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DNA Barcoding Of Important Medicinal Plant(Artemisia pallens,Stevia rebaudiana,Spilanthes acmella) From Asteraceae Family

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Abstract – DNA barcoding is a technique in which species identification and discovery are performed by using short and standard fragments of DNA sequences. In this study, three species of asteraceae family includes Spilanthesacmella, Stevia rebaudiana, Artemisiapallens were sampled. The gene trnH-psbA is used as a DNA marker that is amplified and sequenced. The PCR amplification and sequencing efficiency, intra- and inter-specific divergence and barcoding gap were used to evaluate different loci, and the identification efficiency was assessed using BLAST1 and Nearest Distance methods. In our work three plant samples were collected and genomic DNA was extracted and quantified. It is then identified by using agarose gel electrophoresis method. In the case of yielding high purity of DNA plant sample were done by genetic analysis. In the recent studies, attempts were made to optimize DNA isolation by using CTAB method and phylogeny. The modified technique was found to be ideal for PCR amplification of pure DNA from the three sample species of Asteracae family. The trnH-psb Aintergenic spacer region has been used in DNA bar coding. In conclusion, trnH-psbA can be used to correctly identify medicinal plants that are closely related evolutionary, and it will be a potential DNA barcode for identifying medicinal plants of other taxa.

KEYWORDS – Spilanthesacmella, Stevia rebaudiana, Artemisia pallens, trnH-psb, DNA barcoding

1. INTRODUCTION

DNA barcoding is a standardized approach to identifying plants and animals by minimal sequences of DNA, called DNA barcodes. DNA barcode – short gene sequences taken from a standardized portion of the genome that is used to identify species. The total number of unique organisms described to the species level is around 1.5 million, but the total number of 'species' is likely to be in the region of 10 million. The overall 'taxonomic deficit' (the ratio of expected taxa to named taxa) is thus approximately sixfold. For, vertebrates the current described species total is likely to be relatively close to the 'true' total. The same is true of most groups whose members have body sizes greater than 10mm. The vast majority of organisms on the earth have body sizes less than 1mm, and for these groups the taxonomic deficit is likely to be several fold worse than for land plants and vertebrates.

DNA barcoding is a normalized way to deal with distinguishing plants and creatures by insignificant groupings of DNA, called DNA scanner tags. DNA scanner tag – short quality arrangements taken from a normalized segment of the genome that is utilized to recognize species. The all out number of novel living beings portrayed to the species level is around 1.5 million, however the absolute number of 'species' is probably going to be in the district of 10 million. The by and large 'ordered deficiency' (the proportion of expected taxa to named taxa) is hence around sixfold. For, vertebrates the current depicted species all out is probably going to be generally near the 'genuine' absolute. The equivalent is valid for most gatherings whose individuals have body sizes more prominent than 10mm. By far most of life forms on the earth have body measures under 1mm, and for these gatherings the ordered shortage is probably going to be a few overlay more regrettable than for land plants and vertebrates.

2. Materials and methods:

Sample collection: Healthy, disease free plant samples were collected from State Forest Research Institute, Vandalur. Fresh samples were transported to the laboratory in sterile ziplock covers with 24 hours of collection.

DNA ISOLATION: Deoxyribonucleic acid (DNA) isolation is an extraction process of DNA from various sources.

REAGENT'S PREPARATION: Plant genomic lysis buffer (CTAB Buffer): 0.3g CTAB 0.82g NaCl 670µl TrisHCl 400µl EDTA 20 µl 2-Mercaptoethanol 2% PVP CTAB, NaCl, TrisHCl and EDTA were added and made upto10ml using distilled water. Then PVP and 2-Mercaptoethanol were added to the mixture and used for the process.

M Tris-HCl pH 8.0(50 ml): 7.88g of Tris base were dissolved in 40ml of water. The pH was adjusted to 8.0 with Conc.HCl. The volume was madeupto 50ml using **0.5M EDTA, pH 8.0(50ml)distilled water**: 9.3g of EDTA were added to 40ml of distilled water. NaOH pellets were added to dissolve EDTA. The pH was adjusted to 8.0 with NaOH. The volume was made up to 50ml using

70% ETHANOL water: 70ml of AR grade ethanol was made upto 100ml using autoclaved sterile distilled water and stored at -20°C

METHOD

1g of the frozen leaf tissue was grinded into a fine powder using liquid nitrogen. To the fine powder pre – heated CTAB buffer was added and kept in water bath for 1 hr at 65˚C. Equal volume of chloroform:isoamyl alcohol was added and centrifuged at10,000 rpm for 10mins at 4˚C. To the supernatant equal volume of isopropanol was added and centrifuged at10000 at 10 min at 4˚C. The supernatant was removed and the pellet was washed with 70% ethanol twice and was air-dried at room temperature. The pellet was dissolved in TE buffer and stored under -20°C

DNA ISOLATION: The genomic DNA for plants Spilanthesacmella, Stevia rebeudiana, Artemisia pallens were isolated and viewed under gel documentation by using 0.8% agarose gel electrophoresis.

