

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, intraand 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 airdried 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.

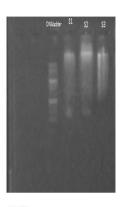
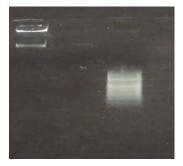


FIGURE 1 Lane1: DNA ladder 1kbp Lane2:spilanthes acmella (sample 1) Lane3:Artemisia pallens (sample 2) Lane4:Stevia rebeudiana (sample 3)



PCR RESULTS

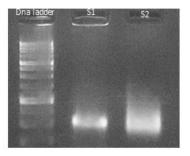


FIGURE.2 Lane1:DNA ladder Lane2:Spilanthes acmella (Sample 1) Lane3:Artemisia pallens (sample 2)

2. Results and discussion:

SEQUENCING OF Spilanthes acmella



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>S1_TrnH-psbA1Forward_9513-1_P1453,Raw

Sequence(740 bp)

CCTTGCAAAAAACCACAACGTCAGTATTACTATAATTTTTCC TTAA

CATAAAAAAAAGCATATTATTTCTTTCTTATTTTATTTAAG AAAT

AAAAAATAAGCAAAATTTTCATTTTTATCTATTTTCGATTGA ACTT

GAATTGGAAATAAAACTTCATAAAAGATTGGGAAAAGTAT ATGA

TATATAAACCTATAATATAAATGAATACAAAGAAAAAACAC GCA

AATCGAACCAAACTATAAAAAGTACTTGTTATTTTTAAAGA AACT

ATGTAAGGGAAATAGTACTAAATAAAAAAAGGAGCAATA ACGCC

CTCTTGATAAAACAAGAGGGAAGCTATTGGTCCTTTTTAA TTCA

AAAACTCCTATACAATCAAACCAAAGTCTTATCCATTTGGA AATG

GGGCTTCGAAACAAGAACCGAGCCGCTCTAAGCTGCTTGT GAAAC

AGTAGTAGCCCTAGTGGCTTCAGCCCACCCCTCCACGCTCG CGGC

CTTGAAGCACGTCTTTATCAATTGTGTTTCTACAGGCGCTT AAACG

AATTGAAAAAATGTCGTGAAACGAAGCTGAAATGGAAAG GAAAG

GTCCAGGCAGGTAACAATGCCACCATGACCTTACCACCGC TGACG AGCCCAAGAAGGGTGAGGTAAA

Graphical Representation

<40	40-50		lignment sco		>=200
<40	40-50		-80 Ouery		>=200
1	60	120	180	240	300

Description Description

Alignment Representation

Score		p 381 <u>GenBank</u> <u>Grap</u> Expect	Identities	Gaps	Strand	Previous Match	
576 bi	its(63		330/337(98%)	0/337(0%)	Plus/Mi	nus	
uery	1	tattatttctttctta	tttttatttaagaaataa	saastaagraasatttt	catttttat	60	
bjct	381	TATIATICITICITA	TITTTATTTAATAAATAA	LAALTALGCALAATTTT	TATTTTAT	322	
uery	61	ctatttcGATTGAAC	TTGAATTGGAAATAAAAC	FTC ataaaagattggga	aaagtatat	120	
bjct	321	CTATITITGATIGAAC	TIGAATIGTAAATAAAAC	TTCATAAAAGATTGGGA	AAGTATAT	262	
uery	121	gatetatasecctate	atatasatgastacasag	BBBBBBCACGCAAATCG	ACCARACT	180	
bjct	261	GATATATAAACCTATA	ATATAAATGAATACAAAG	AAAAAACACGCAAATCG	ACCAAACT	202	
uery	181	ATAAAAAGTACTTGTT	attittaaagaaactatg	TAAGGGAAATAGTACTA	ATasasas	240	
bjct	201	ATAAAAAGTACTTGTI	ATTTTTAAAGAAACTATG	TAAGGCAAATAGTACTA	ATAAAAAA	142	
uery	241	BGGAGCAATAACGCCC	TCTTGATAAAACAAGAGG	SAAGCTATTIGGTCCTTT	TTTAATTCA	380	
bjct	141	AGGAGCAATAACGCCC	TCTTGATAAAACAAGAGG	GAAGCTATTGCTCCTTT	ITTAGTICA	82	
uery	301	AAAACTECTATACAAT	CAAACCAAAGTETTATEC	ATT 337			
bjct	81	AAAACTCCTATACAAT	CAGACCAAAGTCTTATCC	ATT 45			



