

Use of *ITS* DNA barcode for identification of Jewels of Opar (*Talinum paniculatum*) collected in Thanh Hoa, Vietnam

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Abstract:

Jewels of Opar (*Talinum paniculatum*) is a herbaceous plant known for its highly medicinal value. This medicinal plant is listed in the Red List of Vietnam's medicinal plants and in the handbook of Vietnam's medicinal plants, both documents warranting its necessity for protection. Jewels of Opar contains bioactive substances such as phytosterols, saponins, flavonoids, tannins, steroids, and many other inorganic substances. Jewels of Opar plants collected in Thanh Hoa, Vietnam, are tree-like herbs, their green trunks grows straight up and then branch out. The shapes of the leaves vary; they are generally oval or ovate-oblong in shape, with wavy veins. The flowers of this tree have five reddish purple wings, two sepals, more than ten stamens, and a spherical ovary. Both the fruits and their seeds are small and black. Tuberos roots are cylindrical with many small roots, and the tubers themselves are sweet and spicy. *Internal transcribed spacer (ITS)* region isolated from *T. paniculatum* plants in Thanh Hoa, Vietnam is 643 bp in length. Based on the nucleotide sequences of the *ITS* region and using the basic local alignment search tool (BLAST) in the National Center for Biotechnology Information (NCBI), the Jewels of Opar samples collected in Thanh Hoa, Vietnam were determined to belong to *T. paniculatum* species, *Talinum* genus, Portulacaceae.

Keywords: DNA barcode, *ITS* marker, Jewels of Opar, medicinal plants, *Talinum paniculatum*.

Classification numbers: 3.3, 3.4

Introduction

Jewels of Opar (*T. paniculatum* Gaertn or *T. crassifolium* Willd or *T. patens* L.) is a species in the *Talinum* genus of the Portulacaceae. A herbaceous plant, it is well known for its medicinal value. Jewels of Opar is one of the medicinal plants included in the Red List of Vietnam medicinal plants and in the handbook of Vietnam's medicinal plants as a species that should be protected at the vulnerable level (VU A1A, c, d) [1]. Jewels of Opar contains substances such as phytosterols, saponins, flavonoids, tannins, steroids and several other inorganic substances. These substances have anti-viral effects and are very effective on Herpes' disease and skin infections. Besides, they can also be used as a supporting medicine for Parkinson's disease and heart disease and for lowering blood cholesterol... [2].

Currently, the identification of medicinal plants has been mainly based on morphological analysis. However, this method often encounters obstacles when the raw plant material has been completely or partially processed. According to Hebert, et al. (2003), the DNA barcode is one of the methods used as a method to identify the species. Some DNA regions within the nucleus genome and the chloroplast genome have been used in the identification of plant species [3]. The nucleus rADN genes are a multi-gene system coding for rRNA sequences that are both conservative and diverse when it comes to distinguishing close species. In the nucleus of the cell, rADN are arranged as random units, including DNA encoding 18S, 5.8S, 28S ribosomal RNA and alternating between non-encoding sequences *ITS1* and *ITS2* (internal transcribed spacers) located on either side of the 5.8S gene segment [4-6]. The coding sequences of three rADN genes, 18S, 5.8S, 28S, are more conservative than the sequences of *ITS1* and *ITS2*. Currently, the *ITS* region of the nuclear genome is considered to be one of the most useful tools for identifying and evaluating plant phylogeny [7]. In this work, the researchers present the results of using the *ITS* DNA barcode to identify the Jewels of Opar samples collected from Quan Hoa district and Bim Son town of the Thanh Hoa province of Vietnam.

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Material and methods

The Jewels of Opar (*T. paniculatum*) samples collected from Quan Hoa district and Bim Son town (Thanh Hoa province) were used for morphological and DNA barcode analysis.

The identification of *T. paniculatum* plants was done according to the method suggested by Nguyen Tien Ban (2013) [8] and Pham Hoang Ho (1999) [9]. The total DNA was isolated from young leaves based on the method proposed by Shaghai, et al. (1984) [10]. The amplification of *ITS* region by PCR with primer pair *ITS-F/ITS-R* was synthesized according to Kress, et al. (2005) [11]. The expected size of the amplified DNA fragment is 665 bp in length.

