Phytotaxa 296 (3): 239–252
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.296.3.3

DNA profiles to identify *Dillenia* species (Dilleniaceae) in Thailand

TIKUMPORN THOOPTIANRAT[1,2], ARUNRAT CHAVEERACH*[1,2], RUNGLAWAN SUDMOON[2,3] & TAWATCHAI TANEE[2,4]

[1]*Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Genetics and Environmental Toxicology Research Group, Khon Kaen University, Khon Kaen 40002, Thailand*
[3]*Faculty of Law, Khon Kaen University, Khon Kaen 40002, Thailand*
[4]*Faculty of Environment and Resource Studies, Mahasarakham University, Maha Sarakham 44150, Thailand*

Abstract

Surveying of the species throughout Thailand revealed 13 identified and one unidentified species including *D. aurea*, *D. excelsa*, *D. grandifolia*, *D. hookeri*, *D. indica*, *D. obovata*, *D. ovata*, *D. parviflora*, *D. pentagyna*, *D. philippinensis*, *D. pulchella*, *D. reticulata*, *D. suffruticosa* and *Dillenia* sp. They were phylogenetically examined based on RAPD profiles of 2,290 discrete characters including a monomorphic and 226 polymorphic characteristics. The phylogenetic relationships calculated from theses banding data show that the intraspecific genetic similarity (S) values ranged from 0.990 to 1.000, and the interspecific S values ranged from 0.520 to 0.790. The RAPD method can be effectively used in *Dillenia* study. However, its quality of unreproducible method, it cannot be reasonable used in any study on specific genes or areas. In additions, sequences from two molecular regions, *rbcL* gene and *psbA-trnH* spacer, were analyzed and determined for genetic distances. The *rbcL* gene sequences were rather ineffective, as all of the paired species displayed no or low genetic distance values. The *psbA-trnH* spacer sequences were rather effective, with only one pair (*D. reticulata* and *D. parviflora*) showing low genetic distance values. The other species pairs indicated rather far genetic distances, ranging from 0.006 (*D. parviflora* and *D. grandifolia*) to 0.376 (*Dillenia* specie and *D. indica*). Intraspecific genetic distance values ranged from 0–0.003 and 0–0.013 for *rbcL* and *psbA-trnH* spacer regions, respectively. Based on the results from both nucleotide variations and DNA fingerprinting, *D. grandifolia* and *D. ovata* were distinct *Dillenia* species.

Keywords: barcode, *rbcL* gene, *psbA-trnH* spacer, flowering plant, genetic similarity

Introduction

The genus *Dillenia* Linnaeus (1753: 535) in the family Dilleniaceae from Thailand were studied by Hoogland (1972) and the Office of the Forest Herbarium (2013), and following 11 species were recognized: *D. aurea* Smith (1806: 65), *D. excelsa* Martelli (1886: 163), *D. grandifolia* Wall. in Hooker & Thomson (1855: 71), *D. hookeri* Pierre ex Gilg (1893: 125), *D. indica* Linnaeus (1753: 535), *D. obovata* (Blume 1825: 6) Hoogland (1951: 173), *D. ovata* Wall. in Hooker & Thomson (1855: 70), *D. pentagyna* Roxburgh (1795: 20), *D. parviflora* Griffith (1854: 704), *D. suffruticosa* Martelli (1886: 163) and *D. reticulata* King (1889: 367). However, *D. grandifolia* was listed as a synonym of *D. ovata* by the Plant List (http://www.theplantlist.org/tpl1.1/record/kew-2768260).

Species of *Dillenia* have been consumed as food and used in traditional medicines in many countries. They are evergreen trees or shrubs, simple and alternate leaves, desolate or acropetalous with five sepals and petals and many of stamens. The fruit is dehiscent or indehiscent, edible, several seeds with or without aril. These species have been used in tribal and folk communities from East Asia for the treatment of multiple ailments and diseases (Dubey *et al*. 2009, Alam *et al*. 2012). Elephant apple, *D. indica* containing β-sitosterol, stigmasterol, and vitamin E showed antimicrobial (Loizou *et al.* 2010, Gabay *et al*. 2010), antioxidant (Yeshwante *et al*. 2009, Muhit *et al*. 2010), anti-inflammatory (Parvin *et al*. 2011), and antidiarrheal (Hussain *et al*. 2009, Sunder *et al*. 2011) properties. Anticancer activity was reported from *D. kerrii* Craib (1911: 8) (Li *et al*. 2009) and *D. philippinensis* Rolfe (1884: 307) which also showed anti-Leishmanial activity (Macahig *et al*. 2011). Additionally, *D. obovata* showed antimicrobial activity (Yap *et al*. 2013). Based on previous researches in other plant groups (Cimanga *et al*. 2002, Mitova *et al*. 2003, Lu *et al*. 2006, Jegadeeswari *et al*. 2012, Mariajancyrani *et al*. 2014), the chemical composition of various *Dillenia* extracts can be successfully determined using gas chromatography-mass spectrometry (GC-MS).