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

Tabular Representation



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.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

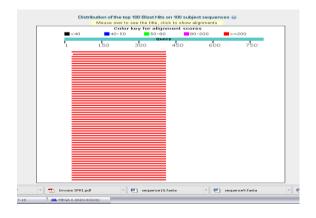
AAGTCTTGCTTGTCATAGCAGACCTCTATTTCAATTTTCTCA GACT

ATTTGAAAAAAGGGGAATCTGGGCGGGGCGGAGCATTAA ACCAA

GAGATATGAGTCAGTAACTCAAACCCTCGGAAAATTCCCG TTTCG

TTACATTCCTAAAAAAACCTGCAGCTGAAAAGCCCTAGGG AACA

ATGGCAGAACTAATGAACATCTACTCTCAAGCGCCATCG Graphical Representation



Tabular Representation

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Description			Query cover		lderit	Accession
Jacobana at bossa chiosaplant 0144 containing antiA-ImH (55: specimen voucher MB 2PL 33873	516	515	47%	5e-142	91%	HER66801.1
Adamisia vulgaris subsp. vulgaris chloroplast DNA containing psb4/mHr105	514	514	46%	2e-141	91%	LN819016
Chreaethernum indicum chloroplast genes for psbA, psbA/triH intergenic spacer, IRNA-His (60.05), isolate, population 1/22, partial cds, complete and partial sequence	514	514	47%	2e-141	91%	AB234764.1
Ademisia indica vojuter WATE psbA-InH intersenci sparar, parlal savojenca, chipropiast	510	510	47%	2e-140	91%	KU555819
Ademisia indica vaucher 997/80 pably tri Hinterantic spacer, partial sequence, chieraplast	510	510	47%	28-140	91%	HU555818
Atemisia indra voucher WVA0 psbAmini interpenis spaces, partial sequence, chimoplast	510	510	47%	2e-140	91%	KUSSAB17
Atemisia indica voucher 99787 pubA treHintersenic spacer, partial sequence, chicoplant	510	510	47%	2=140	91%	HU555816
Atemisia indica inputier WVAD pate-briki internetic space: partial sequence, chiuriplant	510	510	47%	2e-140	91%	HU555812
Atemisia indica voucher 97/82 pab&/httl interpretic spacer, partial sequence: chiosophot	510	510	47%	2+140	91%	HU555811
Atemicia indica voluter W/AL psbA/mH interpent spaces patial sequence chloroplast	510	510	47%	2e-140	91%	HUSSERIO.
Ademisia activise, pracilla veucher CVAII pably IniHinderpenis, spacer, partial sequence, chioropiant	510	510	47%	2e-140	91%	HU555E03
Atemicia and via practic souther (36 path-Imi) interpent: space, partial sequence, chiproplast	510	510	47%	2e-140	91%	HUSSES08
Ademisia and ser, gracilis souther CDAI pably/InH ofersenx spacer, partial sequence, othersplant	510	510	47%	26-140	91%	HU555108
Atemisia acta var. grabilis veucher CNRD abbil-ImH intergenic statuer, partial sequence, inforcebatt	510	510	47%	2e-140	91%	KU555805
Ademicia arosi soucher A40 usb3-trem intercenti, spacer, partial sequence, chiproplast	510	510	47%	2e-140	91%	HU555802
Ademisia acta stucher A38 asbAdmilinteroenis spacer, patial sequence, chistoplast	510	510	47%	2e-140	91%	10/555602
Atemisia and souther ASE usbA-thrif intercents spaces, partial sequence, chiptoplast	510	510	47%	29-140	91%	KUSSSED1
Atemisia anté soucher A38 auto-4milinteroania apacer, partial sequence, chiengelarti	510	510	47%	20140	91%	HU555793
Ademisia aron voucher A33 asbA-tremintergenic spoler, partial sequence, chiproplest	510	510	47%	2e-140	91%	KU154796
Attentisia ange voucher A32 oxbArtnii intergenic spaces, patial separance chieroplast	510	510	47%	20-140	91%	10,555795
Atemicia anal-mocher A31 asbA-mit interpenic aparen, partial bequence, chiptoplast	510	510	47%	2e-140	91%	KU555794
Attemisia anni voscher A30 astA-Imilintergenic spacer, partial seguence, chieroplast	510	510	47%	20-140	91%	HU555791
Ademinia anna souther A29 aisti-A3111 intervenic apacar, partial sequence, chiantoliast	510	510	47%	26		
Attemisia arg/ voucher A28 psbA-tmH intergenic spaces, partial sequence, chiptoplast	510	510	47%	24	Que	stions/co

