore, *Arnebia szechenyi*, *Arnebia euchroma* and *Arnebia guttata* can be distinguished by ITS2 region. The result approved that the original plant of Inner Mongolia *Arnebia Radix* was *Arnebia szechenyi*.

Discussion

In our study, the methods of morphological characteristics and DNA barcoding were used to identify Inner Mongolia *Arnebia Radix*. The morphological identification of Inner Mongolia *Arnebia Radix* was according to *Flora of China*[40] and *Flora Reipublicae Popularis Sinnicae*[41]. Compared the morphological traits of Inner Mongolia *Arnebia Radix* with identification key to genera of Boraginaceae and species of *Arnebia*, the result showed that Inner Mongolia *Arnebia Radix* was morphologically similar with both *Arnebia guttata* and *Arnebia szechenyi*. The morphological traits of *Arnebia guttata* conformed to the description of *Arnebia guttata* in *Flora of China*, leaves were spatulate-linear to linear, densely hirsute, hairs discoid at base, apex obtuse, bracts linear-lanceolate, limb 7-12mm wide, stigma reniform. The morphological traits of Inner Mongolia *Arnebia Radix* conformed to the description of *Arnebia szechenyi* in *Flora of China*, leaves were narrowly ovate to linear-oblong, short strigose and minutely

hispid, hairs discoid at base, margin obtusely serrate, apex acute, bracts same shape as leaves, limb 5-7mm wide, stigma hemispher.

Chen et al. proposed that ITS2 was the most promising universal DNA barcode for identifying medicinal plants[31,39,43]. The ITS2 region is sufficiently short in length and relatively easy to amplify even for the amplification of degraded DNA[31,39]. The success rate of amplification for ITS2 region was 100% in our study. The ITS2 sequences' lengths of Inner Mongolia *Arnebia Radix* (ZC-1 to ZC-5) were 224 bp, and the ITS2 sequences' lengths of *Arnebia guttata* (ZC-6 and ZC-7) were 223 bp, there was a 1 bp difference between the ITS2 sequences' lengths of Inner Mongolia *Arnebia Radix* and *Arnebia guttata*. An ideal barcode should possess sufficient variation among the sequences for the discrimination of species, and should be sufficiently conserved to guarantee the variability within species is less than between species[44,45]. In our study, the phylogenetic analysis result showed that Inner Mongolia *Arnebia Radix* and *Arnebia guttata* clustered in two separately clades, Inner Mongolia *Arnebia Radix* (ZC-1 to ZC-5) clustered together with EF199863.1, an ITS2 sequence of *Arnebia szechenyi*. *Arnebia guttata* (ZC-6 and ZC-7) clustered together with JX976805.1, an ITS2 sequence of *Arnebia guttata*. The result of neighbor-joining (NJ) tree strongly endorsed the opinion that Inner Mongolia *Arnebia Radix* should be contained in *Arnebia szechenyi*, which was consistent with the identification result of Morphological characteristics. Determination of genetic divergence confirmed that the ITS2 region possesses high interspecific divergence and is well separated. Mean inter-specific divergence of the ITS2 region is significantly higher than its mean intra-specific variation[31]. In our study, the genetic divergence showed that the inter-specific distance between *Arnebia guttata* and *Arnebia szechenyi* was 0.147, the intra-specific distance of *Arnebia guttata* and *Arnebia szechenyi* were 0.000 and 0.003, separately. Our results showed that the distributions of intra- and inter-specific variation of ITS2 region exhibited distinct gaps. Inner Mongolia *Arnebia Radix* which was identified as *Arnebia szechenyi* could be distinguished with *Arnebia guttata* by ITS2 region. The result approved that the original plant of Inner Mongolia *Arnebia Radix* was *Arnebia szechenyi*.

The herbal medicines have been more and more popular to be used worldwide. It has been practiced for centuries that the herbal materials were used for healthcare and therapeutic purposes[46-51]. Herbal medicines are not completely harmless. Adulteration and misidentification are the internal issues which are affecting the quality of herbal medicines[47-50]. The quality problems of herbal medicines may lead to ineffective, toxicity[52], cancer[53] or even death[54]. With the increase popularity of using herbal supplements for therapeutic purposes, there are > 920 dietary supplement adulteration cases has found by US Food and Drug Administration (FDA) Tainted Supplements Report from 2007 to 2018[51,55]. Despite this, herbal medicines have often contributed to the discovery of certain drugs that have advanced human health. A considerable body of therapeutic drugs were discovered and developed under the backbone of traditional herbal medicines use[56]. Since it is essential that systematic toxicologic studies and the same professional surveillance and strict quality control standards are regularly used for conventional medicines[57], authentication is important to ensure the safety, efficacy and quality control on herbal medicines.

According to the results of morphological characteristics and DNA barcoding, there were some distinguishes between Inner Mongolia *Arnebia Radix* and *Arnebia guttata*, *Arnebia guttata* was the official original plant of Inner Mongolia *Arnebia Radix*, but the identification results of Inner Mongolia *Arnebia Radix* were much matched up with *Arnebia szechenyi*. Therefore, we identified *Arnebia szechenyi* was the original plant of Inner Mongolia *Arnebia Radix*. So we inferred that the differences between authenticated official standards and the using history in the original of Inner Mongolia *Arnebia Radix* was the real reason for none of Inner Mongolia *Arnebia Radix* in the medicine market.

Conclusions

In this study, the methods of morphological characteristics and DNA barcoding were used to identify Inner Mongolia *Arnebia Radix*, and the results showed that Inner Mongolia *Arnebia Radix* were much matched up with *Arnebia szechenyi*. Therefore, we suggested that *Arnebia szechenyi* was the original plant of Inner Mongolia *Arnebia Radix*. Herbal medicines plays an important role in China and even the world, more accurate authentication technology is needed to ensure the safety ensure the safety, efficacy and quality control on herbal medicines in the future.

