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Short Communication

Molecular phylogeny and authentication of selected meliaceae members based on chloroplast *rbcL* sequencing studies

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Abstract

Plants are used as a source of medicine in Traditional Medicinal Systems in India from time immemorial. The demand in medicinal plants leads to the adulteration in pharmaceutical industries. Low grade materials replaced with original one and most of the medicinal plants are sold as root, powder, leaf, fruit or stem, it leads to problem in plant identification. In the present investigation molecular phylogenetic analysis and authentication of medicinal plants Cipadessa baccifera and Melia azedarach belonging to the family Meliaceace was carried out using rbcL sequencing studies. The rbcL sequencing of the selected plants analysed with BLAST for similar sequence studies and the alignment was done with T-coffee software. The phylogenetic relationship between the species was constructed by MEGA software. BLAST results indicated the number of similar sequences present in the Genbank repository. The phylogenetic tree represented the degree of similarity and variation with other species and other accessions of same species. The result of the study was useful for the authentication of selected meliaceae members. The phylogenetic tree showed their relationship with other meliaceae members.

Keywords: *Cipadessa baccifera, Melia azedarach*, *rbcL* sequencing, phylogenetic relationship.

Introduction

India is endowed with rich flora and fauna represented; it is one of the megabiodiversity countries of the world with 16 agroclimatic condition. The vegetation of the country represented different variety of plants with medicinal values[1]. Meliaceae is a mahogan family and it is comes under the order Sapindales. These angiospermic plants are widely distributed in subtropical and tropical areas. These plants are also present in rain forests, mangrove swamps and semi deserts[2]. Limenoids are available in the family Meliaceae (meliacins) used for antifeedent, antimalarial, antimicrobial, cytotoxic and growth-regulating activities[3]. *Cipadessa baccifera* Miq. and *Melia azedarach* Linn. both plants are comes under the family Meliaceae. The chemicals constituents Melianoniol, melianol, melianone, meliandiol, vahillin and vanillic acid are present in the fruits of *Melia azedarach* Linn[4]. *Melia azedarach* and *Cipadessa baccifera* consists of many phytoconstituents in leaves, seeds etc. and used to treat various diseases like diabetes, diarrhoea, headache and piles problem[5,6].

The DNA barcoding studies will helpful for the authentication of medicinal plants like plant identification, biodiversity monitoring, molecular phylogeny and evolutionary relationship studies[7,8]. In phylogenetic relationship, *rbcL* gene is a precious tool. It is present in the chloroplasts of the photosynthetic organisms[9]. The *rbcL* based sequencing studies will helpful for the phylogenetic study of medicinal plants in medicinal plant industries. The *rbcL* gene consists of large subunit of RuBP (ribulose 1, 5 bisphosphatecarboxylase) is code for the *rbcL* gene[10]. For the authentification or for the correct identification of herbal products, there is no standard practices are available now a days. So the pharmaceutical industries suffered a lot by fraud and unethical practices used in medicinal plants[11,12]. The morphological character based identification of medicinal plants leads to misidentification and replacement of other materials in the pharmaceutical industries due to the usage parts of the plants, powder or processed materials. This present study framed to investigate the molecular phylogeny and authentication selected Meliaceae members based on chloroplast *rbcL* sequencing studies.

Materials and methods

The Meliaceae members *Melia azedarach* Linn and *Cipadessa baccifera* Blume were selected for the present study. The taxonomic features of the species have been confirmed with Flora of Presidency of Madras[13]. The healthy, young leaf samples of *Cipadessa baccifera* and *Melia azedarach* collected from the Western Ghats of south India for *rbcL* sequencing studies.

DNA isolation and Agarose Gel Electrophoresis: About 100mg of plant tissue is homogenized using liquid nitrogen and the powdered tissue is transferred to micro centrifuge tube. The DNA was isolated using NucleoSpin® Plant II Kit (Macherey-

Nagel). The isolated DNA was checked using 1% agarose gel. The banding pattern viewed under UV transilluminator. The PCR analysis carried out using thermal cycler. The PCR products checked in 1.2% agarose gel electrophoresis and viewed under transilluminator and photographed using gel documentation system. Forward primer ATGTCACCACAAAC AGAGACTAAAGC and reverse primer GTAAAATCAAGT CCACCRCG were used for the study. PCR amplification profile for *rbcL* was 98^0C - 30 sec, 98^0C - 5 sec, 60^0C - 10 sec, 72^0C - 15 sec, 72^0C - 60 sec and stored at 4^0C.

Sequence analysis was done in thermal cycler using Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems). The PCR profile for sequencing consisted following cycles 96^0C for 2 minutes followed by 96^0C for 30 sec, 50^0C for 40sec and 60^0C for 4 minutes for all primers for 30 cycles.

