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Application of a Synthetic DNA Reference Material for Optimisation of DNA Extraction and Purification for Molecular Identification of Medicinal Plants

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Abstract : DNA barcoding is increasingly used for identification of medicinal plants worldwide. In the last decade, a large number of DNA barcodes have been generated, and their application in species identification explored. The success of DNA barcoding process relies on the accuracy of the results from polymerase chain reaction (PCR) amplification step which could be negatively affected due to a presence of inhibitors or degraded DNA in herbal samples. An established DNA reference material can be used to support molecular characterisation protocols and prove system suitability, for fast and accurate identification of plant species. The present study describes the use of a novel reference material, the trnH-psbA British Pharmacopoeia Nucleic Acid Reference Material (trnH-psbA BPNARM), which was produced to aid in the identification of Ocimum tenuiflorum L., a widely used herb. During DNA barcoding of O. tenuiflorum, PCR amplifications of isolated DNA produced inconsistent results, suggesting an issue with either the method or DNA quality of the tested samples. The trnH-psbA BPNARM was produced and tested to check for the issues caused during PCR amplification. It was added to the plant material as control DNA before extraction and was co-extracted and amplified by PCR. PCR analyses revealed that the amplification was not as successful as expected which suggested that the amplification is affected by presence of inhibitors co-extracted from plant materials. Various potential issues were assessed during DNA extraction and optimisations were made accordingly. A DNA barcoding protocol for O. tenuiflorum was published in the British Pharmacopoeia 2016, which included the reference sequence. The trnH-psbA BPNARM accelerated degradation test which investigates the stability of the reference material over time demonstrated that it has been stable when stored at 56 °C for a year. Using this protocol and trnH-psbA reference material provides a fast and accurate method for identification of O. tenuiflorum. The optimisations of the DNA extraction using the trnH-psbA BPNARM provided a signposting method which can assist in overcoming common problems encountered when using molecular methods with medicinal plants.

Keywords : degradation, DNA extraction, nucleic acid reference material, trnH-psbA
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