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PLUMERIA FLOWERS IDENTIFICATION BY DNA BARCODING AND HPLC SPECIFIC CHROMATOGRAM

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ABSTRACT
Identification DNA Barcoding And HPLC Specific Chromatogram Were Used To Identify Three Kinds Of Plumeria Flowers Respectively. DNA'S Extracted From The Three Plumeria Species Were Amplified By Pcr With Universal Primers, And The Psba-Trnh Region Was Selected. All The Amplified Products Were Sequenced And The Results Were Analyzed By Mega 5.0. Chemometric Methods Including Principle Components Analysis And Hierarchical Clustering Analysis Were Conducted On The Sas 9.0 Software To Demonstrate The Variability Among Samples. In Conclusion, The Psba-Trnh Of All Samples Were Successfully Amplified From Total DNA And Sequenced. These Three Varieties Of Plumeria Can Be Differentiated By The PsbaTrnh Region And Clustered Into Three Groups Respectively Through Building Neighbor Joining Tree, Which Conformed To Their Germplasm Origins. However, It Was Hard To Distinguish Them By HPLC Specific Chromatograms Combined With Chemometrics Analysis. These Indicated That DNA Barcoding Was A Promising And Reliable Tool For The Identification Of Three Kinds Of Plumeria Flowers Compared To HPLC Specific Chromatogram Generally Used. It Could Be Treated As A Powerful Complementary Method For Traditional Authentication, Especially For Those Varieties Which Are Difficult To Be Identified By Conventional Chromatography.

KEYWORDS: Plumeria DNA Barcoding HPLC Specific Chromatogram Chemometrics Analysis.

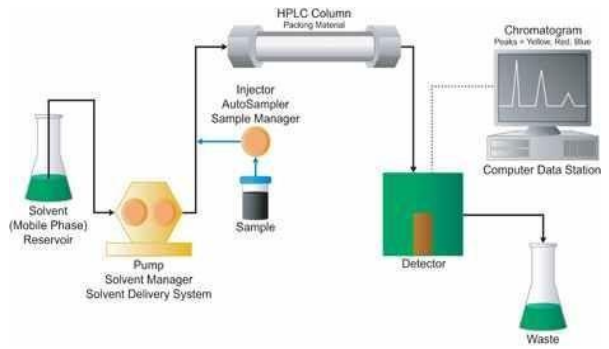
INTRODUCTION
Plumeria Is A Kind Of Deciduous Tree Belonging To Apocynaceae. It Has A Significant Medicinal Value And Can Be Used For The Treatment Of Various Ailments, Such As Sore Throat, Heatstroke, Bellyache, Cough, And Dysentery.[1] The Main Chemical Components Of Plumeria Are Iridoids, Triterpenes, Flavonoids, Essential Oil And So On. Mansour Et Al.[2] Found That The Iridoids In Plumeria Have A Toxic Effect On A Series Human Tumor Cells. The Extracts Of The Flowers Can Significantly Inhibit Streptococcus Faecalis, Bacillus, And Corynebacterium Pyogenes.[3] Gupta Et Al.[4] Indicated That The Flowers Extracts Of Plumeria Had The Antipyretic- Analgesic Effect. As A Medicine With Dual-Purpose Of Drug And Food, The Flowers Of Plumeria Are Also Popular Among People In Making Herbal Tea To Relieve The Summer Heat.

There Are Many Varieties Of Plumeria, Such As Plumeria Rubra L., Plumeria Rubra Var. Alba, And Plumeria Rubra 'Acutifolia'. Some Researches Have Indicated That The Chemical Components Vary From Different Plumeria Flowers, Some Of Which May Have Good Bioactivities.[5,6,7,8] However, It Is Difficult To Identify Plumeria Flowers Because They Are Very Alike After Dried And Processed. Thus, Authenticity Assurance Is Crucial For Their Quality Control. We Intended To Use DNA Barcoding Technique And HPLC Specific Chromatograms To Identify Three Kinds Of Plumeria.

