DNA barcoding of labisia pumila (kacip fatimah) varieties

ABSTRACT

DNA barcoding is a useful tool for molecular evolutionary studies based on nucleotide diversity of conserved sequences. It has been used for authentication of the medicinal and herbal plant products. In this study, three varieties of Labisia pumila (var. pumila, lanceolate and alata) were chosen where four segments of their DNA regions (ITS, trnH-psbA, matK and rbcL) were amplified and sequenced. These three varieties are bound within the same species but may constitute different nucleotide sequences. Basic Local Alignment Tool (BLAST) is used for biological sequence homology analysis and divergence of the amplified sequences. These plants were extracted using direct lysate extraction and amplified with Polymerase Chain Reaction (PCR) using universal primers followed by DNA sequencing. BLAST result showed that the four sequences showed more than 95% of identity with E-value near to zero. Pairwise analysis suggested that L. pumila var. lanceolata and L. pumila var. alata has the highest sequence divergence with 16 % of differences for trnH-psbA gene while L. pumila var. alata and L. pumila var. pumila shows highest divergence value between them for ITS and matK gene region, with 1.4 % and 17 % respectively. Divergence obtained was 4 % after comparing rbcL gene sequences between L. pumila var. pumila and L. pumila var. lanceolata. Multiple sequence alignment analysis indicates that most of the genes used are conserved based on their nucleotide and amino acid aligned using Multiple Sequence Comparison by Log Expectation (MUSCLE) algorithm. Phylogenetic analysis implied that the three varieties shared similar or some homology nucleotide sequences based on the conserved sequences as low p-distance values are acquired. Barcoding of Life Data System (BOLD) pattern barcode shows that matK region could be used to differentiate these varieties as more differences can be seen after comparing their conserved region.