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Identifying Efficiency in Herbal Medicine *Cinnamomum* Species (Lauraceae) Using Banding Patterns and Sequence Alignments of *rpo*B, *rbc*L and *mat*K Regions

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ABSTRACT

Genus *Cinnamomum* is an important plant group containing aromatic oils in its leaves and bark. These oils are widely used in flavoring agents for foods, cosmetic and pharmaceutical preparations, and in other traditional and prepared forms. The *Cinnamomum* diversity in Thailand was investigated and identified in areas where it naturally grows. There were seven known species, seven unknown species, one traditional form, and two prepared forms included in this research. The unknown species which had no reproductive parts were identified firstly by comparing their random amplified polymorphic DNA (RAPD) banding patterns to the known species. The 15 different primers produced a total of 983 bands, which were successfully used to construct a dendrogram that distinctly separates species with genetic similarity values ranging from 0.56 to 0.84 accordingly to show different leaf characters. DNA barcodes with *mat*K, *rbc*L, and *rpo*B gene regions were constructed in all studied samples. Compared to some sequences from GenBank, the genetic distances in the regions were 0.00 to 0.52, 0.00 to 0.36, and 0.00 to 0.30, respectively. Considering these values, some different species showed no genetic distances. To summarize, the three regions cannot be used to identify these *Cinnamomum* species as there are no distinct sequence variations between many different species.

Keywords: *Cinnamomum*, DNA barcode, herbal medicine, RAPD

1. INTRODUCTION

Cinnamomum is an important large genus of evergreen aromatic trees and shrubs belonging to the family Lauraceae. Some *Cinnamomum* species contain aromatic oils in their leaves and bark which are widely used in flavoring agents for food, as well as in cosmetic and pharmaceutical preparations [1]. There are a great number of economically important species, that are used in several of the aforementioned ways, and examples of such species are *C. aromaticum* (cassia oil), *C. camphora* (sassafras and ho leaf oils), *C. bejolghota*, *C. tamala*, *C. zeylanicum* (*C. verum*) and *C. burmannii*. The barks of these plants are often used in pieces and powder form for food flavoring, or distilled for aromatic oils. In Thailand there are about 19 species [2], but the one species used primarily as a flavoring agent is *C. burmannii*, which is available from natural sources only. It's dried bark in both powdered and in stick forms has long been used for flavoring in confectionery, desserts, pastries, pork, chicken and meat, and is also specified in many curry recipes. Consequently, these species may become rare or endangered due to the peeling of their bark. They can survive only in the deep forest, and they can also be grown in national parks under the protection of law enforcement. The other species *C. bejolghota*, *C. tamala*, and *C. zeylanicum* are always used for their barks and as flavoring agents, and more over are important for spices. Many species provide aromatic oils from their roots, bark, leaves, twigs and flowers that are used in several aspects including pharmaceutical, food flavoring, cosmetics, repellent industries [3,4,5,6].

The conservation and sustainability of the *Cinnamomum* species must be progressed by making it a national agenda. The species diversity and their DNA marker construction are the works for the purpose. Many years ago, there were 19 species recorded in the country, however with these problems it is hard to calculate the exact number of species still alive.

Molecular analysis leads to the discovery of invaluable knowledge that is useful for identification, conservation, and sustainability uses. Various molecular approaches, DNA fingerprints, and partial sequences called barcodes are used for the aforementioned purposes. Random amplified polymorphic DNA (RAPD) is one effective method that produces diverse banding patterns as a DNA fingerprint. This is widely used to resolve problems related to the genome which are independent from environmental factors and more numerous than phenotypic characters because it provides a clearer indication of the underlying variation in the genome, as demonstrated in numerous publications. Finger *et al*. [7] studied *Capsicum chinensis* genetic diversity of 49 accessions based on RAPD markers and morphological and agronomic traits; Souza *et al*. [8] used RAPD for genetic diversity evaluation of mango (*Mangifera indica*) germplasm; and Barakat *et al*. [9] indicated that there was genetic differentiation among apricot clones using RAPD technique.

For DNA barcoding, a species-specific marker is essential for further plant part identification, such as with leaves, short trunks, pods, chopped plants or powdered plants. This is especially true in traditional medicines, processed foods, fossil remains, or herbarium specimens which possess highly degraded DNA or a short reliable DNA region. There is much extensive research on DNA barcoding done in plants beginning in 2003 by Dr. Paul D.N. Hebert, a population geneticist at the University of Guelph, Ontario [10]. Since then, there have been many studies testing standard regions in plant groups aiming to provide rapid,

accurate, and automatable species identification by using a standardized DNA region as a tag [11]. Chase *et al*. [12] proposed using two barcoding region options as a standard protocol for barcoding all land plants: the three combined regions of the *rpo*C1, *mat*K, and *psb*A-*trn*H spacer regions, or the *rpo*B, *mat*K and *psb*A-*trn*H spacer regions. Newmaster *et al*. [13] proposed using the *mat*K and *psb*A-*trn*H spacer regions to identify plants in Myristicaceae. Finally, at the Consortium for the Barcode of Life (CBOL), CBOL Plant Working Group [14] recommended *rbc*L + *mat*K as the core DNA barcode regions for land plants. Since then, there have been many publications seeking to determine a suitable standard region affected for a plant group. Monkheang *et al*. [15] used only one region, *psb*A-*trn*H spacer, which became the barcode for the *Senna* species. Kwanda *et al*. [16] carried out the barcode of parasitic plants which included eight species of two families, *Dendrophthoe lanosa*, *D. pentandra*, *Helixanthera parasitica*, *Macrosolen brandisianus*, *M. cochinchinensis* and *Scurrula atropurpurea* of Loranthaceae; *Viscum articulatum* and *V. ovalifolium* of Viscaceae, studying the *rbc*L and *psb*A-*trn*H spacer regions. In the *Cinnamomum* species, *trn*L-*trn*F intergenic spacer and *trn*L intron regions were efficiently used to construct barcodes for identification in *C. burmannii*, *C. cassia*, *C. sieboldii*, and *C. zeylanicum*. The research indicated that the standardized DNA regions were used as tags for further plant identification in processed medicine [17]. Moreover, in 2007, there was research done using ITS2 region for identification of medicinal herbs as traditional Chinese drugs for treating many diseases. The herbs were 55 processed medicinal herbs belonging to 48 families [18].

This study aimed to investigate the species diversity of *Cinnamomum* used in traditional medicines, flavoring agents for foods, and distilled for aromatic oils in Thailand. Thai traditional medicines are always available to patients in dried piece sets, as a powder, or chopped plant parts, and each form should have molecular markers for plant sample testing. As short standardized chloroplast DNA is efficient for standard sequences, the *rbc*L and *rpo*B regions were selected and used as tag sequences for this study. They were deposited in the GenBank database as species-specific markers to be used for further rapid, accurate, and automatable species identification.