DNA Barcoding Is A Technique For Identifying Biological Specimens Using Short DNA Sequences From Either Nuclear Or Organelle Genomes.[9] This Technique Has Three Advantages As Follows. Firstly, The Results Have Good Repeatability Compared To Other Molecular Identification Methods. Secondly, DNA Barcoding Technique Is Highly Universal. The Traditional Taxonomic Method Requires That The Leaves, Flowers, Fruits And Other Organs Of Plants Must Exist. However, DNA Barcoding Technique Is Not Limited By Growth Stage, Organ, Tissue Difference Or The External Environment. And A Small Number Of Samples Are Enough For The Identification. Lastly, It Can Establish A Unified Database And Identification Platform To Realize Digital Species Identification.[10]

DNA Barcoding Technique Has Been Applied To Identification Of Animals, Gymnosperms, Angiosperms, Fungi And So On.[11]

The CP DNA (Cpdna) Has Been Commonly Used For DNA Barcoding Studies In Plants. The CpDNA Contains Variable DNA Regions, Among Which The Most Commonly Used Cpdna Intergenic Spacer Is Psba-Trnh, Which Has Shown High Variability And Can Be Used To Elucidate Genetic Relationships At The Intraspecific Level.[12,13] In This Pioneering Study, We Selected Psba-Trnh To Identify Three Varietas Of Plumeria, At The Same Time, The Hplc Specific Chromatogram That Is Commonly Used For Identification Was Also Used For Distinguishing These Three Varieties Of Plumeria To Make Comparison. We Hope That Our Established Method Will Be Helpful For The Future Quality Control Of Plumeria Flowers.



High performance liquid chromatography

2. Experimental

2.1 Materials And Reagents

Twelve Samples Of Plumeria Flowers Were Collected From Three Districts In Guangzhou, Guangdong, China (Table 1). They Were Authenticated By Associate Professor Lin Jiang, Sun Yat-Sen University, China. Methanol Was Of Analytical Grade And Manufactured By Tianjin Zhiyuan Chemical Reagent Factory (Tianjin, China). Tae Buffer, Agarose, Pvp-40 And Taq Pcr Master Mix (2×, Blue Dye) Were Purchased From Sangon Biotech (Shanghai, China). Dna Secure Plant Kit Was Purchased From Tiangen Biotech (Beijing, China). Goldview (Mym Biological Technology Co., Ltd., Usa) Was Used For Agarose Examination. Acetonitrile Was Of Hplc Grade Manufactured By Sk Chemicals (Korea). Ultrapure Water Was Obtained From A Milli-Qrg Purification Unit (Millipore, Bedford, Ma, Usa).

Table 1: Information of The Collected Sample.

R1	Plumeria Rubra L. Sun Yat-Sen University Campus · Panyu · Guangzhou
R2	Plumeria Rubra L. Sun Yat-Sen University Campus · Panyu · Guangzhou
R3	Plumeria Rubra L. Sun Yat-Sen University Campus · Panyu · Guangzhou
R4	Plumeria Rubra L. Sun Yat-Sen University Campus · Panyu · Guangzhou
P1	Plumeria Rubra Var. Alba Sun Yat-Sen University Campus · Panyu · Guangzhou
P2	Plumeria Rubra Var. Alba Liwan-Lake Park · Liwan · Guangzhou
P3	Plumeria Rubra Var. Alba Tianhe Park · Tianhe · Guangzhou
P4	Plumeria Rubra Var. Alba Tianhe Park· Tianhe · Guangzhou
W1	Plumeria Rubra 'Acutifolia' Sun Yat-Sen University Campus · Panyu · Guangzhou

2.2 Apparatus

An Electronic Balance (Kern Abt 220-5dm, 0.1 Mg, Germany), An Ultrasonic Machine (Sb2512dtd, Xinzhi Biotechnical Ltd., Ningbo, China) And An Eppendorf Centrifuge 5417r (Eppendorf Ag, Hamburg, Germany) Were Used For Sample Preparation And Dna Extraction. Pcr Amplification Was Performed On The K960 Thermal Cycler (Hangzhou Jingge Scientific Instrument Co., Ltd, Hangzhou, China). Hplc Analysis Was Performed On The Shimadzu Lc-15c High Performance Liquid Chromatograph (Shimadzu, Japan) With A Dikma Diamonsil C18 Column (250 Mm × 4.6 Mm, 5 µm; Dikma, Beijing, China) And A Guard Column (15 Mm × 4.6 Mm, 5 µm; Dikma, Beijing, China).