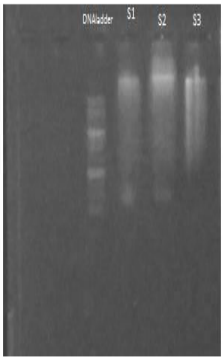
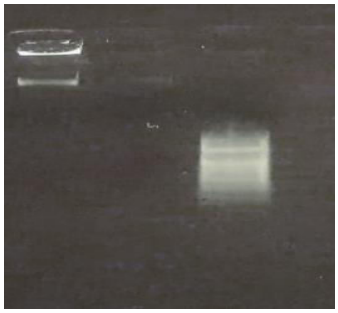


FIGURE.1
Lane1: DNA ladder 1kbp
Lane2:spilanthes acmella (sample 1)
Lane3:Artemisia pallens (sample 2)
Lane4:Stevia rebeudiana (sample 3)



PCR RESULTS

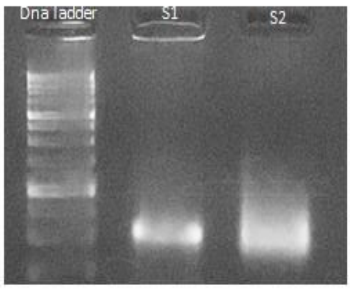


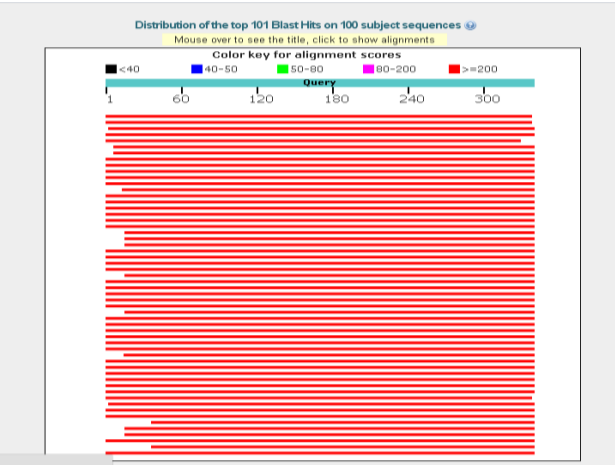
FIGURE.2
Lane1:DNA ladder
Lane2:Spilanthes acmella (Sample 1)
Lane3:Artemisia pallens (sample 2)

2. Results and discussion:

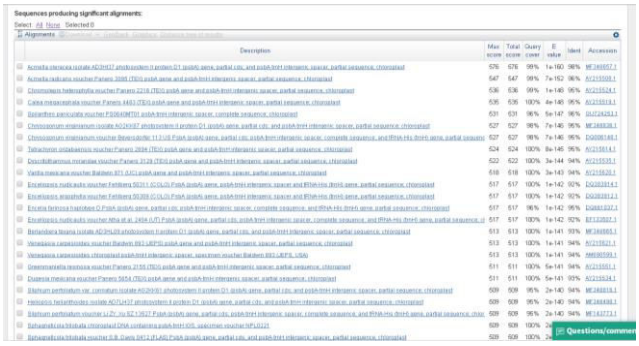
SEQUENCING OF Spilanthes acmella

>S1_TrnH-psbA1Forward_9513-1_P1453,Raw Sequence(740 bp)
CCTTGCAAAAAACCACAACGTCAGTATTACTATAATTTTTCC
TTAA
CATAAAAAAAAGCATATTATTTCTTTCTTATTTTTATTTAAG
AAAT
AAAAAATAAGCAAAATTTTCATTTTTATCTATTTTCGATTGA
ACTT
GAATTGGAAATAAAACTTCATAAAAGATTGGGAAAAGTAT
ATGA
TATATAAACCTATAATATAAATGAATACAAAGAAAAAACAC
GCA
AATCGAACCAAACTATAAAAAGTACTTGTTATTTTTAAAGA
AACT
ATGTAAGGGAAATAGTACTAAATAAAAAAAGGAGCAATA
ACGCC
CTCTTGATAAAACAAGAGGGAAGCTATTGGTCCTTTTTTAA
TTCA
AAAACTCCTATACAATCAAACCAAAGTCTTATCCATTTGGA
AATG
GGGCTTCGAAACAAGAACCGAGCCGCTCTAAGCTGCTTGT
GAAAC
AGTAGTAGCCCTAGTGGCTTCAGCCCACCCCTCCACGCTCG
CGGC
CTTGAAGCACGTCTTTATCAATTGTGTTTCTACAGGCGCTT
AAACG
CAATCGAATAACAAACAGGCTAAAAAAATCATCTGTTGAA
CAAACATACTTAGTAAAAGAAAAAAAAAGTTATAGATCTA
AATGAGCG
AATTGAAAAAATGTCGTGAAACGAAGCTGAAATGGAAAG
GAAAG
GTCCAGGCAGGTAACAATGCCACCATGACCTTACCACCGC
TGACG AGCCCAAGAAGGGTGAGGTAAA

