Molecular identification of *Uncaria gambir* [Hunter] Roxb. through DNA barcoding

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Abstract. Gambir [*Uncaria gambir* [Hunter] Roxb.] is an important source of traditional medicinal from West Sumatera. Different types of this plant give different catechin content as one of the active compounds. The identification of the correct species and types are important for quality control of medicinal materials. The internal transcribed spacer [ITS] of nuclear ribosomal DNA is one of the most commonly used DNA markers in plant phylogenetic and DNA barcoding analyses, and it has been recommended as a core plant DNA barcode. Four types of gambir [Riau Gadang, Mancik, Cubadak, and Udang] and ITS barcode named ITS4 and ITS5 were used in this study. This barcode gives 100% amplification efficiency and length of products 592-690 bp. variation sites detected for gambir Cubadak and gambir Riau Gadang. The results are able to discriminate 2 types in *Uncaria gambir* [Hunter] Roxb. Species which are essential for the investigation of adulterants and misidentifications of gambir.

Keywords: *Uncaria*, gambir, ITS, DNA barcoding

1. Introduction
Gambir [*Uncaria gambir* [Hunter] Roxb.] belongs to the *Rubiaceae* family and has the characteristics of shrubs, vines and woody plants which are specific commodities from West Sumatra [1]. gambir is widely used as raw material for the pharmaceutical and food industry, including raw material for liver disease drugs with the patent name "catergen", and candy raw material which soothes the throat for smokers in Japan [2]. From the various types of gambir in production centers [Siguntur and Pesisir Selatan], there are 3 good quality gambir variants, namely Udang, Cubadak, and Riau types [3]. But now, there has been the addition of a new variant, namely Mancik.

So far, plant identification uses a morphological method which is identified from its physical form [flowers, leaves, stems, branches and seeds]. However, by the advancement of molecular technology, a new method of identification of plant and animal species has been developed, namely barcode DNA technology that uses standard short pieces of DNA [4].

Internal Transcribed Spacer [ITS] is an RNA sequence from the main transcription process that is between the ribosomal precursor subunits and is removed in the splicing process when the RNA precursors a structural molecular sign processed into a ribosome. The ITS region lies between the repeating sequences of the 18S and 28S DNA ribosomal nucleus, which is a versatile genetic marker [5].

Previously, this research had been carried out on the DNA barcodes of the gambir plant based on the *rbcL* and *matK* genes, but the ability of the DNA barcodes was still low. *Rubiaceae*, can
distinguish to the level of the genus only [6]. This research will be carried out to identify gambir plant using ITS-2 gene as DNA barcode, for the molecular identity of the gambir plant from West Sumatra.

2. Material and Methods

2.1 Plant material
Gambir leaves were taken at Siguntur Pesisir Selatan. The morphology identification of 4 samples of gambir leaves [Cubadak, Riau, Udang, Mancik] were carried out at the Herbarium, Department of Biology, Andalas University.

2.2 DNA isolation and amplification
The DNA isolation of samples was carried out using the CTAB method with a slight modification. PCR reaction using MyTaq™ HS Red Mix Bioline. The final conditions for each reaction are: MyTaq™ HS Red Mix Bioline 12.5 µL, reverse primer [ITS4] 1 µL, forward primer [ITS5] 1 µL, DNA template 1 µL, Nuclease-Free Water / ddH2O 9.5 µL and final reaction conditions 25 µL. The results of DNA isolation were examined using 1.5% agarose gel and the results were documented with GelDoc. The sequencing process was carried out in 1st Base Singapore.

2.3 Data analysis
The sequenced DNA was edited using BioEdit. For the accuracy of target gene amplification tested by predicting amino acid sequences based on the ITS-2 gene, allignment was used to identify the sequence types in 4 types of gambir and molecular identification was carried out through BLAST to NCBI [National Center for Biotechnology Information].

3. Result
One of the things that must be considered in DNA isolation activities is the effectiveness of isolation, because certain plant tissues have different physicochemical structure specificities, too long in the crushing process will activate the DNase enzyme so that it will break down DNA molecules [7].

Figure 1. Electrophoresis of DNA Isolation Results, Notes: 1. Marker, 2. Results
The same DNA band between marker and result showed the presence of DNA in the isolation results. The results of DNA isolation were amplified in order to multiply the formed DNA, amplification was carried out by a PCR machine. In the amplification activity, the thing that must be considered is the comparison concentration between the sample DNA as the template and the primary concentration. Gradient PCR was used to get the accurate temperature used in annealing [Figure 2].
Figure 2. Gradient ITS4 and ITS5, note: 1 = Marker, 2 & 3 = DNA gambir riau gadang + barcod, 4 & 5 = DNA gambir mancik + barcod, 6 & 7 = DNA gambir cubadak + barcod, 8 & 9 = DNA gambir Udang + barcod

ITS-2 barcode was able to amplify four types of gambir [Figure 2]. This result was not given by rbcL and matK barcode. Alignment is the first step in DNA barcoding, no result in amplification means it could not be sequenced and the barcoding process could not be reached. Alignment results showed ITS2 barcode only can discriminate Cubadak type from other types of gambir [Figure 3]. ITS2 showed high power to differentiate Uncaria gambir species that can not be given by another barcode DNA.

Figure 3. Alignment result of 4 gambir types, note: DNA 1 = gambir Riau, DNA 2 = gambir Mancik, DNA 3 = gambir Cubadak, DNA 4 = gambir Udang

4. Conclusion
Based on the amplification results on 4 samples of DNA gambir [Uncaria gambir [hunter] Roxb] with ITS-2 barcode gene gives amplification result length from 592-690 bp. The alignment results using ITS-2 barcode were able to distinguish between 1 type of gambir [gambir Cubadak]. The difference showed at 20 bp and 68 bp. This difference can be used as a marker [barcode] for the Cubadak type.

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