2. MATERIALS AND METHODS

2.1 Plant Material

Cinnamomum species were explored and collected in many provinces of northeastern and northern Thailand in 2010-2011. They were identified following Chayamarit [19] and Xiwen *et al*. [20]. Young leaves were collected and immediately kept at -20°C for DNA extraction. All collected samples underwent DNA extraction, DNA fingerprinting and DNA barcoding. Additionally, the dried pieces of bark for *Cinnamomum* sp. 8 and the prepared capsules of *Cinnamomum* sp. 9 and *Cinnamomum* sp. 10 (Figure 1) were kept for *Cinnamomum* species testing.

2.2 DNA Extraction

Genomic DNA was extracted from all collected samples using the Qiagen DNeasy mini-kit. Extracted DNA was examined by subjecting it to 0.8% agarose gel electrophoresis stained with ethidium bromide. The quality and quantity of DNA were determined with a gel documenting instrument. Then DNA samples were diluted to a final

Cinnamomum sp.8 *Cinnamomum* sp.9 *Cinnamomum* sp.10

Figure 1. Dried bark pieces of *Cinnamomum* sp. 8 and the prepared capsules of *Cinnamomum* sp. 9 and *Cinnamomum* sp. 10.

concentration of 20 ng/μl in TE (10 mM Tris, 1 mM EDTA), and these dilutions were used as a DNA template in the PCR reaction.

2.3 DNA Fingerprinting by RAPD Marker and Dendrogram Construction for Unknown Species Identification and Comparing It to Known Species

Amplifications were carried out on all *Cinnamomum* samples studied, including known and unknown species, except for traditional and prepared forms. The samples were each examined in 25 μl reactions consisting of GoTaq Green Master Mix (Promega), 0.5 μM primer and a 10 ng DNA template. Sixty six RAPD primers were screened and the 15 primers that successfully amplified clear bands are as follows (5′ to 3′): TGCCGAGCTG, AATCGGGCTG,GGGTAACGCC, CAATCGCCGT,GTTGCGATCC, CAAACGTCGG,GTCCCGACGA, TCAGTCCGGG,CACCATCCGT, TCCCAACAAC,CCCCAACAAC, CCCAAGGTCC,ACCGCCTGCT, AGCGAGCAAG and AGGCCCGATG. The reaction mixture was incubated at 94°C for 3 min and the amplification was performed with the following 35 thermal cycles of denaturation for 1 min at 94°C, annealing for 2 min at 40°C, extension for 2 min at 72°C, and then final extension for 7 min at 72°C using a Swift Maxi Thermal Cycler (Esco Micro Pte. Ltd.). Amplification products were detected by 1.2% agarose gel electrophoresis in a TAE buffer and visualized using ethidium bromide staining. The resulting RAPD bands were used to construct a dendrogram using the NTSYSpc 2.1 software [21]. Identification of the unknown species was compared to the known species using the results from the constructed dendrogram.

2.4 DNA Barcode Amplification and Sequence Analysis for Identification of Unknown or Traditional and Prepared Forms

DNA barcoding construction was performed in all studied samples including known species, unknown species, and traditional and prepared forms.

PCR was performed using primer pairs (5′ to 3′) ATCCATCTGGAAATCTTAGT TC and CTTCCTCTGTAAAGAATTC of *mat*K gene region, ATGCAACGTCAAG CAGTTCC and ATCCCAGCATCACAAT TCC of *rpo*B gene region, and ATGT CACCACAAACAGAGACTAAAGC and GTAAAATCAAGTCCACCRCG of *rbc*L gene region (http://www.kew.org/barcodin/update.html; 28 January 2009). The reaction mixture was done in 30 μl consisting of GoTaq Green Master Mix (Promega), 0.25 μM of each primer, and 10 ng DNA template. The reaction mixture was incubated at 94°C for 1 min and the amplification was performed with the following 35 thermal cycles: denaturation

for 30 s at 94°C, annealing for 40 s at 53°C, extension for 40 s at 72°C, and then final extension for 5 min at 72°C. The amplified products were detected by 1.2% agarose gel electrophoresis in a TAE buffer and visualized with ethidium bromide staining. The amplified specific fragments of the studied samples were sequenced and the sequences were submitted to the GenBank database.

All the sequences of the plants in the research and sequences of some species quoted by Smitinand and Larsen [2] brought from GenBank database were compared (sequence alignment) using MEGA5 [22] indicating genetic distances for identification of unknown and traditional and prepared forms.

3. RESULTS AND DISCUSSION

The samples collected were identified through morphological characters and revealed seven species, namely *C. aromaticum* Nees, *C. bejolghota* (Buch.-Ham.) Sweet, *C. camphora* (L.) J.Presl, *C. iners* Reinw. ex. Blume, *C. loureiroi* Nees, *C. parthenoxylon* (Jack) Meisn. and *C. verum* J.Presl, and seven unknown species, *Cinnamomum* sp. 1-7, that lacked reproductive parts. The plant *C. loureiroi* was not contained in the Smitinand and Larsen 1972's lists [2], probably because the plant was unexplored at that time.

The 15 different polymorphism primers produced a total of 983 bands, 288 characters ranging in sizes from 300 to 2,500 bp. Examples of banding patterns are shown in Figure 2. The total RAPD data was successfully used to construct genetic similarity values and a dendrogram (Figure 3) that distinctly separates each species on a different branch. Additionally, genetic similarity values, and the highest value of 0.84 belonged to a pair, *C. verum* and *C. iners*, as well as the lowest similarity value of 0.56 between *C. camphora* and *Cinnamomum* sp. 1 are shown in Table 1.

Molecular markers using DNA barcodes with *rpo*B, *rbc*L and *mat*K regions were constructed in all studied samples, including the seven known species *C. aromaticum*, *C. bejolghota*, *C. camphora*, *C. iners*, *C. loureiroi*, *C. parthenoxylon* and *C. verum*, seven unknown species, unknown 1 to unknown 7 and traditional and prepared samples of *Cinnamomum* sp.

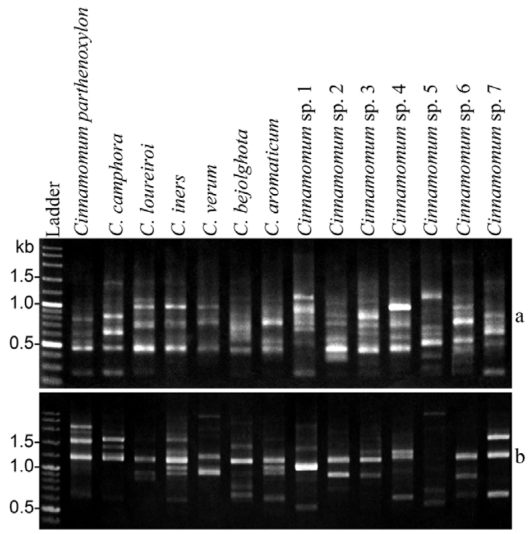

Figure 2. Examples of RAPD fingerprints of all plant samples studied from primers (a) TCCCAACAAC and (b) GGGTAACGCC.