Graphical Representation

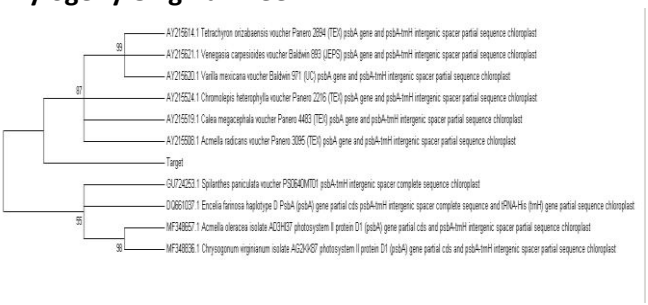


Tabular Representation



Alignment Representation

Phylogeny Original Tree



Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-3681.98) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the NeighborJoining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 515 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

3. CONCLUSIONS

SEQUENCING OF Artemisia pallens

.4.1 Sequence >S2_TrnH-psbA1Forward_9513-2_P1453,Raw Sequence(799 bp)

GAAAAGTGAAAGGTATAGGATTAGTTGGGCTAGATTTTTA
CCTCA
TTGTAAAAGAGAACAGATTATTTCCTTTTTATTTTGGAAAA
CCAA
GAAAGAAATAAGGCAAAATTTTTTTTTTTATATATTTTGGGT
TGAA
ATTGAATTGGAAAACAAACTTCATAAAAAATTTGGAATAA
AATAT
ACTAACCTTTAATATAAATGAATATGAATACAAAGAGAAA
ACCC
GCGAATCGAACCTTACTAAAAAATATTTTTAAAGAAACTGG
GGAA
GGCAAATAGTACTAAATAAAAAAAGGAGCTGTAACGCCCT
CTTG
ATAAAACAAGAGGAAAGCTATTGCTCCTTTTTTAGTTCAAA
AACT
ACTCCTAAACAATCAGACCAAAGTCTTATCCATTTGTAGAT
GGGG
CTTCGAGCAAGCGGATGGATCAAGGAAGAAACGGTGCTT
CCACC
CCCTGATGGAAGGGGCAGTAATCGAGCTTCTTGCTCTCCA
ATTTC
TTTTAAAGGAACCTTCGTTCCCCGCAGCCCGCTACGCCACT
GGTC
GAAATAATGAACGCGCCTAAAACAACTGACTCCCGCTAAA
CAAA
AAGTCTTGCTTGTCATAGCAGACCTCTATTTCAATTTTCTCA
GACT
ATTTGAAAAAAGGGGAATCTGGGCGGGGCGGAGCATTAA
ACCAA
GAGATATGAGTCAGTAACTCAAACCCTCGGAAAATTCCCG
TTTCG
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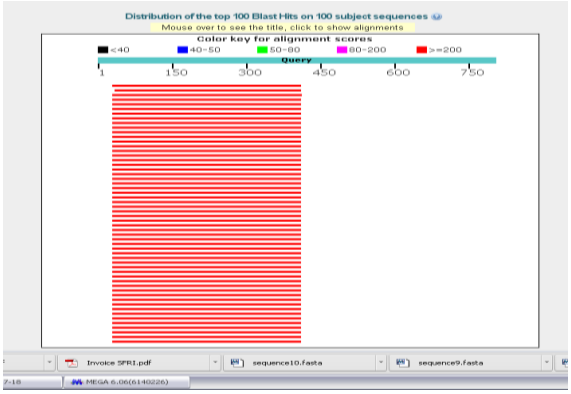
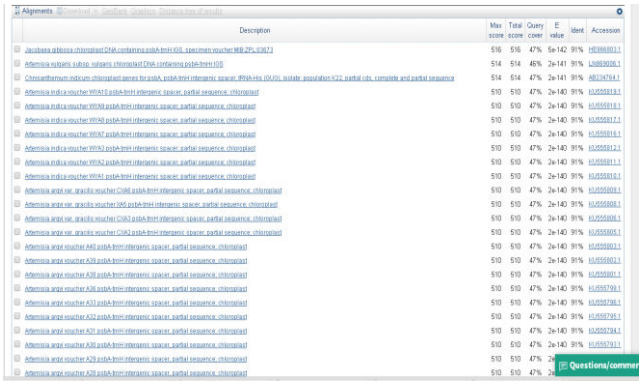
AACA
ATGGCAGAACTAATGAACATCTACTCTCAAGCGCCATCG