2.3 Sample pre-treatment for DNA extraction and total DNA extraction

The fresh flowers were washed up and wiped with 75% alcohol aqueous. About 100 mg of each sample was grinded with 1% (m/m) PVP-40. Then, DNA secure Plant Kit was used for total DNA extraction. The process followed the instruction of the Kit.

2.4. PCR amplification and DNA sequencing

DNA barcodes were amplified by polymerase chain reaction using universal primers (fwd: 5′-GTTATGCATGAACGTAATGCTC-3′ and rev: 5′-CGCGCATGGATTCACAATCC-3′). Each 25µL reaction mixture contained 12.5µL Taq PCR Master Mix, 1µL 1µL of each 10µM primer, 1µL $MgCl_2$ solution and 8.5 µL ddH_2O The PCR conditions for amplification were 1 cycle 94 °C for 5 min; 40 cycles of 94 °C 30 s, 56 °C 30 s, 72 °C 45 s; and 1 cycle 72 °C for 10 min, and hold 4 °C. To detect amplified products successfully, PCR products were examined on 2% agarose gels stained with Goldview and visualized under ultraviolet light.

2.5. Sequence Alignment And Analysis

All The Amplified Products Were Sent To Sangon Guangzhou For Sequencing. The Sequences Were Analyzed By Mega 5.0.

2.6. Preparation Of Sample Solution For HPLC Analysis

Test Solutions Were Prepared By Extracting 0.5G Dried And Pulverized Herbs With 10 Ml 70% Methanol Aqueous Under Ultrasonic Condition At Room Temperature For 30 Min. After Cooling, The Extracted Solution Was Added With 70% Methanol Aqueous To The Original Weight. The Extracts Were Filtered Through A 0.45 µm Filter Before Used For Hplc Analysis.

2.7. HPLC Conditions

Chromatographic Separation Was Carried Out On A Diamonsil C18 Column Along With A Guard Column. The Separation Was Conducted At 35 °C With A Flow Rate Of 0.7 Ml/Min. 0.5% Acetic Acid Aqueous Solution (A) And Acetonitrile (B) Were Used As The Mobile Phase In Gradient Elution Mode. The Elution Gradient Was Set As Follows: 0–10 Min, 10% (B); 10–30 Min, 10%→15% (B); 30–70 Min, 15%→20% (B); 70–85 Min, 20%→65% (B); 85–90 Min, 65%→90% (B); 90–95 Min, 90% (B). The Detection Wavelength Was 240 Nm. The Injection Volume Was 20Ml.

2.8. Chemometric Analysis

PEINCPLE COMPONENTS ANALYSIS (PCA) And HIERARCHIAL CLUSTERING ANALYSIS (HCA) Were Conducted On The Sas 9.0 Software To Demonstrate The Variability Among The 12 Samples.

3. RESULTS AND DISCUSSIONS

3.1. DNA Barcode Result Analysis

Authenticity Assurance Is Crucial For Quality Control Of Natural Products. It Is Essential To Develop Different Approaches To Authenticate The Natural Products As Each Approach Has Advantages That Complementary To One Another.[14] A Desirable DNA Barcode Should Process High Interspecific Divergences And Low Intraspecific Variations. The Consortium For The Barcode Of Life (Cbol) Suggested Comparing The Interspecies Distance (Dinter) And Intraspecies Distance (Dintra) To Estimate The Identification Effectiveness Of The Selected Barcode. The Interspecific Divergences Of An Ideal 'Barcoding Gap' Should Be Significantly Larger Than Intraspecific Divergences. If Dinter/Dintra Is Smaller Than 1, It May Not Be A Suitable DNA Barcode.[10]

