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# PHYLOGENETIC ANALYSIS, OF *CASSIA* SPECIES IN KERALA

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***Abstract***
*Since the advent of modern drug treatments, traditional treatment medicines have greatly receded in occidental societies. Cassia species is used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite production being most probably responsible for the plant's beneficial effects (1). The construction and interpretation of phyogentic tree are used to classify the evolutionary relationships between homologous genes represented in the genomes of the species. High sequence identity suggests that the sequences in question have a comparatively young most commonly recent ancestor, while low identity suggests that the divergence is more ancient. Therefore, it does not account for possible difference among organisms or species in the rates of DNA repair or the possible functional conservation of specific regions in a sequence (2). More statistically accurate methods allow the evolutionary rate on each branch of the phylogenetic tree to vary, thus producing better estimates of the sequenced species. Cassia alata ,Cassia tora and Cassia fistula was subjected to sequencing to determine the distinctive medicinal properties and to determine genetic variability.*

## Introduction & Methodology

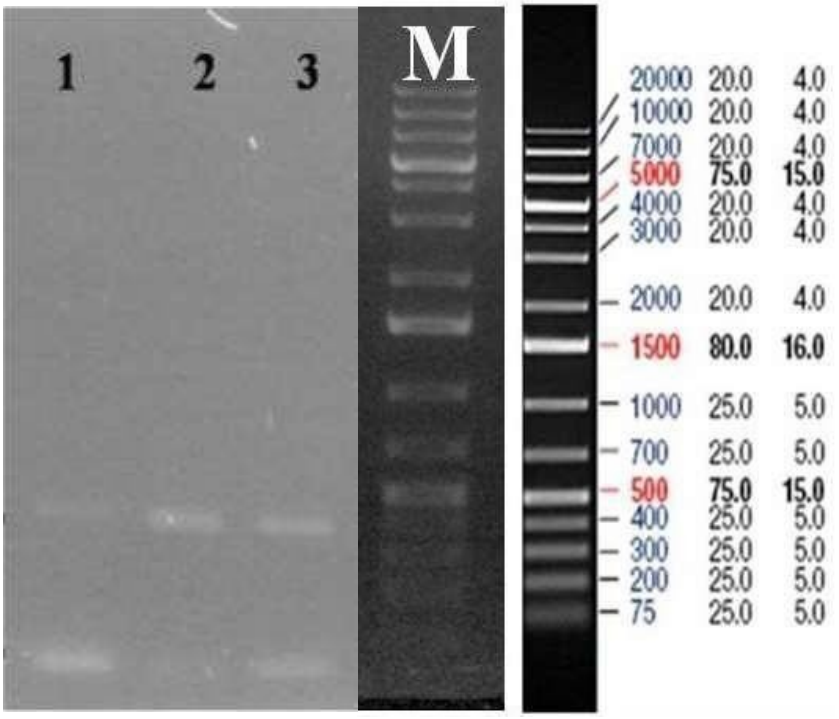
Three species of *Cassia* (*Cassia tora and Cassia allata ,Cassia fistula*), were collected from Trivandrum district in Kerala. These samples were photographed. For the molecular analysis of phylogenetic variability of the *Cassia* species used in the present study, sequencing done with the conserved region specific gene of the chloroplast genome (RuBiSCo gene, rbcL). For this purpose, DNA isolation is a prerequisite wherein the isolated DNA can be PCR amplified using rbcL specific primers and the products can be sequenced to know the variability and can be used for constructing the dendrogams.

For the same, good quality total genomic DNA was isolated from the collected leaf samples using CTAB method.The integrity and quality of the extracted DNA was checked in agarose gel electrophoresis.

In order to analyse the phylogenetic variability of the three *Cassia* species used in the present study, sequencing of conserved genes is the most appropriate method. For the identification and variability analysis of plant species, , rbcL gene was selected and used in the present study.From the total genomic DNA isolated from leaves of each samples, DNA at a concentration of 25 ng/□l were used for Polymerase Chain Reaction (PCR) using rbcL gene specific primers.

## Result

**PCR product (700bp) of genomic DNA, isolated from leaf samples of three species of *Cassia* using rbcL primer set.** 1- *Cassia fistula*, 2-*Cassia tora, 3 –Cassia alata* - non template control, M- 1kb plus DNA marker.




**Summary and Conclusion**

The present study deals with the DNA bar-coding of three Cassia species namely, *Cassia tora* ,*Cassia alata* and *Cassia fistula* using rbcL gene in order to analyze the phylogenetic variability among these species by constructing dendrograms. Good amplifications were obtained by Gradient PCR amplification . The amplification of Gradient PCR product was strong enough for the isolation of bands or direct sequencing. BLAST was used to detect and confirm the sequences of the present study species and with their related genus or species sequences available on NCBI database. The phylogenetic analysis was done by N-J method using MEGA5. All the sequences were supported with high bootstrap value.

In the dendrogram of the present study, *Senna tora* and *Hevea brasiliensis* (Outgroup) came under the same subgroup whereas *Senna alata* forms a different clade. Analysis of the sequence clearly explains that the species with which the samples collected are the same as morphologically observed. Also the dendrogram analysis states that *Cassia tora* is more genetically similar to *Hevea brasiliensis*.This explains that the species of Cassia were originated from a common ancestor but evolved into entirely different species. Both the clades have a single origin indicating that these two species have a common ancestor. It can be interpreted that genetic changes may occurred 50 in all two species due to the geographical separation between them. Therefore, by this study it can be stated that the three species used in the present study namely *Cassia tora , Cassia alata* and *Cassia fistula* have not exhibited any genotypic variations in the conserved domain. Furthermore analysis is needed to validate the extent of conserved genes in the respective species.

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