Declarations

Authors' contributions

Junli Wang, Jian Zheng and Shuangcheng Ma conceived the project. Jian Zheng, Junli Wang, Jie Liu, Linong Guo, and Chunyan Zhao prepared the samples and performed sequence analyses. Chunyan Zhao, Linong Guo, Jie Liu, Jian Zheng performed the morphological examination. Jie Liu, Linong Guo, Junli Wang and Jian Zheng prepared the manuscript. Jian Zheng, Junli Wang and Shuangcheng Ma participated in discussions and provided suggestions. All authors read and approved the final manuscript.

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Availability of data and materials

The DNA barcoding data of *Arnebia guttata*, *Arnebia szechenyi* and *Arnebia euchroma* are available online. The materials of *Arnebia guttata*, *Arnebia szechenyi* and *Arnebia euchroma* are available from *National Institute for Food and Drug Control*.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

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Tables

Table 1 Samples particulars

No.	origin	No.	origin
ZC-1	Urad Back Banner, Inner Mongolia (Inner Mongolia <i>Arnebia Radix</i>)	ZC-8	Medicine market in Yulin, Guangxi (<i>Arnebia euchroma</i>)
ZC-2	Bayannaoer League, Inner Mongolia (Inner Mongolia <i>Arnebia Radix</i>)	ZC-9	Medicine market in Yulin, Guangxi (<i>Arnebia euchroma</i>)
ZC-3	Altay Prefecture, Sinkiang (Inner Mongolia <i>Arnebia Radix</i>)	ZC-10	Bayanbulak, Sinkiang (<i>Arnebia euchroma</i>)
ZC-4	Mongolian hospital in Urad Back Banner, Inner Mongolia (Inner Mongolia <i>Arnebia Radix</i>)	ZC-11	Bayanbulak, Sinkiang (<i>Arnebia euchroma</i>)
ZC-5	Urad Back Banner, Inner Mongolia (Inner Mongolia <i>Arnebia Radix</i>)	ZC-12	Medicine market in Guangzhou, Guangdong (<i>Arnebia euchroma</i>)
ZC-6	Altay Prefecture, Sinkiang (<i>Arnebia guttata</i>)	ZC-13	Medicine market in Guangzhou, Guangdong (<i>Arnebia euchroma</i>)
ZC-7	Altay Prefecture, Sinkiang (<i>Arnebia guttata</i>)		

Table 2 Sequences download from NCBI database

Sequence No.	Latin name	Submitted agency
EF199863.1	<i>Arnebia szechenyi</i>	Hong Kong Baptist University
JX976805.1	<i>Arnebia guttata</i>	Xinjiang Institute of Ecology and Geography

Table 3 The intra-specific and inter-specific distance with each species of *Arnebia szechenyi*, *Arnebia euchroma* and *Arnebia guttata*

Latin Name	Distance
<i>Arnebia szechenyi</i>	0.003
<i>Arnebia euchroma</i>	0.005
<i>Arnebia guttata</i>	0.000
<i>Arnebia szechenyi</i> VS. <i>Arnebia euchroma</i>	0.075
<i>Arnebia szechenyi</i> VS. <i>Arnebia guttata</i>	0.147
<i>Arnebia euchroma</i> VS. <i>Arnebia guttata</i>	0.153

Figures

Figure 1

The whole plant of Inner Mongolia Arnebia Radix.

Figure 2

The ovary's morphological characteristics of Inner Mongolia *Arnebia Radix*. (A) Ovary and style. (B) Fruit. (C) Amplified of fruit.

Figure 3

The flower's anatomical structure of Inner Mongolia *Arnebia Radix*. (A) Anatomical surface of flower. (B) Corolla. (C) Anther. (D) Stigma. (E) Stamens.

Figure 4

The leaf of Inner Mongolia Arnebia Radix.

Figure 5

The morphological comparison of Inner Mongolia *Arnebia Radix* and *Arnebia guttata* on leaves. (A) The leaf of Inner Mongolia *Arnebia Radix*, red circle showed apex acute. (B) Margin bristly of the leaf of Inner Mongolia *Arnebia Radix* ×26. (C) Short strigose and minutely hispid on the upper surface of the leaf of Inner Mongolia *Arnebia Radix* ×50. (D) The leaf of *Arnebia guttata*. (E) The long strigose of the leaf of *Arnebia guttata* ×26.

Figure 6

The morphological characteristics of the flower and appendices of *Arnebia guttata*. (A) Inflorescences. (B) Flower. (C) The flower's anatomical structure. (D) Calyx. (E) Bracts. (F) Stigma. (G) Anther. (H) Ovary.

Figure 7

The morphological characteristics of the flower and appendices of Inner Mongolia *Arnebia Radix*. (A) The flower and appendices. (B) Bracts. (C) Calyx. (D) Flower. (E) Hirsute and hirtellous.

Figure 8

The nutlets morphological characteristics of Inner Mongolia *Arnebia Radix*. (A) Lateral view of the fruit. (B) Underside view of the fruit. (C) Tuberculate. (D) Short appressed pubescent

Figure 9

The morphological comparison of Inner Mongolia *Arnebia Radix* and *Arnebia guttata* on inflorescences. (A) The inflorescences of *Arnebia guttata*. (B) The inflorescences of Inner Mongolia *Arnebia Radix*.

Figure 10

Phylogram obtained from the Neighbor-Joining analysis of ITS2 with 15 sequences.