Accepted by Yunfei DENG: 29 Jan. 2017; published: 20 Feb. 2017

Medicinal plants have been used in natural and modified forms, such as dried sliced plant parts, powder and capsules, which are difficult to recognize by morphological characters. Therefore, reliable identification methods for these plant forms should be developed. DNA barcoding is the most reliable and applicable method for overcoming this problem (Hebert *et al*. 2003, CBOL Plant Working Group 2009, Hollingsworth *et al*. 2011). Several research groups suggested appropriate DNA barcoding regions in some plant groups, such as the *matK* gene (Siripiyasing *et al*. 2012, Tanee *et al*. 2012), the *rbcL* gene (Tanee *et al*. 2012, Kwanda *et al*. 2013), and the *psbA-trnH* spacer region (Chaveerach *et al*. 2011). Standard barcodes used for most plant groups are *matK* gene, *rbcL* gene, *psbA-trnH* spacer, and ITS region identified by the CBOL Plant Working Group (2009), Chaveerach *et al*. (2011), Hollingsworth *et al*. (2011) and Monkheang *et al*. (2011).

Another molecular technique used for plant systematics is DNA fingerprinting based on various methods, especially random amplified polymorphic DNA (RAPD). These genetic variation markers are generally independent of environmental factors and more numerous than phenotypic characteristics, thereby providing a clearer indication of the underlying variation in the genome. The banding patterns reveal genetic variations/relationships through cladogram construction. Homology, a concept critical to cladistics, can be defined as the similarity/distance resulting from common ancestry (Simpson, 2006). This method has been used for many plant species as shown by Siripiyasing *et al*. (2013), Chaveerach *et al*. (2014), Sanubol *et al*. (2014) and Sudmoon *et al*. (2016).

This study aims to construct a species specific DNA barcode for *Dillenia* species that is a reliable tool for labeling species using species-specific identification and ownership. Effective RAPD marker is concurrently being used to assess genetic relationships of the *Dillenia* species.

Materials and Methods

Plant Materials

The *Dillenia* species from Thailand have been found throughout Thailand and young leaves have been collected. Species identification was completed using the appropriate literature (Hoogland 1972), and the plant names have been verified using The Plant List (http://www.theplantlist.org). Herbarium specimens were prepared for all of the collected species. The voucher specimens are kept at the Department of Biology, Faculty of Science, Khon Kaen University, Thailand (KKU). The plant information including voucher specimen numbers, collected areas and date of collection is shown in the Table 1.

TABLE 1. Plant information showing plant names, voucher specimen numbers, collected areas and dates.

Species	Voucher specimen number	Collected location	Collected date
Dillenia species 1.1	A. Chaveerach 911.1	Ongkharak district, Nakhon Nayok province	7 December 2013
D. species 1.2	A. Chaveerach 911.2	Mueang Khon Kaen district, Khon Kaen province	25 February 2014
D. aurea 1	A. Chaveerach 911.1	Mae Rim district, Chiang Mai province	20 January 2011
D. aurea 2	A. Chaveerach 911.2	Mae Rim district, Chiang Mai province	9 January 2013
D. excelsa 1	A. Chaveerach 912.1	Lan Saka district, Nakhon Si Thammarat province	21 March 2014
D. excelsa 2	A. Chaveerach 912.2	Phanom district, Surat Thani province	25 March 2014
D. grandifolia 1	A. Chaveerach 913.1	Su-ngai Kolok district, Narathiwat province	11 November 2014
D. grandifolia 2	A. Chaveerach 913.2	Su-ngai Kolok district, Narathiwat province	11 November 2014
D. hookeri 1	A. Chaveerach 914.1	Phanom district, Surat Thani province	19 March 2014
D. hookeri 2	A. Chaveerach 914.2	Buntharik district, Ubon Ratchathani province	27 March 2014
D. indica 1	A. Chaveerach 915.1	Mueang Khon Kaen district, Khon Kaen province	14 March 2013
D. indica 2	A. Chaveerach 915.2	Sai Yok district, Kanchanaburi province	8 May 2013
D. obovata 1	A. Chaveerach 916.1	Sai Yok district, Kanchanaburi province	10 May 2013
D. obovata 2	A. Chaveerach 916.2	Takua Pa district, Phang nga province	23 May 2011
D. ovata 1	A. Chaveerach 917.1	Mae Rim district, Chiang Mai province	15 January 2013
D. ovata 2	A. Chaveerach 917.2	Mueang Si Sa Ket district, Si Sa Ket province	9 September 2014