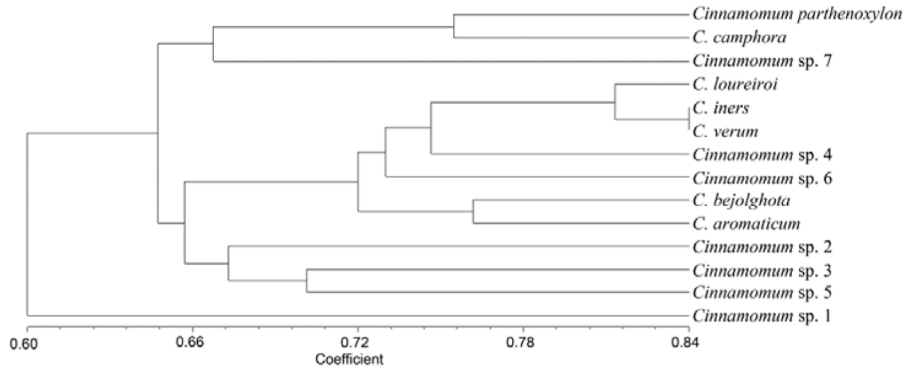

Figure 3. The dendrogram of the seven known and seven unknown species constructed from 15 RAPD primers produced totally 983 bands by the NTSYSpc 2.10p.

Table 1. Similarity coefficient (S) of seven known and unknown species analyzed by RAPD fingerprint data from 15 primers.

	C. parthenoxylon	*C. camphora*	*C. loureiroi*	*C. iners*	*C. verum*	*C. bejolghota*	*C. aromaticum*	*Cinnamomum* sp.1	*Cinnamomum* sp.2	*Cinnamomum* sp.3	*Cinnamomum* sp.4	*Cinnamomum* sp.5	*Cinnamomum* sp.6	*Cinnamomum* sp.7
Cinnamomum parthenoxylon	1.00													
C. camphora	0.75	1.00												
C. loureiroi	0.64	0.65	1.00											
C. iners	0.63	0.61	0.82	1.00										
C. verum	0.66	0.65	0.80	0.84	1.00									
C. bejolghota	0.66	0.62	0.72	0.69	0.73	1.00								
C. aromaticum	0.69	0.60	0.72	0.73	0.76	0.76	1.00							
Cinnamomum sp.1	0.59	0.56	0.59	0.57	0.63	0.63	0.63	1.00						
Cinnamomum sp.2	0.66	0.66	0.63	0.58	0.66	0.68	0.67	0.63	1.00					
Cinnamomum sp.3	0.66	0.62	0.69	0.66	0.67	0.74	0.68	0.62	0.69	1.00				
Cinnamomum sp.4	0.68	0.65	0.76	0.72	0.75	0.74	0.70	0.63	0.70	0.73	1.00			
Cinnamomum sp.5	0.63	0.60	0.62	0.57	0.62	0.66	0.66	0.60	0.66	0.70	0.63	1.00		
Cinnamomum sp.6	0.66	0.60	0.73	0.69	0.77	0.68	0.73	0.59	0.64	0.70	0.72	0.64	1.00	
Cinnamomum sp.7	0.68	0.66	0.65	0.62	0.66	0.72	0.67	0.58	0.65	0.65	0.66	0.66	0.69	1.00

8-9. The barcode fragments were successfully amplified in all but the traditional and prepared samples, *Cinnamomum* sp. 8-9 with *mat*K region. Consequently the results are lacking these sequence alignments.

The Thailand *Cinnamomum* barcode sequences, with asterisks, were compared

through MEGA5 with some sequences of identical species from the GenBank database: three species of the *rpo*B region, *C. bejolghota*, *C. camphora*, and *C. verum*; six species of the *rbc*L region, *C. aromaticum*, *C. bejolghota*, *C. burmannii*, *C. camphora*, *C. subavenium*, and *C. verum*, and one species, *C. parthenoxylon* of the *mat*K region. The results were shown by dendrograms and tables of genetic distances of each gene region (Figures 4-6 and Tables 2-4). The genetic distances of each pair species in the *rpo*B region are 0.00 in the species group of *C. parthenoxylon* JQ410177*, *C. camphora* JQ410178*, EF590433.1, EU153889.1, *C. loureiroi* JQ410179*, *C. verum* EU153895.1, EF590434.1, *C. bejolghota* GQ248745.1, EU153891.1, *C. aromaticum* JQ410183*, *Cinnamomum* sp. 3 to 0.30 in *Cinnamomum* sp. 9* and *Cinnamomum* sp. 5*.

The genetic distances of each pair species in the *rbc*L region are 0.00 in the species group of *C. camphora* HQ427259.1, GQ436752.1, *C. verum* JX843243*, JX414039.1, *C. bejolghota* GQ248569.1, *C. aromaticum* JX843245*, JF950023.2, HM019459.1, *C. burmannii* HM019455.1, HM019454.1, *C. subavenium* HQ427266.1, *Cinnamomum* sp. 8* to 0.36 in *Cinnamomum* sp. 10* and *Cinnamomum* sp. 6*.

The genetic distances of each pair species in the *mat*K region are in the species group of 0.00 in *C. parthenoxylon* GQ434288.1, *C. camphora* HM019317.1, HM019316.1, *C. verum* JX414035.1, EF590398.1, *C. bejolghota* JQ435503*, GQ248098.1, EU153831.1, *C. aromaticum* GQ434289.1, HM019318.1, *C. burmannii* HM019314.1, HM019315.11, GQ434290.1, *C. subavenium* HQ427408.1 to 0.52 in *Cinnamomum* sp. 5* and *C. verum*

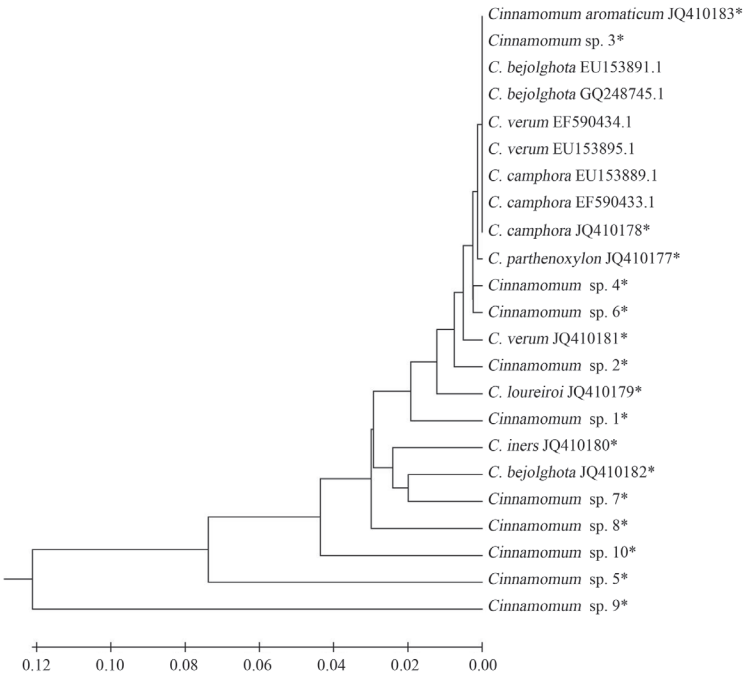

Figure 4. Dendrogram constructed from *rpo*B sequences of the seven known and unknown Thailand species with asterisks and the others species from GenBank database.