Graphical Representation

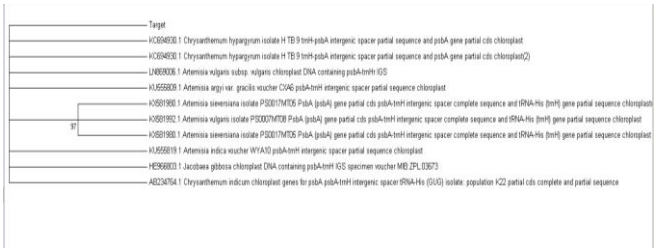


Tabular Representation



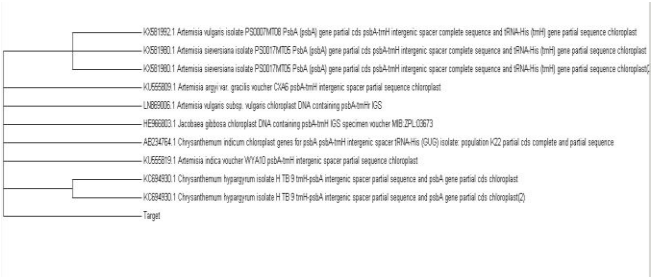
Alignment Representation

Phylogeny Original Tree



The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-3681.98) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree 53 for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 515 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

BOOTSTRAP



The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Initial treefor the heuristic search were obtained by applying the Neighbor-Joining method to a matrix ofpairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The analysis involved 13 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 515 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

SEQUENCING OF Stevia rebeudiana

S3_TrnH-psbA1Forward_9513-2_P1453,Raw Sequence

CCTCTACTATTATCTAGTATTATTTTTCCATTAACATAATACA
TAA
CAAAAACAGATTTTTTCTTTCTTATTTAATAAATCAAATCAA
ATCA
AAGTAATAAATAAATAAATCAAAGTAATAAATAAATAAAT
CAAA
GTAATAAATAAGCCAAATTTTCATTTTGATCTATTTTCGATT
GAAA
TTGAATTGGAAATAAAACTTTATAAAAGATTGGTAAAAGT
ATATT
ATATAGAATAGAAACCTATAATATAAATAAATACAAAGAA
AAAA
GACACAAATCGACCCAAACTATAAAAAGTCCTTTTTATTTA
TAAA
GAAACTATATAAGGCAAATAGTACTAAATAAAAAAAGGAG
CAAT
AACGCCCTCTTGATAAAACAAGAGGGGAGCTATTGCTCCT
TTTTT
AGTTCAAAAACTCCTATACAATCAGACCAAAGTCTTATCCA
TTTG
TAGATGGAGCTTCAATAGCAGCTAAGTCTAGAGGGAAGAC
TTTGG
TCTGATTGTATAGGAGTTTTTGAACTAAAAAAGGAGCAAT
AGCTC
CCCTCTTGTTTTATCAAGAGGGCGTTATTGCTCCTTTTTTTA
TTTAG
TACTATTTGCCTTATATAGTTTCTTTATAAATAAAAAGGACT
TTTT
ATAGTTTGGGTCGATTTGTGTCTTTTTTCTTTGTATTTATTTA
TATT
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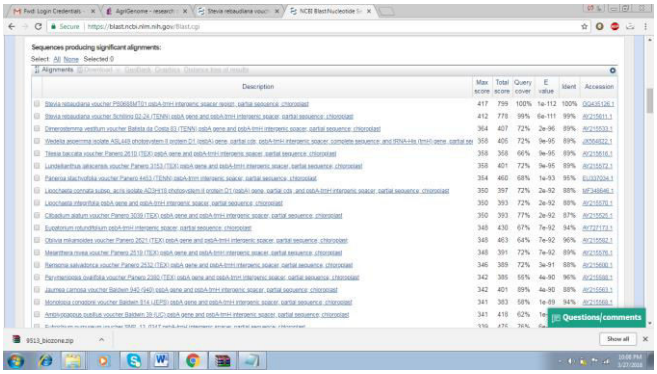
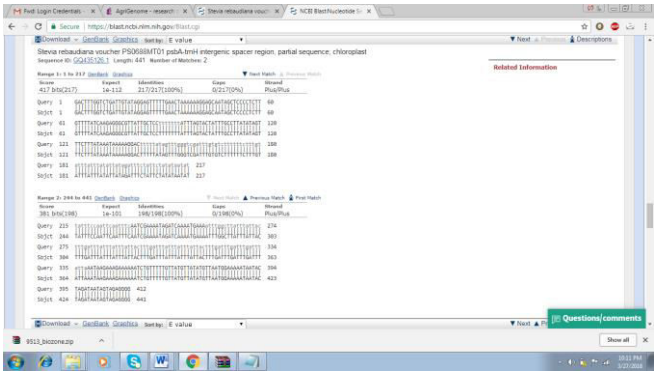
AAA GTTTTATTTCCAATTCAATTTCAATCGAAA