...continued on the next page

TABLE 1. (Continued)

Species	Voucher specimen number	Collected location	Collected date
D. parviflora 1	A. Chaveerach 918.1	Mae Rim district, Chiang Mai province	9 January 2013
D. parviflora 2	A. Chaveerach 918.2	Mueang Mae Hong Son district, Mae Hong Son province	12 June 2014
D. pentagyna 1	A. Chaveerach 919.1	Mueang Khon Kaen district, Khon Kaen province	17 May 2013
D. pentagyna 2	A. Chaveerach 919.2	Takua Pa district, Phang nga province	1 May 2011
D. philippinensis 1	A. Chaveerach 920.1	Mueang Prachin Buri district, Prachin Buri province	9 September 2013
D. philippinensis 2	A. Chaveerach 920.2	Mueang Khon Kaen district, Khon Kaen province	14 March 2013
D. pulchella 1	A. Chaveerach 921.1	Sikao district, Trang province	20 February 2014
D. pulchella 2	A. Chaveerach 921.2	Sikao district, Trang province	3 February 2015
D. reticulata 1	A. Chaveerach 922.1	Lan Saka district, Nakhon Si Thammarat	20 February 2014
D. reticulata 2	A. Chaveerach 922.2	Lan Saka district, Nakhon Si Thammarat	20 February 2014
D. suffruticosa 1	A. Chaveerach 923.1	Mae Rim district, Chiang Mai province	28 October 2011
D. suffruticosa 2	A. Chaveerach 923.2	Ongkharak district, Nakhon Nayok province	7 December 2014

DNA extraction

Whole genomic DNA was extracted using a Plant Genomic DNA Extraction Kit (RBC Bioscience) according to the manufacturer's protocols.

DNA fingerprinting by RAPD marker and dendrogram construction

Amplifications were carried out in 25 µl reaction mixtures consisting of GoTaq Green Master Mix (Promega), 0.5 µM primer and 5 ng DNA template. Fifty RAPD primers were screened and the 14 primers that successfully amplified clear bands were as follows (5′ to 3′): TGCCGAGCTG, GGGTAACGCC, GTTGCGATCC, CTGCTGGGAC, GTAGACCCGT, GGAGGGTGTT, TTTGCCCGGA, CCGCATCTAC, GATGACCGCC, TGTCTGGGTG, GGACCCAACC, GTCGCCGTCA, TCTGGTGAGG and TGAGCGGACA. The amplification was performed using a thermal cycler (Swift™ Maxi Thermal Cycler, Esco Micro Pte. Ltd.) with an initial denaturation at 94°C for 3 min; then followed by 35 thermal cycles of denaturation for 1 min at 94°C, annealing for 2 min at 40°C and extension for 2 min at 72°C; lastly, final extension for 7 min at 72°C was added. Amplification products were detected by 1.2% agarose gel electrophoresis in Tris-acetate EDTA (TAE) buffer and visualized using ethidium bromide staining. The resulting RAPD bands were used to construct a dendrogram using NTSY Spc version 2.1 (Rohlf 1998).

Amplification of the DNA fragments of rbcL and psbA-trnH spacer regions

Polymerase chain reactions (PCR) were performed with the following primer pairs (5′ to 3′): ATGTCACCACAAACAGAGACTAAAGC and GTAAAATCAAGTCCACCRCG (Wang *et al*. 2010) for the *rbcL* gene and TTATGCATGAACGTAATGCTC and CGCGCATGGTGGATTCACAATCC (Tate and Simpson 2003) for the *psbA-trnH* spacer region. The reaction mixture (30 µl) consisted of GoTaq Green Master Mix (Promega), 0.5 µM primers, and 10 ng DNA template. The following amplification conditions were used: a pre-denaturation at 94°C for 1 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The amplified products were subjected to 2% agarose gel electrophoresis in TAE buffer with ethidium bromide staining.