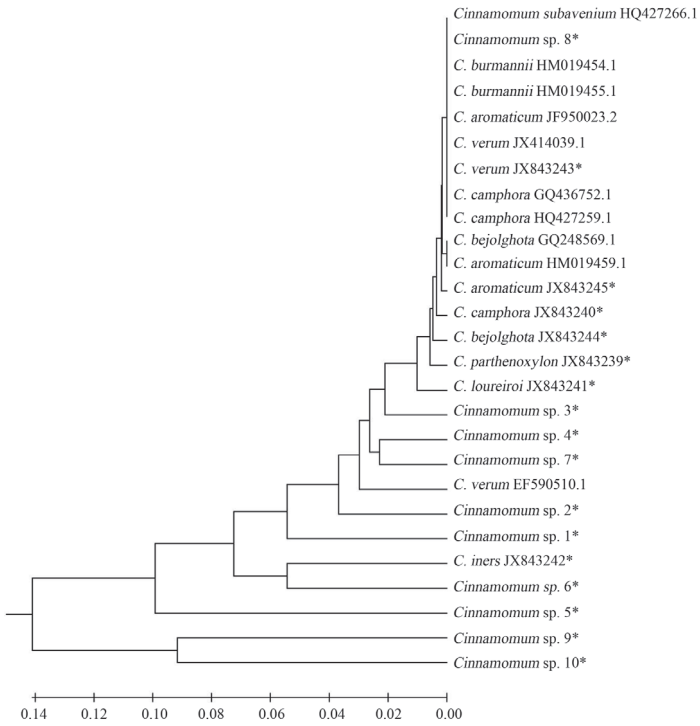

Figure 5. Dendrogram constructed from *rbc*L sequences of the seven known and unknown Thailand species with asterisks and the others species from GenBank database.

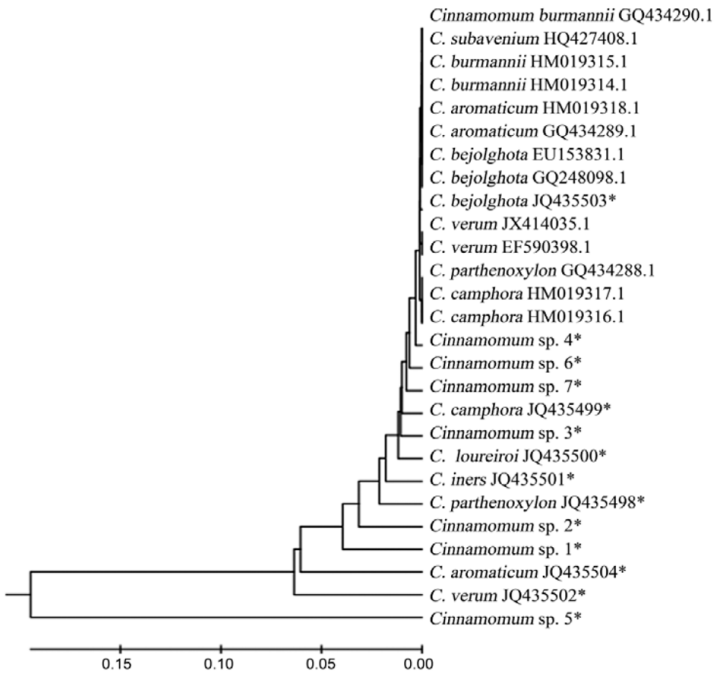

Figure 6. Dendrogram constructed from *mat*K sequences of the Thailand species with asterisks and the others species from GenBank database.

Table 2. Sequence variations constructed from *rpo*B sequences of the Thailand species with asterisks, unknown species, traditional and prepared medicinal forms and the others species from GenBank database.

	C. parthenoxylon JQ410177*	*C. camphora* JQ410178*	*C. camphora* EF590433.1	*C. camphora* EU153889.1	*C. loureiroi* JQ410179*	*C. iners* JQ410180*	*C. verum* JQ410181*	*C. verum* EU153895.1	*C. verum* EF590434.1	*C. bejolghota* JQ410182*	*C. bejolghota* GQ248745.1	*C. bejolghota* EU153891.1	*C. aromaticum* JQ410183*	*Cinnamomum* sp. 1*	*Cinnamomum* sp. 2*	*Cinnamomum* sp. 3*	*Cinnamomum* sp. 4*	*Cinnamomum* sp. 5*	*Cinnamomum* sp. 6*	*Cinnamomum* sp. 7*	*Cinnamomum* sp. 8*	*Cinnamomum* sp. 9*	*Cinnamomum* sp. 10*
Cinnamomum parthenoxylon JQ410177*	0.00																						
C. camphora JQ410178*	0.00	0.00																					
C. camphora EF590433.1	0.00	0.00	0.00																				
C. camphora EU153889.1	0.00	0.00	0.00	0.00																			
C. loureiroi JQ410179*	0.00	0.02	0.02	0.02	0.00																		
C. iners JQ410180*	0.05	0.05	0.05	0.05	0.05	0.00																	
C. verum JQ410181*	0.01	0.01	0.01	0.01	0.03	0.05	0.00																
C. verum EU153895.1	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00															
C. verum EF590434.1	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00	0.00														
C. bejolghota JQ410182*	0.05	0.06	0.06	0.06	0.04	0.05	0.06	0.06	0.06	0.00													
C. bejolghota GQ248745.1	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00	0.00	0.06	0.00												
C. bejolghota EU153891.1	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00	0.00	0.06	0.00	0.00											
C. aromaticum JQ410183*	0.00	0.00	0.00	0.00	0.02	0.05	0.01	0.00	0.00	0.06	0.00	0.00	0.00										
Cinnamomum sp. 1*	0.04	0.04	0.04	0.04	0.06	0.09	0.05	0.04	0.04	0.09	0.04	0.04	0.04	0.00									
Cinnamomum sp. 2*	0.02	0.01	0.01	0.01	0.04	0.05	0.01	0.01	0.01	0.06	0.01	0.01	0.01	0.05	0.00								
Cinnamomum sp. 3*	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.00	0.00	0.06	0.00	0.00	0.00	0.04	0.01	0.00							
Cinnamomum sp. 4*	0.01	0.01	0.01	0.01	0.03	0.05	0.01	0.01	0.01	0.06	0.01	0.01	0.01	0.04	0.01	0.01	0.00						
Cinnamomum sp. 5*	0.14	0.14	0.14	0.14	0.16	0.19	0.14	0.14	0.14	0.20	0.14	0.14	0.14	0.14	0.13	0.14	0.14	0.00					
Cinnamomum sp. 6*	0.01	0.01	0.01	0.01	0.03	0.06	0.02	0.01	0.01	0.06	0.01	0.01	0.01	0.04	0.02	0.01	0.01	0.14	0.00				
Cinnamomum sp. 7*	0.06	0.06	0.06	0.06	0.05	0.05	0.07	0.06	0.06	0.04	0.06	0.06	0.06	0.09	0.07	0.06	0.06	0.20	0.06	0.00			
Cinnamomum sp. 8*	0.06	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0.06	0.07	0.06	0.06	0.06	0.09	0.06	0.06	0.06	0.17	0.05	0.07	0.00		
Cinnamomum sp. 9*	0.24	0.24	0.24	0.24	0.23	0.26	0.24	0.24	0.24	0.26	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.30	0.24	0.25	0.24	0.00	
Cinnamomum sp. 10*	0.07	0.07	0.07	0.07	0.10	0.13	0.08	0.07	0.07	0.13	0.07	0.07	0.07	0.09	0.08	0.07	0.07	0.15	0.07	0.13	0.13	0.23	0.00