ISSN: 2582-3930

Alignment Representation

Download + <u>GenBank</u> Graphics	🔻 Next 🔺 Previous 🛓 Descriptio
lacobaea globosa chloroplast DNA containing psb4-tmH IGS, specimen voucher MIB ZPL:03873	
Sequence ID: <u>HE968803.1</u> Length: 427 Number of Matches: 1	
Range 1: 16 to 393 GenBank, Graphics 🔰 Rent Natch 🛦 Previous Natch	Related Information
Score Expect Identities Caps Strand 516 bits[279) 5e-142 345[378](91%) 0[378](9%) PLus[Minus	
DETY 30 CTAGATTTTTACCTCATTGTAAAAGAGAACAGATTATTTCCTTTTTATTTTGGAAAACCA 89	
bjet 393 CIETETTTTECTCAACETAAAAAGGCETETETTTCTTTTTTATTETGAAATCAA 334	
(hery 90 AGAILGAATAAGGAALAATTTTTTTTTTTTTTTTTTGGATGAAATTGAALAA 149	
ibjet 333 Affiatlaatlaafaaaaattaattittattittittatattitteettitaaatteaatteaatteeaat 214	
bery 150 CAMETTCATAAMAAATTTIGGAATAAMATAATTAACTIAACTITTAATATAAATGAATAATGAATAATGAAT	
bjet 273 CAAACTTCATAAAAATTTIGGAATAAAATATACAAACTIGTAATATAAATGAATATGAAT 214	
hery 210 ACALAGAGAAACCCCCCAATCGAACCTTACTAAAAAATATTTTTAAGAAACTGGGGAA 269	
bjet 213 Araansasaaaraceesaatosaaccaaaratataaarattiitaaasaarteiseaa 154	
(DECY 270 GECALATASTICTALATIONSONSGARCTISTLAGECCOTOTISATALAACAAGAGAAA 329	
Rejet 153 GECHAATASTRITAAATAAAAGGARGARTAADSGCCTUTTGATAAAAGAAGAGGAAA 94	
(pery 330 ΟΓΙΑΠΙΟΓΙΟΙΤΗΤΗΑΠΙΟΑΑΑΑΙΤΑΓΙΟΓΙΑΑΑΑΑΠΙΑΓΙΟΓΙΑΑΑΟΑΑΑΑΠΙΟΙΟ 389	
bjet 93 GCTATTGCTCCTTTTTAFTICAAAAACTACTCCTATACAAFCAGACCAAAFTCTTATCC 34	
Derry 390 ATTISTAGATISSSCTTC 407	
Bjet 33 ATTIGTAGATHGAGCTTE 16	

Phylogeny Original Tree

	Target
	KIC894930.1 Chrysanthemum hypargynum isolate H TB 9 tmH-psbA intergenic spacer partial sequence and psbA gene partial cds chlosoplast
	KC894930.1 Chrysanthemum hypargynum isolate H TB 9 tmH-pobA intergenic spacer partial sequence and psbA gene partial cds chloroplast(2)
	UNEE0006 1 Artemisia vulgaris sobsp. vulgaris chloroplast DNA containing psbA-tmHr IGS
	KU555808 1 Artemisia argyl var. gracilis voucher CX46 psb4-tmH intergenic spacer partial sequence chloroplast
	NUSB1952.1 Artemisia vulgaris isolate PS0007MT00 PsbA (psbA) gene partial cds psbA-tmH intergenic spacer complete sequence and ISPUA-His (bmH) gene partial sequence chloroplast
37	
	HE966803 1 Jacobasa olibora chloroplast DNA containing psbA-tmH KSS specimen voucher MB ZPL 03673
	AB234754.1 Chrysanthemum indicum chloroplast genes for pobA pobA-tmH intergenic spacer 19144-His (GUG) isolate: population K22 partial cds complete and partial sequence