The Results Of DNA Barcoding Showed A Good Differentiation. The Psba-Trnh Of All Samples Were Successful y Amplified From Total DNA And Sequenced. Properties Of The Psba-Trnh Region Are Summarized In Table 2. The Genetic Distance Was Calculated By Mega 5.0, Based On Kimura-2-Parameter Model. The Intraspecies Distances Of Plumeria Rubra L. And Plumeria Rubra 'Acutifolia' Were 0.001 And 0.004, Respectively. There Was No Intraspecies Distance For Plumeria Rubra Var. Alba. The Interspecies Distance Was 0.007 Between Plumeria Rubra L. And Plumeria Rubra Var. Alba, 0.034 Between Plumeria Rubra L. And Plumeria Rubra 'Acutifolia', 0.033 Between Plumeria Rubra Var. Alba And Plumeria Rubra 'Acutifolia'. Results Of Each Dinter/Dintra Were Larger Than 1, Which Indicated That The Psba-Trnh Region Was Suitable For The Identification Of Three Varietas Of Plumeria. The Neighbor Joining Tree Was Built By Mega 5.0 By Repeated 1000 Times Bootstrap (Fig. 1). The Neighbor Joining Tree Showed That The Samples Of Plumeria Rubra L., Plumeria Rubra Var. Alba And Plumeria Rubra 'Acutifolia' Can Be Clustered Into Three Groups, Respectively. Therefore, The Psba-Trnh Region Was An Appropriate DNA Barcode For Identifying These Three Varietas Of Plumeria.

3.2 HPLC ANALYSIS

Fig. 2 Shows The Hplc Specific Chromatograms Of Samples R1 (Plumeria Rubra L.), P1 (Plumeria Rubra Var. Alba) And W1 (Plumeria Rubra 'Acutifolia'), Their Hplc Chromatograms Were So Similar That It Was Difficult To Separate The Three Varietas Visually.

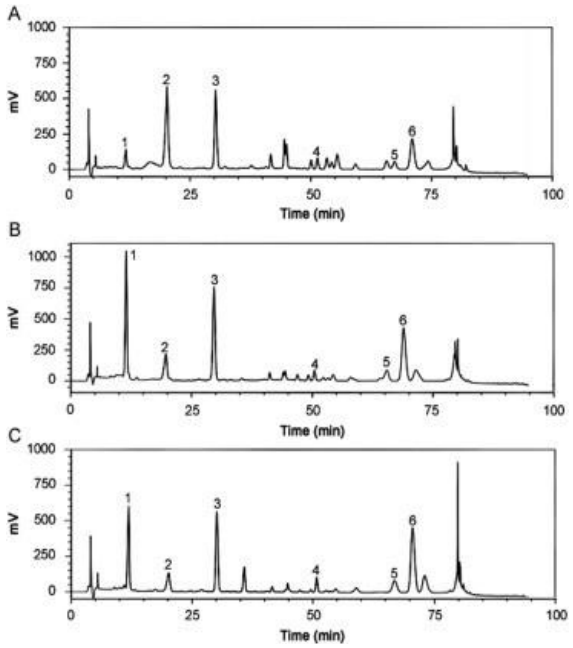


Fig. 2: The Liquid Chromatograms Of The Three Plumeria (A: Plumeria Rubra L., B: Plumeria Rubravar. Alba, C: Plumeria Rubra 'Acutifolia').

3.3. PRINCIPLE COMPONENT ANALYSIS (PCA)

PCA, A Multivariate Analysis Technique, Could Visualize Similarities Or Differences Within Multivariate Data.[15] It Was Employed To Analyze The Differences Among These 12 Samples. The Peak Areas Of 6 Characteristic Peaks Were Set As Variables, While 12 Samples Were Set As Observations. Pc1 Explained 41.2% Of The Total Variance In The Data Set While Pc2 Explained 32.4%. The Cumulative Proportion Of Pca As Well As The Loading Diagram Is Shown In Fig. 3. According To The Loading Diagram, Pc1 Showed A Strong Correlation With Peak 5 And Peak 6. Pc2 Showed A Strong Correlation With Peak 2 And Peak 4. Pc3 Showed A Strong Correlation With Peak1 And Peak 5. The Distinguished Results Of Hplc Specific

Chromatograms Combined With Pca Were Not As Accurate As That Of Dna Barcoding.