The *ITS* primer sequences: *ITS-F*: 5' ACGAATTCATGGTCCGGTGAAGTGTTTCG 3'; *ITS-R*: 5' TAGAATTCCTCCGGTTCGCTCGCCGTTACT 3'.

The thermal cycle of PCR occurs after the initial 94°C denaturation step over a duration of 4 minutes. The reaction was carried out followed over 40 cycles at 94°C for 30 seconds, 58°C for 60 seconds (annealing), 72°C for 60 seconds, and a final extension 72°C for 10 minutes. The PCR products were detected by electrophoresis on 1.0% agarose gel. Later, QIAquick Gel Extraction Kit was used to purify the PCR products.

The nucleotide sequence of the *ITS* region was determined by the ABI PRISM® 3100 sequencing Avant Genetic Analyzer using the BigDye® Terminator v3.1 Cycle Sequencing Kit. The *ITS* nucleotide sequence was analyzed using BLAST, BioEdit, and DNASTAR software.

Results and discussion

Morphological characteristics of Jewels of Opar (*T. paniculatum*) plants

Figure 1 presents the morphological characteristics of Jewels of Opar. Jewels of Opar is a tree-like herb that grows straight up to about 30-50 cm in height. The trunk is green, smooth, and branching at the bottom. The leaves of Jewels of Opar are staggered; they are generally oval, ovate-oblong, or egg back shaped. They are thick, glossy, and wavy vein, without hairs. The flowers of the plants have five reddish purple wings, two sepals, more than ten stamens, and a spherical ovary. The fruits are small, and the ripe fruit is ash gray in color. The seeds are very small, slightly flat, and black. The tuberous roots are cylindrical, with many small dark brown roots. The shape of the tuber resembles a humanoid. The tubers are about 3-6 cm long and pinkish white. On drying out, the tubers turn black in color. Both the roots and the tubers are sweet and spicy.

The seeds of Jewels of Opar collected from Quan Hoa district and Bim Son town (Thanh Hoa) were cultivated and analyzed on the basis of the morphological characteristics of roots, tubers, stems, leaves, flowers, fruits, and seeds (Fig. 1).

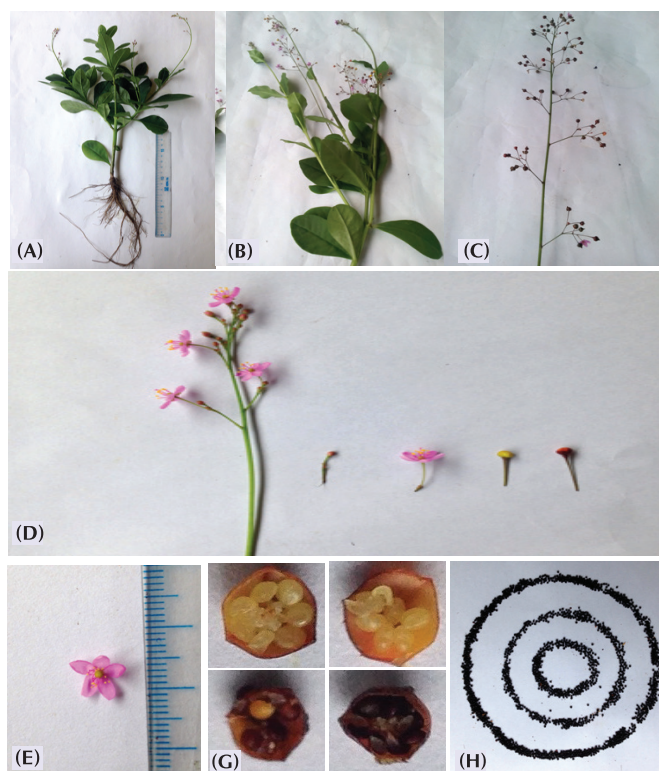


Fig. 1. Morphology of Jewels of Opar (*T. paniculatum*). (A): Jewels of Opar plant; (B): branches bearing flowers; (C): branches bearing fruit; (D, E): buds and flowers; (G): sliced fruit; (H): seeds.

