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Optimization of DNA extraction protocol and DNA barcoding of *Hedyotis quinquinervia* in Sri Lanka

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Hedyotis quinquinervia, has been identified as a critically endangered possibly extinct (CR(PE)) endemic plant by the National Red List 2012 of Sri Lanka. However, in 2014 it was rediscovered from Thotupolakanda montane forests. It has a high potential as an ornamental plant due to compact, mosaic arrangement of curved shiny leaves with prominent veins. Thick leaf cuticle of this plant, hindered the extraction of good quality DNA for barcoding. Therefore, the aim of the present research was to establish an efficient DNA extraction protocol for *H. quinquinervia* in the absence of liquid N_2. *H. quinquinervia* leaves were stored at -80 ℃ or in silica gel for 48 hours prior to the DNA extraction. Three DNA extraction protocols were tested and agarose gel electrophoresis and spectroscopic method were used for the evaluation of quality and quantity of extracted DNA. All the extracted DNA samples were subjected to Polymerase Chain Reaction (PCR) for the amplification of nuclear rDNA-ITS region for barcoding. Out of three methods, classical CTAB protocol with 0.2% β-mercaptoethanol and 2% polyvinylpyrrolidone in CTAB buffer was successful, only after removing the cuticle of the adaxial surface of the leaf. The cuticle removal was achieved simply by using a clear tape and it was confirmed by staining the clear tape with safranin and observing it under the microscope. No DNA extraction was successful with the cuticle present on the leaves. PCR amplification was successful from the extracted DNA and rDNA-ITS sequences were obtained. A sequence of 435 bp, exhibited 98% query cover and 100% identity to *H. quinquinervia* of the NCBI database. Sequences were deposited in the GenBank under the accession numbers MT373692 and MT373691. It was also found that with the removal of cuticle, the amount of leaves required for good quality DNA extraction was five times less than that of the leaves with cuticle. Modified CTAB buffer and the cuticle removal from fresh leaves of *H. quinquinervia* were quick and easy modifications to obtain good quality DNA in the absence of liquid N_2. Cuticle removal method using clear tapes and the storage at -80 ℃ prior to DNA isolation could be recommended for identification of *H. quinquinervia* and other plant species with thick cuticle layers on the leaves.

Keywords: Cuticle, DNA extraction, DNA barcoding, *Hedyotis quinquinervia*, ITS-rDNA