Table 3. Sequence variations constructed from *rbc*L sequences of the Thailand species with asterisks, unknown species, traditional and prepared medicinal forms and the others species from GenBank database.

	C. parthenoxylon JX843239*	*C. camphora* JX843240*	*C. camphora* HQ427259.1	*C. camphora* GQ436752.1	*C. loureiroi* JX843241*	*C. iners* JX843242*	*C. verum* JX843243*	*C. verum* JX414039.1	*C. verum* EF590510.1	*C. bejolghota* JX843244*	*C. bejolghota* GQ248569.1	*C. aromaticum* JX843245*	*C. aromaticum* JF950023.2	*C. aromaticum* HM019459.1	*C. burmannii* HM019455.1	*C. burmannii* HM019454.1	*C. subavenium* HQ427266.1	*Cinnamomum* sp.1*	*Cinnamomum* sp.2*	*Cinnamomum* sp.3*	*Cinnamomum* sp.4*	*Cinnamomum* sp.5*	*Cinnamomum* sp.6*	*Cinnamomum* sp.7*	*Cinnamomum* sp.8*	*Cinnamomum* sp.9*	*Cinnamomum* sp.10*
Cinnamomum parthenoxylon JX843239*	0.00																										
C. camphora JX843240*	0.02	0.00																									
C. camphora HQ427259.1	0.01	0.01	0.00																								
C. camphora GQ436752.1	0.01	0.01	0.00	0.00																							
C. loureiroi JX843241*	0.02	0.03	0.02	0.02	0.00																						
C. iners JX843242*	0.12	0.12	0.11	0.11	0.12	0.00																					
C. verum JX843243*	0.01	0.01	0.00	0.00	0.02	0.11	0.00																				
C. verum JX414039.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00																			
C. verum EF590510.1	0.06	0.06	0.05	0.05	0.07	0.16	0.05	0.05	0.00																		
C. bejolghota JX843244*	0.02	0.02	0.01	0.01	0.03	0.11	0.01	0.01	0.06	0.00																	
C. bejolghota GQ248569.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00																
C. bejolghota JX843245*	0.01	0.01	0.00	0.00	0.02	0.10	0.00	0.00	0.05	0.01	0.01	0.00															
C. aromaticum JF950023.2	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.00	0.00														
C. aromaticum HM019459.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.01	0.00	0.00													
C. burmannii HM019455.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00												
C. burmannii HM019454.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00											
C. subavenium HQ427266.1	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00										

Table 3. Continued

	C. parthenoxylon JX843239*	*C. camphora* JX843240*	*C. camphora* HQ427259.1	*C. camphora* GQ436752.1	*C. loureiroi* JX843241*	*C. iners* JX843242*	*C. verum* JX843243*	*C. verum* JX414039.1	*C. verum* EF590510.1	*C. bejolghota* JX843244*	*C. bejolghota* GQ248569.1	*C. aromaticum* JX843245*	*C. aromaticum* JF950023.2	*C. aromaticum* HM019459.1	*C. burmannii* HM019455.1	*C. burmannii* HM019454.1	*C. subavenium* HQ427266.1	*Cinnamomum* sp. 1*	*Cinnamomum* sp. 2*	*Cinnamomum* sp. 3*	*Cinnamomum* sp. 4*	*Cinnamomum* sp. 5*	*Cinnamomum* sp. 6*	*Cinnamomum* sp. 7*	*Cinnamomum* sp. 8*	*Cinnamomum* sp. 9*	*Cinnamomum* sp. 10*
Cinnamomum sp. 1*	0.11	0.11	0.11	0.11	0.12	0.17	0.11	0.11	0.14	0.12	0.11	0.11	0.11	0.11	0.11	0.11	0.00										
Cinnamomum sp. 2*	0.07	0.07	0.07	0.07	0.09	0.13	0.07	0.07	0.12	0.08	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.00								
Cinnamomum sp. 3*	0.05	0.05	0.04	0.04	0.05	0.13	0.04	0.04	0.09	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.11	0.08	0.00							
Cinnamomum sp. 4*	0.06	0.06	0.05	0.05	0.07	0.12	0.05	0.05	0.10	0.06	0.06	0.06	0.05	0.06	0.05	0.05	0.05	0.11	0.06	0.04	0.00						
Cinnamomum sp. 5*	0.20	0.20	0.19	0.19	0.19	0.25	0.19	0.19	0.22	0.20	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.20	0.18	0.19	0.20	0.00					
Cinnamomum sp. 6*	0.17	0.17	0.17	0.17	0.17	0.11	0.17	0.17	0.23	0.17	0.17	0.16	0.17	0.17	0.17	0.17	0.17	0.21	0.18	0.16	0.16	0.30	0.00				
Cinnamomum sp. 7*	0.06	0.05	0.05	0.05	0.07	0.13	0.05	0.05	0.10	0.06	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.12	0.05	0.06	0.05	0.18	0.17	0.00			
Cinnamomum sp. 8*	0.01	0.01	0.00	0.00	0.02	0.11	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.07	0.04	0.05	0.19	0.17	0.05	0.00		
Cinnamomum sp. 9*	0.27	0.27	0.27	0.27	0.26	0.29	0.27	0.27	0.32	0.26	0.26	0.27	0.27	0.26	0.27	0.27	0.27	0.28	0.26	0.28	0.27	0.31	0.35	0.27	0.27	0.00	
Cinnamomum sp. 10*	0.27	0.28	0.28	0.28	0.28	0.29	0.28	0.28	0.31	0.29	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.30	0.29	0.29	0.30	0.32	0.36	0.29	0.28	0.18	0.00

Table 4. Sequence variations constructed from *mat*K sequences of the Thailand species with asterisks, unknown species, traditional and prepared medicinal forms and the others species from GenBank database.