An analysis of the growth and development of Jewels of Opar from seedling to flowering stage show that the seeds started to germinate after 6-7 days of their cultivation and the first leaves appeared after about 15 days. After 50 days, the roots of Jewels of Opar began to bulge and produce tubers. From the 65th day onwards, Jewels of Opar went on to produce flowers and fruits (Fig. 2).

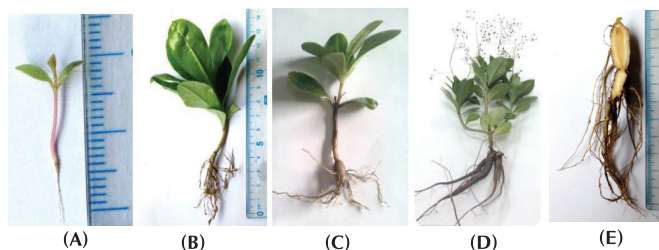


Fig. 2. The growth and development of Jewels of Opar. (A): 15-day-old seedling; (B): 40-day-old plant; (C) the roots began to bulge and produce tubers after 50 days; (D) the plant started to flower and produce fruits after 65 days; (E): tubers of 113-day-old Jewels of Opar.

According to Do Tat Loi, Jewels of Opar is a wild, herbaceous plant. The plants are cultivated in many places because of the medicinal value they carry [12]. Jewels of Opar are distributed across many localities such as Ha Giang, Tuyen

Quang, Hoa Binh, Lang Son, Cao Bang, Hai Phong, Thanh Hoa, and Nghe An... [9, 12]. Jewels of Opar can be planted by using the seed or the root. They are fast-growing trees, they can be harvested after a year, and the perennial Jewels of Opar will have larger tubers. Thus, it can be determined through the analytical results of the morphological characteristics and monitoring of growth and development of Jewels of Opar that the samples collected from Bim Son and Quan Hoa (Thanh Hoa) belong to the species *T. paniculatum*. Based on morphological characteristics, Pham Hoang Ho (1999) [9] and Do Tat Loi (2004) [12] classified Jewels of Opar as a type of ginsengs called Dong Duong ginseng. Its scientific name is *T. paniculatum*, belonging to *Talinum* genus, the Portulacaceae, Caryophyllales, and Magnoliopsida. However, if the plant is in its growing stage without flowers, it is easy to confuse it with the same species of *T. fruticosum*. In addition, the classification of Jewels of Opar encounters obstacles if the plants have been completely or partially processed. As a result, it is necessary to use an extra method and criterion for the classification. The DNA barcoding method with the *ITS* region can be used to accurately identify the Jewels of Opar samples without confusion with other herbs.

Characteristics of the *ITS* region isolated from Jewels of Opar plants

The total DNA was extracted from the leaves of Jewels of Opar and used to perform PCR with *ITS-F/ITS-R* primer pairs. PCR products were separated by electrophoresis on agarose gel with 1 kb DNA marker. The results showed that the PCR products of all samples obtained a DNA band of about 0.65 kb in size, which was similar to the predicted size of the *ITS* region (Fig. 3).

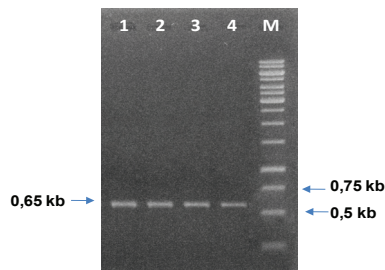


Fig. 3. PCR analysis of *ITS* region from genome of Jewels of Opar (M: DNA marker 1 kb; 1, 2: PCR products of two Jewels of Opar samples collected from Quan Hoa, Thanh Hoa; 3, 4: PCR products of two Jewels of Opar samples collected from Bim Son, Thanh Hoa).

