Chapter - 06

SUMMARY & CONCLUSION
Plants are a rich source of secondary metabolites that have medicinal and aromatic properties. Majority of herb products are packaged in the form of powders or shredded slices, which no longer bear the original features of the plant, thus, rendering their authentication by morphological and histological techniques very difficult. One of the most reliable methods for identification of medicinal materials is by analyzing DNA that is present in all organisms. Many DNA-based methods, including restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and sequence characterized amplified region (SCAR), have been in use for the authentication of medicinal plants. In the present work, we have developed a new DNA-based identification method, the LAMP, for the identification of authentic medicinal plants.

The LAMP (loop-mediated isothermal amplification) method can amplify a few copies of DNA to $10^9$ copies with high specificity and efficiency in less than an hour under isothermal conditions. Furthermore, no special apparatus is needed, which makes it more economical and practical than PCR. The LAMP operation is quite simple; it starts with the mixing of buffer, primers, DNA lysates, and DNA polymerase in a tube, and then the mixture is incubated at 65°C for a certain period. There is no need for a thermal cycler because there is no heat denaturation step of the template DNAs with this method. The only equipment needed for the LAMP reaction is a regular laboratory water bath or a heat block that furnishes a constant temperature of 65°C. Visual judgment eliminates the need for any laborious and time-consuming post amplification operations.
Summary and Conclusion

such as hybridization and electrophoresis as well as the need for special equipment. One of the most attractive characteristics of LAMP is the visual judgment of nucleic acid amplification. In order to facilitate the field application of the LAMP assay, the monitoring of amplification by the LAMP assay was also carried out through naked-eye inspection. Following amplification, the tubes were inspected for white turbidity with the naked eye after a pulse spin to deposit the precipitate in the bottom of the tube. This can be achieved due to the high specificity and high amplification efficiency of LAMP. Although this is a quite simple approach, detecting a small amount of the white precipitate by the naked eye is not always easy; therefore, the detection limit is apparently inferior to that of electrophoresis. To increase the rate of recognition by the naked eye, we added SYBR Green I to the reaction solution. By this approach, the detection limit of LAMP could be improved so as to approach that of electrophoresis. This amplification is not only rapid (results can be obtained in less than 1 h), but is also easy to perform and low in cost, with only simple incubators without any high costly instrument.

The LAMP-based marker was developed in this work for the identification of Catharanthus roseus, Hedychium spicatum and Polyandrum hexandrum. Development of LAMP marker starts with the RAPD analysis of these plants with 25 random decamers. A few primers produced good quality and reproducible fingerprint patterns. Several specific RAPD amplicons of high intensity and reproducibility were eluted, cloned and sequenced. Nucleotide sequence of 610 bp, 825 bp and 429 bp, specific for all the six accessions of C. roseus, H. spicatum and P. hexandrum, respectively were used for designing primers for LAMP reaction for respective plant. LAMP reaction requires four primers, two outer primers
(F3 & B3) and two inner primers (FIP & BIP). The design of the two outer primers, F3 and B3, is the same as that of regular PCR primers, while the design of the two inner primers, FIP and BIP, is different from that of PCR. FIP consists of the sense sequence of F2 at the 3' end and the F1c region at the 5' end that is complementary to the F1 region. BIP consists of a B2 region at the 3' end that is complementary to the B2c region and the same sequence as the B1c region at the 5' end. LAMP primers were cathF3, cathB3, cathFIP, cathBIP for C. roseus, hyF3, hyB3, hyFIP hyBIP for H. spicatum and pdF3, pdB3, pdFIP, pdBIP for P. hexandrum. LAMP reaction for these plants, containing genomic DNA, LAMP primers, dNTPs, Bst DNA polymerase, MgSO₄ and betaine, was conducted at 65 °C for one hour. SyBr Green dye was added at the end of the reaction. The products of LAMP reactions were visualized by naked eyes and under UV light. The amplified product produced the green fluorescence, indicating the successful amplification of the desired sequence of all the three plants. No fluorescence was seen in the negative control (NC). Validation of LAMP reaction was carried out by real time PCR. In real time PCR the outer forward and outer backward LAMP primer pairs (pdF3 and pdR3) were used for the real time analysis by using SYBR Green-I chemistry. LAMP primer pairs and selected target for LAMP-based marker was verified by RT-PCR. In-house designed LAMP-markers for C. roseus, H. spicatum and P. hexandrum were tested for authentication testing of market samples of respective plants. For the analysis of these market samples, same procedure was applied. The LAMP-based method successfully identified the genuine plant drug of C. roseus, H. spicatum and P. hexandrum.

In conclusion, the LAMP-based marker can be used for identification of C. roseus, H. spicatum and P. hexandrum. The assay is more