	C. parthenoxylon JQ435498*	*C. parthenoxylon* GQ434288.1	*C. camphora* JQ435499*	*C. camphora* HM019317.1	*C. camphora* HM019316.1	*C. loureiroi* JQ435500*	*C. iners* JQ435501*	*C. verum* JQ435502*	*C. verum* JX414035.1	*C. verum* EF590398.1	*C. bejolghota* JQ435503*	*C. bejolghota* GQ248098.1	*C. bejolghota* EU153831.1	*C. aromaticum* JQ435504*	*C. aromaticum* GQ434289.1	*C. aromaticum* HM019318.1	*C. burmannii* HM019314.1	*C. burmannii* HM019315.1	*C. burmannii* GQ434290.1	*C. subavenium* HQ427408.1	*Cinnamomum* sp. 1*	*Cinnamomum* sp. 2*	*Cinnamomum* sp. 3*	*Cinnamomum* sp. 4*	*Cinnamomum* sp. 5*	*Cinnamomum* sp. 6*	*Cinnamomum* sp. 7*
Cinnamomum parthenoxylon JQ435498*	0.00																										
C. parthenoxylon GQ434288.1	0.04	0.00																									
C. camphora JQ435499*	0.04	0.02	0.00																								
C. camphora HM019317.1	0.04	0.00	0.02	0.00																							
C. camphora HM019316.1	0.04	0.00	0.02	0.00	0.00																						
C. loureiroi JQ435500*	0.06	0.02	0.04	0.02	0.02	0.00																					
C. iners JQ435501*	0.06	0.04	0.04	0.04	0.04	0.05	0.00																				
C. verum JQ435502*	0.14	0.11	0.13	0.11	0.11	0.14	0.15	0.00																			
C. verum JX414035.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00																		
C. verum EF590398.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00																	
C. bejolghota JQ435503*	0.04	0.00	0.02	0.00	0.00	0.02	0.04	0.11	0.00	0.00	0.00																
C. bejolghota GQ248098.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00															
C. bejolghota EU153831.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00														
C. aromaticum JQ435504*	0.14	0.11	0.11	0.11	0.11	0.12	0.13	0.21	0.11	0.11	0.11	0.11	0.11	0.00													
C. aromaticum GQ434289.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00												
C. aromaticum HM019318.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00											
C. burmannii HQ019314.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00										
C. burmannii HQ019315.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00									
C. burmannii GQ434290.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00								
C. subavenium HQ427408.1	0.04	0.00	0.02	0.00	0.00	0.02	0.03	0.11	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00							
Cinnamomum sp. 1*	0.11	0.07	0.09	0.07	0.07	0.09	0.10	0.19	0.07	0.07	0.07	0.07	0.07	0.19	0.07	0.07	0.07	0.07	0.07	0.07	0.00						
Cinnamomum sp. 2*	0.09	0.06	0.07	0.06	0.06	0.08	0.08	0.18	0.06	0.06	0.06	0.06	0.06	0.14	0.06	0.06	0.06	0.06	0.06	0.06	0.13	0.00					
Cinnamomum sp. 3*	0.05	0.02	0.03	0.02	0.02	0.03	0.04	0.13	0.02	0.02	0.02	0.02	0.02	0.12	0.02	0.02	0.02	0.02	0.02	0.02	0.09	0.07	0.00				
Cinnamomum sp. 4*	0.04	0.01	0.02	0.01	0.01	0.02	0.03	0.12	0.00	0.00	0.01	0.01	0.01	0.12	0.01	0.01	0.01	0.01	0.01	0.01	0.07	0.06	0.02	0.00			
Cinnamomum sp. 5*	0.41	0.36	0.38	0.36	0.36	0.40	0.40	0.52	0.37	0.37	0.37	0.37	0.37	0.55	0.37	0.37	0.37	0.37	0.37	0.37	0.45	0.39	0.37	0.00			
Cinnamomum sp. 6*	0.04	0.01	0.03	0.01	0.01	0.03	0.04	0.12	0.01	0.01	0.01	0.01	0.01	0.13	0.01	0.01	0.01	0.01	0.01	0.01	0.08	0.07	0.03	0.01	0.38	0.00	
Cinnamomum sp. 7*	0.06	0.01	0.03	0.01	0.01	0.04	0.05	0.13	0.02	0.02	0.02	0.01	0.01	0.13	0.01	0.01	0.01	0.01	0.01	0.01	0.09	0.07	0.03	0.02	0.38	0.03	0.00

JQ435502*.

The voucher specimen numbers and the barcode tag sequences of the known Thailand species are kept at GenBank with the accession numbers shown in the Table 5. Accession numbers of sequences

reported in GenBank that are included in the analysis are listed in Table 6.

Generally the *Cinnamomum* species diversity of Thailand has been expected to have 19 species, but the plants are disturbed by unsustainable uses, habitat destruction, and environmental change due to global warming, which are all contributing to its number loss. There are four economically popular trade species, *C. burmannii*, *C. tamala*, *C. bejolghota*, and *C. verum*, the latter two of which were examined in this study. The most popularly used species, *C. burmannii*, can presently be found only in the jungle. Thailand often uses the bark of these species for spices, flavoring agents, traditional and prepared forms medicine, and for distillation into aromatic oils. When bark, including phloem is peeled out, the plants generally die. Therefore, these species should be seriously planted, coupled with studying how to use the less vital leaves as a substitute.

The unknown species lacking reproductive parts were identified first by comparing the RAPD banding patterns to the seven known species that were to be identified. The dendrogram (Figure 3),

Table 5. *Cinnamomum* species with *rpo*B, *rbc*L and *mat*K barcode regions and GenBank accession numbers.

Voucher specimen number	Species	GenBank accession number		
		*rpo*B	*rbc*L	*mat*K
A. Chaveerach 737	*Cinnamomum parthenoxylon*	JQ410177	JX843239	JQ435498
A. Chaveerach 738	*C. camphora*	JQ410178	JX843240	JQ435499
A. Chaveerach 739	*C. loureiroi*	JQ410179	JX843241	JQ435500
A. Chaveerach 740	*C. iners*	JQ410180	JX843242	JQ435501
A. Chaveerach 741	*C. verum*	JQ410181	JX843243	JQ435502
A. Chaveerach 742	*C. bejolghota*	JQ410182	JX843244	JQ435503
A. Chaveerach 743	*C. aromaticum*	JQ410183	JX843245	JQ435504

Table 6. Accession numbers and references for the sequences from GenBank included in this research.