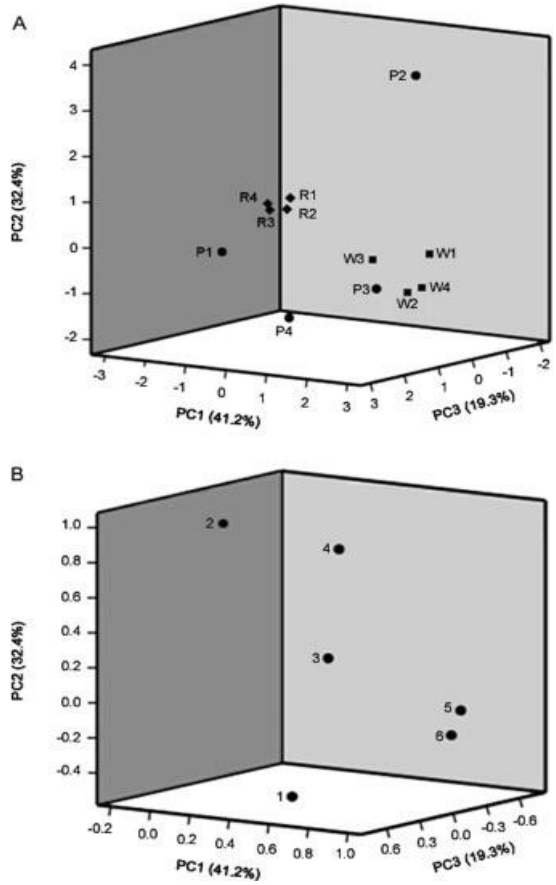


Fig. 3: Score Plot (A) And Loading Plot (B) Of Pca.

3.4. HIERARCHIAL CLUSTER ANALYSIS (HCA)

HCA, One Of The Most Commonly Used Unsupervised Pattern Recognition Methods, Is A Useful Multivariate Statistic Technique To Assign A Data Set Into Groups By Creating A Cluster Tree Or Dendrogram According To Similarity.[15] In Order To Assess The Resemblance And Differences Of These Samples, Hca Of Plumeria Samples Was Performed Based On The Peak Area Of The 6 Characteristic Chromatographic Peaks By Sas 9.0 Software. The Ward's Method Was Applied As The Amalgamation Rule And The Squared Euclidean Distance Was Selected To Measure The Resemblance And Classify The 12 Samples. The Result Is Shown In Fig. 4. Samples R1–R4 (Plumeria Rubra L.) Were Categorized Into One Cluster, W1–W4 (Plumeria Rubra 'Acutifolia') Were Categorized Into Another Cluster. However, Samples P1–P4 (Plumeria Rubra Var. Alba) Cannot Cluster Together. Samples R1–R4 And W1–W4 Were Collected From The Same District, But Samples P1–P4 Were From Three Different Districts. Therefore, We Speculated That The Environment Affected The Chemical Component Which Affected The Result Of PCA and HCA.

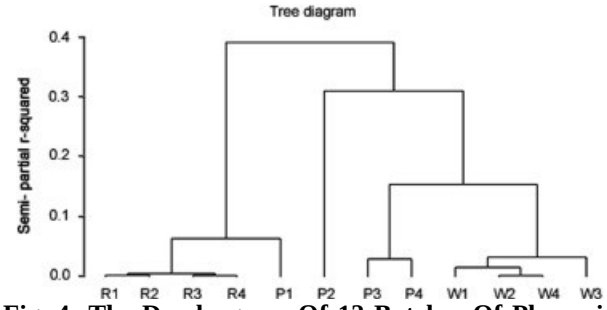


Fig. 4: The Dendrogram Of 12 Batches Of Plumeria By Hca. R1-R4: Plumeria Rubra L., P1P4: Plumeria Rubra Var. Alba, And W1-W4: Plumeria Rubra 'Acutifolia'.

4. CONCLUSION

This Study Has Shown That DNA Barcoding Combined With Chemometrics Analysis Can Distinguish Plumeria Rubra L., Plumeria Rubra 'Acutifolia' And Plumeria Rubra Var. Alba. While The HPLC Method Can Not Identify These Three Varietas Because The Specific Chromatograms Were Similar. DNA Barcoding Technique Is Hopeful For Automation As It Is More Stable, Accurate And Not Affected By Growth Stage, Tissue Difference Or External Environment. This Technique Is An Effective Supplement For Traditional Authentication Methods, Especially When Different Species Are Mixed Together. This Work Also Provides An Experimental Reference For Identification Of Natural Medicines By DNA Barcoding technique.