Graphical Representation

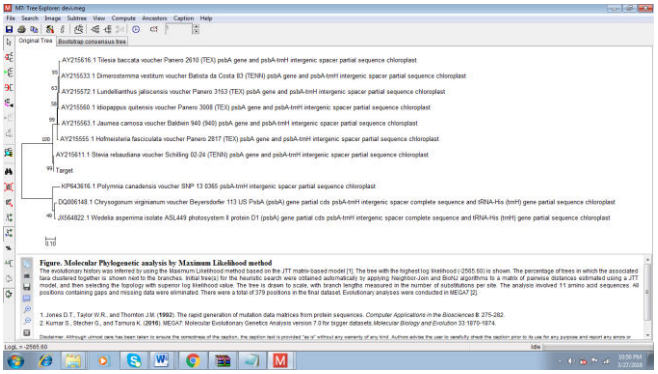
Tabular Representation



Alignment Representation

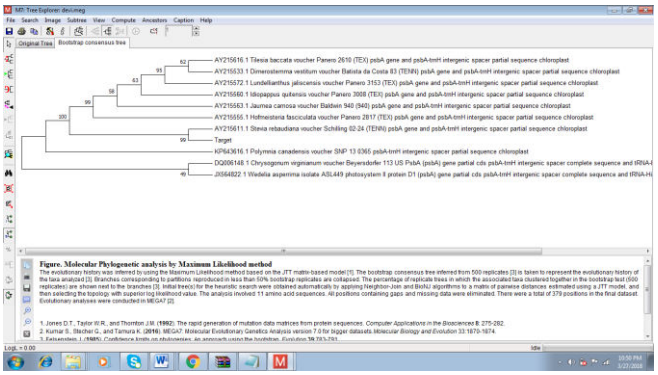


Phylogeny Original Tree



The evolutionary history was inferred by using the Maximum Likelihood 57 method based on the JTT matrix-based model [1]. The tree with the highest log likelihood (-2565.60) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 379 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Bootstrap



The evolutionary history was inferred by using the Maximum Likelihood 58 method based on the JTT matrix-based model [1]. The bootstrap consensus tree inferred from 500 replicates [3] is taken to represent the evolutionary history of the taxa analyzed [3]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [3]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The analysis involved 11 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 379 positions in the final dataset. Evolutionary analyseswere conducted in MEGA7.

SUMMARY AND CONCLUSION

The current study was taken with an aim of developing DNA barcodes for selected medicinal plants of the Asteraceae family. DNA barcoding is a technique in which species identification and discovery are performed by using short and standard fragments of DNA sequences. In this study, two species of asteraceae family includes Spilanthesacmella, Stevia rebaudiana, Artemisia pallenswere sampled. The gene trnH-psbA is used as a DNA marker that are amplified and sequenced. The PCR amplification and sequencing efficiency, intra- and inter-specific divergence and barcoding gap were used to evaluate different loci, and the identification efficiency was assessed using BLAST1 and Nearest Distance methods. In our work three plant samples were collected and genomic DNA was extracted and quantified. It is then identified by using agarose gel electrophoresis method. In the case of yielding high purity of DNA plant sample were done by genetic analysis. In the recent studies, attempts were made to optimize DNA isolation by using CTAB method and phylogeny. The modified technique was found to be ideal for PCR amplification of pure DNA from the three sample species of Asteracae family. The trnH-psbAintergenic spacer region has been used in DNA bar coding. In conclusion, trnH-psbA can be used to correctly identify medicinal plants that are closely related evolutionary, and it will be a potential DNA barcode for identifying medicinal plants of other taxa.

6. REFERENCES

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