Species	Accession number of each gene with reference in brackets		
	*rpo*B	*rbc*L	*mat*K
Cissus aromaticum		HM019459.1 [26]	GQ434289.1 [28]
		JF950023.2 [27]	HM019318.1 [26]
C. bejolghota	EU153891.1 [24]	GQ248569.1 [14]	EU153831.1 [24]
	GQ248745.1 [14]		GQ248098.1 [14]
C. burmannii		HM019454.1 [26]	GQ434290.1 [28]
		HM019455.1 [26]	HM019314.1 [26]
			HM019315.1 [26]
C. camphora	EF590433.1 [25]	GQ436752.1 [28]	HM019316.1 [26]
	EU153889.1 [24]	HQ427259.1 [29]	HM019317.1 [26]
C. parthenoxylon			GQ434288.1 [28]
C. subavenium		HQ427266.1 [29]	HQ427408.1 [29]
C. verum	EF590434.1 [25]	EF590510.1 [25]	EF590398.1 [25]
	EU153895.1 [24]	JX414039.1 [30]	JX414035.1 [30]

showing monophyletic groups by common ancestors and polyphyletic groups, coupled with the S in the Table 1 starting from the highest value 0.84 of a *C. verum* and *C. iners* to the lowest value 0.56 of *C. camphora* and *Cinnamomum* sp. 1, indicates that there is no identical species between the known and unknown species of this study. Accordingly, all seven unknown and known species showed the greatest difference in leaf characters. Although the S of 0.84 of *C. verum* and *C. iners* is high, the leaf characters of these two species are different, as quoted above. Ultimately, there were at least 14 species in Thailand commonly grown at national parks, the University's farms, and domestic gardens collected for this research.

Plant parts in dried form have not been used in RAPD studies or other fingerprinting methods, due to shearing DNA obtained through the DNA extraction process. In the case of DNA fingerprinting profiles, DNA fragment products may be pieces of significance. Amplification in chloroplast or mitochondrial DNA partial regions can be successfully performed as a barcode marker because of their short sequences and many copies. Consequently, shearing DNA from dried traditional, processed, or prepared medicinal forms can be used for plant identification with DNA barcode method.

As Thai *Cinnamomum* traditional medicines are always available to patients in dried piece sets, powder, and the other prepared forms (examples shown in the Figure 1), the method to be efficiently used for automatable, accurate and rapid identification was constructed using the *rpo*B and *rbc*L gene regions, aiming at identification of seven unknown fresh species and traditional and prepared forms claiming that they are *Cinnamomum* plants. The seven known and unknown Thailand species sequences as well as the traditional form and prepared form sequences were aligned to some *rpo*B, *rbc*L and *mat*K species sequences one to three individuals as far as possible from GenBank database. These species were as follows: *C. bejolghota*, *C. camphora*, *C. verum* in *rpo*B; *C. aromaticum*, *C. bejolghota*, *C. burmannii*, *C. camphora*, *C. subavenium*, *C. verum* in *rbc*L; and *C. aromaticum*, *C. bejolghota*, *C. burmannii*, *C. camphora*, *C. parthenoxylon*, *C. subavenium*, *C. verum* in *mat*K. Other species found to be indigenous in Thailand [2], included *C. crenulicupulum*, *C. deschampsii*, *C. glaucescens*, *C. ilicioides*, *C. kerrii*, *C. mollissimum*, *C. puberulum*, *C. rhynchophyllum*, *C. sintoc*, *C. tamala*, and *C. tavoyanum*, have not been provided with the two same gene regions in the GenBank. The genetic distances of each pair species in *rpo*B region are 0.00 in *C. verum*, *C. camphora*, *C. bejolghota*, *C. parthenoxylon*, *C. aromaticum*, *Cinnamomum* sp. 3, *C. loureiroi* to 0.30 between *Cinnamomum* sp. 9 and *Cinnamomum* sp. 5. The genetic distances of each pair species in *rbc*L region are of 0.00 in *C. bejolghota*, *C. aromaticum*, *C. burmannii*, *C. subavenium*, *C. camphora*, *C. verum*, *Cinnamomum* sp. 8 to 0.36 between *Cinnamomum* sp. 10 and *Cinnamomum* sp. 6. The genetic distances of each pair species in the *mat*K region are of 0.00 in *C. parthenoxylon*, *C. camphora*, *C. verum*, *C. bejolghota*, *C. aromaticum*, *C. burmannii*, *C. subavenium* to 0.52 between *Cinnamomum* sp. 5* and *C. verum*. The result shows that, many different species both from GenBank database and Thailand have no nucleotide variations leading to not to be used as standard to identify unknown species.

The regions, *trn*L-*trn*F intergenic spacer and *trn*L intron regions efficiently used to construct barcodes for

identification in *C. cassia*, *C. zeylanicum*, *C. burmannii* and *C. sieboldii* species *C. cassia*, *C. zeylanicum*, *C. burmannii* and *C. sieboldii* by Kojoma et al. [17]. should be selected for further study in this plant group, however, the regions tested on only a small number species may lead to changing the results with a high number of species. Additionally, the ITS2 region is also powerful for 55 processed medicinal herbs belonging to 48 families, and the identification and authentication of five sets of easily confusable Chinese herbal materials studied by Chiou *et al.* [18]. However, the consideration point is that these processed medicinal herbs are in a different species and have dominantly distinguishing morphological characters which are an expressed part in the genome. Accordingly, the standard sequences as their barcode suggests, should have nucleotide variations enough to separate 48 families agreeing with the research by Taberlet *et al.* [23] which has used the chloroplast *rbc*L gene, showing in most cases the identication of families, not the genera or species.

4. CONCLUSIONS

In summary, the three regions, *rpo*B, *rbc*L and *mat*K cannot be used to identify the *Cinnamomum* species group. This is due to the fact that there are no sequence variations, as indicated above, between the different and many species. Consequently Thailand *Cinnamomum* sp. 3 cannot be assumed to be *C. parthenoxylon* or *C. aromaticum* using the *rpo*B barcode. *Cinnamomum* sp. 9 and *Cinnamomum* sp. 5 are different species with a nucleotide variation of 0.30. For the same reasons *Cinnamomum* sp. 8 cannot be assumed to be *C. bejolghota* or *C. aromaticum* or *C. burmannii* or *C. subavenium* or *C. camphora* or *C. verum*. Thailand species *Cinnamomum* sp. 10 and *Cinnamomum* sp. 6 are different species with a high nucleotide variation of 0.36. *Cinnamomum* sp. is a different species from *C. verum* with a high nucleotide variation of 0.52.

From this research, the seven unknown species may be the expected Thailand species in addition to Smittinand and Larsen 1972's list. The unknown samples from the traditional form, *Cinnamomum* sp. 8, prepared forms, *Cinnamomum* sp. 9 and *Cinnamomum* sp. 10 are not any species known to exist in Thailand or abroad. The study on species diversity in Thailand should be completed leading to barcode species identification, allowing the right indigenous medicine to be used for the right diseases. Additionally, the other regions for barcode constructions in the *Cinnamomum* group should be examined.

Additionally, Thai people should realize the importance of species diversity, conservation and sustainable uses and not continue to kill existing species. This is especially true for a case such as *C. burmannii*, which is widely used in Thailand, but cannot be found in general areas such as public places and domestic farms. This species can only be found in the jungle, and may be endangered due to its heavy use and minimal natural habitat.

REFERENCES

[1] European Medicines Agency, Assessment report on *Cinnamomum verum* cortex and cortices aetheroleum. EMA/HMPC/246773/2009, accessed 2012, available from http:// www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2011/08/WC500110090.pdf.