The PCR products of *ITS* region were purified and then were sequenced. The results of the sequencing indicated an *ITS* segment of 643 bp in size. Using the BLAST tool in NCBI, the *ITS* sequences isolated from Jewels of Opar in Thanh Hoa province of Vietnam were 97-98% homologous to the three *ITS* sequences of *T. paniculatum* in GenBank, which had an accession number JF508608 [13], EU410357 [14], and L78094 [15]. The plant shares a 93% similarity with the *ITS* sequence of the *T. fruticosum*, *Talinum* genus with an accession number KJ380908 [16] in GenBank (Fig. 4).

| Description | Max score | Total score | Query cover | E value | Ident | Accession |
|---|-----------|-------------|-------------|---------|-------|----------------------------|
| <i>Talinum paniculatum</i> genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate Quanhoa | 1188 | 1188 | 100% | 0.0 | 100% | L78094.1 |
| <i>Talinum paniculatum</i> genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, isolate Bimson | 1138 | 1138 | 100% | 0.0 | 99% | L78094.1 |
| <i>Talinum paniculatum</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal | 1083 | 1083 | 100% | 0.0 | 97% | EU410357.1 |
| <i>Talinum paniculatum</i> 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal | 1013 | 1013 | 91% | 0.0 | 98% | L78094.1 |
| <i>Talinum paniculatum</i> voucher Cicampo & Morales 1458 (RSN) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 | 985 | 985 | 91% | 0.0 | 97% | JF508608.1 |
| <i>Talinum fruticosum</i> voucher LCH42 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene | 802 | 802 | 86% | 0.0 | 93% | KJ380908.1 |

Fig. 4. Analytical results of similarities between *ITS* sequences of Jewels of Opar samples from Bim Son and Quan Hoa (Thanh Hoa, Vietnam) compared with four *ITS* sequences in GenBank by BLAST tool.

As can be seen in Fig. 5, the comparative analysis of two *ITS* nucleotide sequences isolated from the Jewels of Opar samples collected from Bim Son and Quan Hoa (Thanh Hoa, Vietnam) and the *ITS* sequence with an accession number EU410357 in GenBank showed that there were 19 different nucleotide positions. If the *ITS* sequence with an accession number EU410357 in GenBank is used as the reference gene region, the mutation may be an explanation for the changes in the two *ITS* sequences isolated from the Jewels of Opar samples (Thanh Hoa, Vietnam), specifically T or G substituted for A; A or C substituted for G; G or T or A substituted for C; G or A substituted for T.

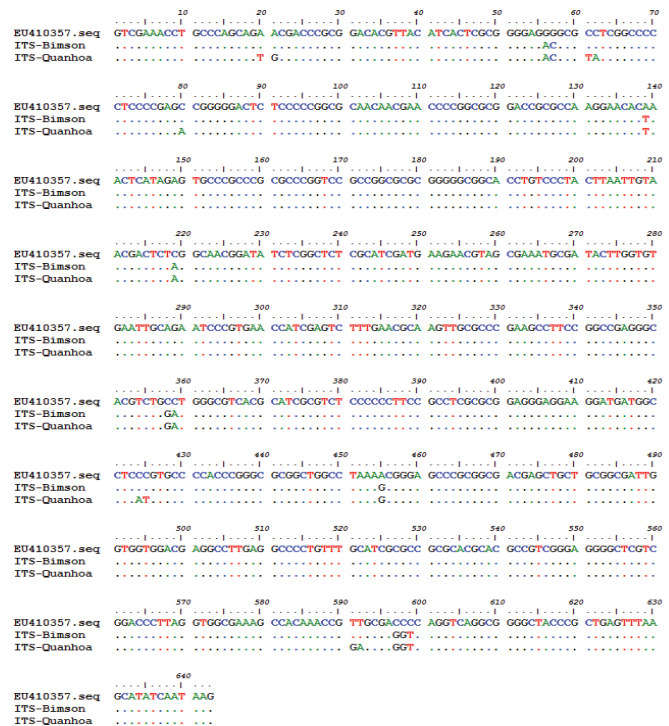


Fig. 5. Two *ITS* nucleotide sequences isolated from Jewels of Opar samples collected in Bim Son and Quan Hoa (Thanh Hoa, Vietnam) and the *ITS* sequence having an accession number EU410357 in GenBank.