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



-VSEYEE1 Adminis vigani sista PSXXXIVXIII BAA (pabA) gane partial do (pAA-trif integenic spacer completa sequence and SRIA-Ho (prif) gane partial sequence chicophat -VSEYEE1 Adminisis senersiana instate PSXXIVXIIIS PAAA (pabA) gane partial do (pAA-trif integenic spacer completa sequence add RNA-Ho (prif) gane partial sequence chicophat
-VXE19801 Attensis severane locke PS0017MTS PbA (sol4) gete partial cls pol4-triff integenic spacer complete sequence and IRNA-fits (triff gete partial sequence chloroplast)
LOEGOID: 1 Antemisia volganis subso, volganis chloroplast DNA containing pobA-mith ICS HEGEOBID: 1 Jacobaes glubosa chloroplast DNA containing pobA-mith ICS specimen wucher MIB ZPL COET3
— 422347641 Okrysanthemum indicum oktomplant genes för päskå påbl-kritt intergenic spacer 1914-Hisi (SUG) solate population X22 partial och complete and partial sequence X155550191 Mennisia indica vocken WYAXD pabAmitt intergenic spacer partial sequence oktomplant
 KC694501 1 Chrysarthemun hypargrum isolate H TB 9 mH-polia hintegenic spacer partial sequence and polia gene partial cds chloroplast – KC694501 1 Chrysarthemun hypargrum isolate H TB 9 mH-polia hintegenic spacer partial sequence and polia gene partial cds chloroplast(2)
 Taget

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

ΑΑGTΑΑΤΑΑΑΤΑΑΑΤΑΑΑΤCAAAGTAATAAATAAATAAAT CAAA

GTAATAAATAAGCCAAATTTTCATTTTGATCTATTTTCGATT GAAA

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ATATAGAATAGAAACCTATAATATAAATAAATACAAAGAA AAAA

GAAACTATATAAGGCAAATAGTACTAAATAAAAAAAGGAG CAAT

AACGCCCTCTTGATAAAACAAGAGGGGGGGGGGCTATTGCTCCT

AGTTCAAAAACTCCTATACAATCAGACCAAAGTCTTATCCA TTTG

TAGATGGAGCTTCAATAGCAGCTAAGTCTAGAGGGAAGAC TTTGG

TCTGATTGTATAGGAGTTTTTGAACTAAAAAAGGAGCAAT AGCTC

CCCTCTTGTTTTATCAAGAGGGCGTTATTGCTCCTTTTTTA

TTTAG

TACTATTTGCCTTATATAGTTTCTTTATAAATAAAAAGGACT TTTT

ATAGATTTCTATTCTATATAATATACTTTTACCAATCTTTTAT AAA GTTTTATTTCCAATTCAATTCAATCGAAA Graphical Representation



Tabular Representation

Sequences producing significant alignments:								
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Wedela assertma solate ASL445 cholosystem 8 action D1 (astA) cere patial pit, both init riterative papers consists sequence and (RNA-Ha (HHA) cere, patial pit	358	405	72%	9e-95	89%	28564822.1		
Tensis Security volume Parens 2010 / TEX apply owne and poly first intervents, security security intervents, chiprovised	358	358	66%	9e-95	.03%	8/215616.1		
Unstallanthus adjointing souther Parkers 2153 (TEX) work one and bold-Intri intervent souter, bartia sequence, choronial	358	401	72%	94-95	89%	Br215572.1		
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Loochasta vheurihila obbi sene and obbi/miri intervenix soacer, cartia sequence unicrossitat	350	393	72%	28-92	80%	AV215570.1		
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Alignment Representation

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Stevia rebaudiana voucher PS0688MT01 psb Seguence (0: GQ435125.1: Length: 441. Number of	A-tmH intergenic spacer region, partial sequence, chloroplast Manham 2	
Renard 1: 1 to 212 Declark Gradua	These Match & Process Statute	Related Information
Scare Expect Identities 417 bts/2175 10-112 217/217(1009	Gaps Strand	
Query 1 excilionicioariorataegesittiteae Sect 1 excilientiteae	TAMANING POCHT INCTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	
Sedet 1 GACTITIGETCTGATIGETATAGGAGTTTTTGAAC Query 61 GTTTTATCAAGAGGGCGTTATTGCTCC111111		
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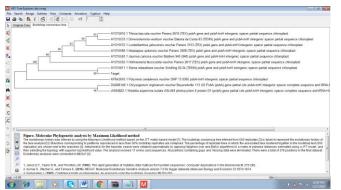
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10	Figure. Molecular Phylogenetic analysis by Maximum Likelihood method	
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5	model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of subditutions per site. The analysis involve	ed 11 amino acid sequences. All
1	positions containing gaps and missing data were eliminated. There were a total of 379 positions in the final dataset. Existence analyses were conducted in MEGA7 [2]	
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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 trnHpsbAintergenic 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.

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