[2] Smitinand T. and Larsen K., *Flora of Thailand volume 2 part 2*, Thailand

Institute of Scientific and Technological Research, Bangkok, 1972.

[3] Siripattanadilok S., 2003. Thai *Cinnamomum* (*C. burmannii* Bl.). http://www.ku.ac.th/e-magazine/april46/agri/plant.html.

[4] Ho C.L., Wang E.I.C. and Su Y.C., Essential oil compositions and bioactivities of the various parts of *Cinnamomum camphora* Sieb. var. *linaloolifera* Fujuta, *The Forestry Research Quarterly Journal*, 2009; **31:** 77-96.

[5] Orwa C., Mutua A., Kindt R., Jamnadass R. and Simons A., 2009. Agroforestree Database: A tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/).

[6] Kumanan R., Manimaran S., Saleemulla K., Dhanabal S.P. and Nanjan M.J., Screening of bark of *Cinnamomum tamala* (Lauraceae) by using α-amylase inhibition assay for anti-diabetic activity, *Int. J. Pharm. Biomed. Res.*, 2010; **1:** 69-72.

[7] Finger F.L., Lannes S.D., Schuelter A.R., Doege J., Comerlato A.P., Goncalves L.S.A., Ferreira F.R.A., Clovis L.R. and Scapim C.A., Genetic diversity of *Capsicum chinensis* (Solanaceae) accessions based on molecular markers and morphological and agronomical traits, *Genet. Mol. Res.*, 2010; **9:** 1852-1864.

[8] Souza I.G.B., Valente S.E.S., Britto F.B., de Souza V.A.B. and Lima P.S.C., RAPD analysis of the genetic diversity of mango (*Mangifera indica*) germplasm in Brazil, *Genet. Mol. Res.*, 2011; **10:** 3080-3089.

[9] Barakat M.N., El-sabagh A.S., Etman A.A. and Genaidy E.A.E., Genetic analysis of some *Apricot* cv. *canino* genotyping using RAPD-PCR technique as a major molecular marker for genetic differentiation among apricot clones, *American-Eurasian J. Agric. Environ. Sci.*, 2012; **12:** 139-143.

[10] Hebert P.D.N., Cywinska A., Ball S.L. and de Waard J.R., Biological identifications through DNA barcodes, *Proc. Royal Soc. London B Biol. Sci.*, 2003; **270:** 313-321.

[11] Hebert P.D.N. and Gregory T.R., The promise of DNA barcoding for taxonomy, *Syst. Biol.*, 2005; **54:** 852-859.

[12] Chase M.W., Cowan R.S., Hollingsworth P.M., van den Berg C., Madriñán S., Petersen G., Seberg O., Jφrgsensen T., Cameron K.M., Carine M., Pedersen N., Hedderson T.A.J., Conrad F., Salazar G.A., Richardson J.E., Hollingsworth M.L., Barraclough T.G., Kelly L. and Wilkinson M., A proposal for a standardised protocol to barcode all land plants, *Taxon*, 2007; **56:** 295-299.

[13] Newmaster S.G., Fazekas A.J., Steeves A.J. and Janovec J., Testing candidate plant barcode regions in the Myristicaceae, *Mol. Ecol. Resour.*, 2008; **8:** 480-490.

[14] CBOL Plant Working Group, A DNA barcode for land plants, *Proc. Natl. Acad. Sci. U.S.A.*, 2009; **106:** 12794-12797.

[15] Monkheang P., Sudmoon R., Tanee T., Noikotr K., Bletter N. and Chaveerach A., Species diversity, usages, molecular markers and barcode of medicinal *Senna* species (Fabaceae, Caesalpinioideae) in Thailand, *J. Med. Plant Res.*, 2011; **5:** 6173-6181.

[16] Kwanda N., Noikotr K., Sudmoon R., Tanee T. and Chaveerach A.,

Medicinal parasitic plants on diverse hosts with their usages and barcodes, *J. Nat. Med.*, 2013; **67:** 438-445.

[17] Kojoma M., Kurihara K., Yamada K., Sekita S., Satake M. and Lida O., Genetic identification of Cinnamon (*Cinnamomum* spp.) based on the *trn*L-*trn*F chloroplast DNA, *Planta Med.*, 2002; **68:** 94-96.

[18] Chiou S.J., Yen J.H., Fang C.L., Chen H.L. and Lin T.Y., Authentication of medicinal herbs using PCR-amplified ITS2 with specific primers, *Planta Med.*, 2007; **73:** 1421-1426.

[19] Chayamarit K., *Thai Herbs*, National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment of Thailand, Bangkok, 1997.

[20] Xiwen L., Jie L. and van der Werff H., *Cinnamomum* Schaeff. *Flora of China*, 2008; **7:** 166-187.

[21] Rohlf F.J., NTSYS_pc: numerical taxonomy and multivariate analysis system version 2.1, Applied Biostatistics Inc, New York, 1998.

[22] Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S., MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol. Biol. Evol.*, 2011; **28:** 2731-2739.

[23] Taberlet P., Coissac E., Pompanon F., Gielly L., Miquel C., Valentini A., Vermat T., Corthier G., Brochmann C. and Willerslev E., Power and limitations of the chloroplast *trn*L (UAA) intron for plant DNA barcoding, *Nucleic Acid Res.*, 2007; **35:** el4.

[24] Madrinan S. and Chacon J., DNA barcodes of Lauraceae, *GenBank, National Center for Biotechnology Information,* 2007; direct submission.

[25] Kress W.J. and Erickson D.L., A two-locus global DNA barcode for land plants: the coding *rbc*L gene complements the non-coding *trn*H-*psb*A spacer region, *PLoS ONE,* 2007; **2:** E508.

[26] Chen K.L. and Liu Z., Application of DNA barcoding in the plants of Lauraceae family, *GenBank, National Center for Biotechnology Information,* 2010; direct submission.

[27] Herrmann F., Romero M.R., Blazquez A.G., Kaufmann D., Ashour M.L., Kahl S., Marin J.J.G., Efferth T. and Wink M., Diversity of pharmacological properties in Chinese and European medicinal plants: Cytotoxicity, antiviral and antitrypanosomal screening of 82 herbal drugs, *Diversity,* 2011; **3:** 547-580.

[28] Chen S., Yao H., Han J., Liu C., Song J., Shi L., Zhu Y., Ma X., Gao T., Pang X., Luo K., Li Y., Li X., Jia X., Lin Y. and Leon C., Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species, *PLoS ONE,* 2010; **5:** E8613.

[29] Pei N.C., Erickson D., Kress W. and Ge X.J., Identification of woody plants in tropics and subtropics with DNA barcodes, *GenBank, National Center for Biotechnology Information,* 2010; direct submission.

[30] Joyner G.T., Hrideek T.K. and Dev S.A., Direct submission, *GenBank, National Center for Biotechnology Information,* 2012; direct submission.