Thus, it can be concluded that the *ITS* region isolated from the Jewels of Opar samples from Bim Son and Quan Hoa (Thanh Hoa, Vietnam) is the *ITS* region of *T. paniculatum* species. The *ITS* sequences have been published in GenBank with the accession numbers LT853590.1 and LT853591.1. Fig. 6 shows the diagram of an *ITS* region isolated from *T. paniculatum* plants in Thanh Hoa, Vietnam.



Fig. 6. An *ITS* region of *T. paniculatum* in Thanh Hoa, Vietnam.

Genetic relationship among the species of the *Talinum* genus

An analysis of the genetic relationship between the species of *Talinum* genus was based on the nucleotide sequences of the *ITS* region in GenBank [17]. DNASTAR software was used to establish a phylogenetic tree of the genetic relationship among *T. paniculatum* samples (Fig. 7).

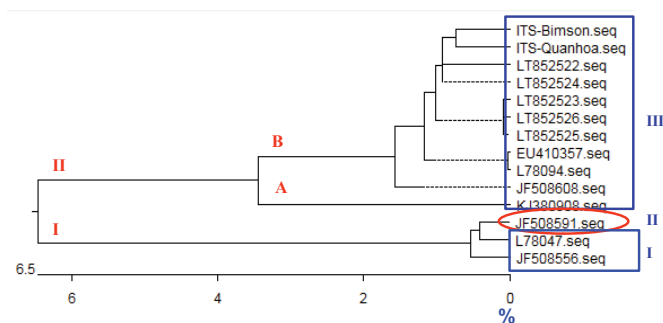


Fig. 7. Phylogenetic tree based on nucleotide sequences of *ITS* region.

Figure 7 shows the genetic relationship between two Jewels of Opar samples collected from Thanh Hoa, Vietnam and the *ITS* sequences of *T. paniculatum* species of the *Talinum* genus that have the following accession numbers: LT852522, LT852523, LT852524, LT852525, LT852526, EU410357, L78094, JF508608, and KJ380908, and the following sequences of Portulacaceae: L78047 (*Portulaca oleracea*), JF508591 (*Portulaca rotundifolia*), JF508556 (*Portulaca intraterranea*). In the diagram, all samples are classified into two large groups, wherein group I contains three *ITS* sequences (JF508591 L78047, JF508556) and group II consists of eleven *ITS* sequences of both genus *Talinum* and *Rotundifolia* of the family Portulacaceae. The genetic distance between the two genus *Talinum* and *Rotundifolia* is 6.5%. Branch II has two sub-branches - A and B. Sub-branch A has an *ITS* sequence with an accession number KJ380908 of *T. fruticosum*, and sub-branch B comprises of ten *ITS* sequences of the same species *T. paniculatum*. The UPGMA method was used to determine the genetic distance between *T. paniculatum* and *Talinum fruticosum*, which was calculated to about 3.5%.

Conclusions

Jewels of Opar (*T. paniculatum*) plants collected from Quan Hoa district and Bim Son town (Thanh Hoa, Vietnam) are tree-

like herbs. The trunk of Jewels of Opar is green and it grows straight up before branching out. The leaves are generally oval or ovate-oblong in shape, with wavy veins. The flower has five reddish purple wings, two sepals, more than ten stamens, and a spherical ovary. The fruits and seeds are both small and black. The tuberous roots are cylindrical with many small roots, while the tubers are sweet and spicy. The *ITS* region isolated from *T. paniculatum* plants in Thanh Hoa, Vietnam is 643 bp in length. Based on the nucleotide sequence analysis of *ITS* region, the Jewels of Opar samples collected from Bim Son and Quan Hoa (Thanh Hoa, Vietnam) were concluded to belong to *T. paniculatum* species, *Talinum* genus, Portulacaceae.

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