REFERENCES

1. Chinese Herbal Medicines Guangxi Standard, 1st Volume, The Guangxi Zhuang Autonomous Region Health Department, Guangxi Science And Technology Press, Nanning, 1990: 59. Google Scholar
2. A.K. Mansour, M.M. Eid, N.S.A.M. Khalilsynthesis And Reactions Of Some New Heterocyclic Carbohydrazides And Related Compounds As Potential Anticancer Agents Molecules, 2003; 8: 744-755 Crossrefview Record In Scopusgoogle Scholar
3. P.A. Egwaikhide, S.O. Okeniyi, C.E. Gimbascreening For Anti-Microbial Activity And Phytochemical Constituents Of Some Nigerian Medicinal Plants J. Med. Plants Res., 2009; 3: 1088-1091. View Record In Scopusgoogle Scholar
4. M. Gupta, U.K. Mazumder, P. Gomathievaluation Of Antipyretic And Antinociceptive Activities Of Plumeria Acuminata Leaves J. Med. Sci., 2007; 7: 452-456 Crossrefview Record In Scopusgoogle Scholar
5. Z. Zahee, A.G. Konale, K.A. Patel, Et Al. Comparative Phytochemical Screening Of Flowers Of Plumeria Alba And Pulmeria Rubra Asian J. Pharm. Clin. Res., 2010; 3: 88-89 Google Scholar
6. N. Tohar, M.A. Mohd, I. Jantan, Et Al.A Comparative Study Of The Essential Oils Of The Genus Plumeria Linn. From Malaysia Flavour Fragr. J., 2006; 6: 304-309 Google Scholar

7. N. Tohar, K. Awang, M.A. Mohd, Et Al. Chemical Composition Of The Essential Oils Of Four Plumeria Specials Grown On Peninsular Malayasia J. Essent. Oil Res., 2006; 6: 613-617. Crossrefview Record In Scopusgoogle Scholar
8. Y.Q. Liu, H.W. Wang, S. Wei, Et Al. Chemical Composition And Antimicrobial Activity Of The Essential Oils Extracted By Microwave-Assisted Hydrodistillation From The Flowers Of Two Plumeria Species Anal. Lett., 2012; 45: 2389-2397. Crossrefview Record In Scopusgoogle Scholar
9. N. Techen, I. Parveen, Z.Q. Pan, Et Al.Dna Barcoding Of Medicinal Plant Material For Identification Curr. Opin. Biotechnol., 2014; 25: 103-110. Articledownload Pdfview Record In Scopusgoogle Scholar
10. S.L. Chen, Dna Barcoding Molecular Markers In Chinese Medicinal Materials, People's Medical Publishing House, Beijing, 2012.
11. S.L. Chen, Y. Hui, J.P. Han, Et Al. Validation Of The Its2 Region As A Novel Dna Barcode For Identifying Medicinal Plant Species Plos One, 2010; 5: E8613. Crossrefgoogle Scholar
12. H. Azum, J.G. García-Franco, V. Rico-Gray, Et Al. Molecular Phylogeny Of The Magnoliaceae: The Biogeography Of Tropical And Temperate Disjunctions Am. J. Bot., 2001; 88: 2275-2285. Google Scholar
13. M.B. Hamilton, J.M. Braverman, D.F. Soria-Hernanzpatterns And Relative Rates Of Nucleotide And Insertion/Deletion Evolution At Six Chloroplast Intergenic Regions In New World Species Of The Lecythidaceae Mol. Biol. Evol., 2003; 20: 1710-1721. Crossrefview Record In Scopusgoogle Scholar
14. P.C. Shaw, J. Wang, P.P.H. But, Authentication Of Chinese Medicinal Materials By Dna Technology, World Scientific, Singapore, 2002: 1–23. Google Scholar
15. Z.S. Xie, S.Z. Lam, J.W. Wu, et al. Chemical fingerprint and simultaneous determination of flavonoids in Flos Sophorae Immaturus by HPLC-DAD and HPLC-DAD-ESI-MS/MS combined with chemometrics analysis A nal. Methods, 2014; 6: 4328-4335.