For post Sequencing PCR Clean up, master mix I of 10µl with 2 µl of EDTA were prepared and mixed well. Master mix II with 2 µl of 3M Sodium acetate and 50 µl of ethanol were prepared and mixed well. The products were incubated and centrifuged for 30 minutes and 100 µl of 70% of ethanol with supernatant was added. The dried pellet was collected after centrifugation. The isolated result was sequenced through ABI 3500 DNA Analyzer (Applied Biosystems) and the quality of the sequence was analysed by Sequence Scanner Software v1. For sequence alignment and altering the obtained sequences were carried out by Geneious Pro v5.1[15].

The species were identified from the representative DNA sequence of the DNA samples using the BLAST search engine. There are ten more similar sequences were identified and it's sequences downloaded in FASTA format using Basic Local Alignment Search Tool of GenBank. These sequences were aligned and compared with *rbcL* sequences generated for our plant samples using T-COFFEE software and MEGA4[15]. Phylogenetic and molecular evolutionary analysis carried out with MEGA.

Results and discussion

The medicinal plants used in pharmaceutical industries are used raw materials for the preparation of drugs and the quality of the materials used in these fields are validated by a set of analyses. The quality of the materials used in these fields authenticated by botanical, physico-chemical and chemical analysed of pharmacopoeias and official compendia[16]. The conventional methods are not suitable when the materials used in different forms like root, stem and leaf or in powder form, so it is lead to adulteration of other plant materials and several studies have revealed species substitutions[17-20].

In the present study *rbcL* based DNA barcoding study was carried out in *Cipadessa baccifera* and *Melia azedarach* belonging to the family Meliaceae. The young and fresh leaf samples collected from the Western Ghats of South India. The DNA was isolated and PCR amplification was carried out using universal *rbcL* primers. After that the sequencing was done with standard protocol in a sequencer. The sequences were obtained in FASTA format and used for BLAST analysis. There are ten similar sequences were obtained from BLAST separately along with their sequences for *Cipadessa baccifera* and *Melia azedarach* in FASTA format. Multiple sequence alignment was done with the help of online T- COFFEE software. Based on the multiple sequence alignment result, a dendrogram was constructed using MEGA software (Figure-1). The dendrogram represent the phylogenetic relationship of Meliaceae members in different clusters. This study is very helpful to know the phylogeny status of selected two plants *Cipadessa baccifera* and *Melia azedarach* in Meliaceae family. The results revealed that other closely related species which are associated with the study plants. All the *Melia azedarach* from different accessions showing more similarity in dendrogram except our accession. There are four more genera also showing close association with *Cipadessa baccifera* species. Consequently, the phylogenetic reconstruction was constrained by limited dataset in the databases. The more sequences of meliaceae members will be helpful to study the comprehensive phylogeny of selected meliaceae members[21]. For the determination of evolutionary relationship the *rbcL* locus is generally applicable. In the present study *Cipadessa baccifera* and *M. azedarch* showed a cluster in the constructed tree based on the sequences available in the database, so the result will provide some useful insights for the authentication of selected meliaceae members[22]. The *rbcL* sequencing studies of *Cipadessa baccifera* and *M. azedarch* will be used for authentification of the species and its relationship with other species.

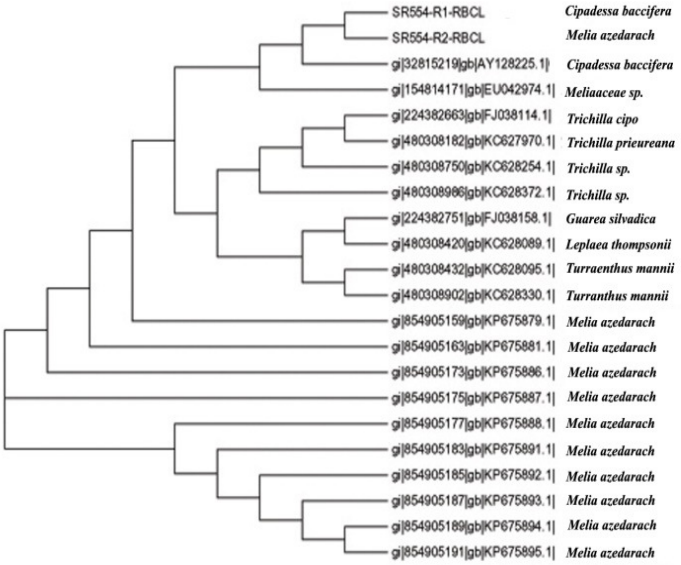

Figure-1: Phylogenetic analysis of *rbcL* sequences of selected Meliaceae members with their similar sequences through BLAST search.

Conclusion

The present study helps to authenticate the selected medicinal plants *Cipadessa baccifera* and *Melia azedarch* in traditional

medicinal systems. The *rbcL* based DNA barcoding studies will help to overcome the problems in plant identification by conventional methods like morphological, anatomical characters. Most of the plants in medicinal industries used as powder form, it leads to adulteration of other low grade material with original drugs. Recently in medicinal plants industries *rbcL* based DNA barcoding studies will be used for the authentication from adulterant and substituted samples which could not be done with other conventional methods. The study also cheapest one compared to other chemical analysis used in medicinal plant industries.

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