DNA sequencing and sequences analysis receiving nucleotide variations

The specific amplified fragments were sent for sequencing at the DNA Sequencing Unit, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand. The sequences were then analyzed using Blast tools (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were aligned for each genome region to determine the genetic distance values by analyzing the nucleotide variations using the MEGA6 program (Tamura *et al*. 2013). The program default parameters were used: evolutionary relationship was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method with bootstrap consensus tree inferred from 1,000 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. All positions containing gaps and missing data were eliminated. Finally, the sequences were submitted to GenBank, and the corresponding accession numbers were given.

Results

In this study, thirteen *Dillenia* species were discovered, namely *D. aurea*, *D. excelsa*, *D. grandifolia*, *D. hookeri*, *D. indica*, *D. obovata*, *D. ovata*, *D. parviflora*, *D. pentagyna*, *D. philippinensis*, *D. pulchella*, *D. reticulata*, and *D. suffruticosa*, and one unidentified species lacking flowers was found (Figure 1). Of these, two species, *D. philippinensis* and *D. pulchella*, were newly reported for Thailand as imported species. Although *D. grandifolia* was treated by some authors as a synonym of *D. ovata*, we still use the name *D. grandifolia* until the identity of the plants has been supported by molecular data.

FIGURE 1. The 13 identified *Dillinia* species and one unidentified species, namely *Dillenia aurea* (A,B), *D. excelsa* (C,D), *D. grandifolia* (E), *D. hookeri* (F), *D. indica* (G,H), *D. obovata* (I), *D. ovata* (J,K), *D. parviflora* (L,M), *D. pentagyna* (N,O), *D. philippinensis* (P,Q), *D. pulchella* (R,S), *D. reticulata* (T,U), *D. suffruticosa* (V,W), and *Dillenia* sp. (X,Y).

TABLE 2. Genetic distance matrix of *Dillenia* species analyzed from RAPD fingerprint data of 14 RAPD profiles.

	D. aurea (1)	*D. aurea* (2)	*D. excelsa* (1)	*D. excelsa* (2)	*D. grandifolia* (1)	*D. grandifolia* (2)	*D. hookeri* (1)	*D. hookeri* (2)	*D. indica* (1)	*D. indica* (2)	*D. obovata* (1)	*D. obovata* (2)	*D. ovata* (1)	*D. ovata* (2)	*D. parviflora* (1)	*D. parviflora* (2)	*D. pentagyna* (1)	*D. pentagyna* (2)	*D. philippinensis* (1)	*D. philippinensis* (2)	*D. pulchella* (1)	*D. pulchella* (2)	*D. reticulata* (1)	*D. reticulata* (2)	*D. suffruticosa* (1)	*D. suffruticosa* (2)	*Dillenia* sp. (1.1)	*Dillenia* sp. (1.2)	*Tetracera loureiri*
D. aurea (1)	1.000																												
D. aurea (2)	1.000	1.000																											
D. excelsa (1)	0.710	0.710	1.000																										
D. excelsa (2)	0.710	0.710	1.000	1.000																									
D. grandifolia (1)	0.660	0.660	0.700	0.700	1.000																								
D. grandifolia (2)	0.660	0.660	0.690	0.690	0.990	1.000																							
D. hookeri (1)	0.610	0.610	0.660	0.660	0.650	0.660	1.000																						
D. hookeri (2)	0.610	0.610	0.660	0.660	0.650	0.660	1.000	1.000																					
D. indica (1)	0.610	0.610	0.640	0.640	0.690	0.700	0.640	0.640	1.000																				
D. indica (2)	0.620	0.620	0.640	0.640	0.690	0.700	0.640	0.640	1.000	1.000																			
D. obovata (1)	0.660	0.660	0.670	0.670	0.650	0.660	0.730	0.730	0.740	0.740	1.000																		
D. obovata (2)	0.660	0.660	0.670	0.670	0.650	0.660	0.730	0.730	0.740	0.740	1.000	1.000																	
D. ovata (1)	0.610	0.610	0.610	0.610	0.600	0.610	0.660	0.670	0.660	0.660	0.790	0.790	1.000																
D. ovata (2)	0.610	0.610	0.610	0.610	0.610	0.600	0.650	0.650	0.650	0.650	0.780	0.780	0.990	1.000															
D. parviflora (1)	0.660	0.660	0.630	0.630	0.610	0.610	0.660	0.660	0.640	0.640	0.720	0.720	0.760	0.770	1.000														
D. parviflora (2)	0.660	0.660	0.630	0.630	0.610	0.610	0.660	0.660	0.640	0.640	0.720	0.720	0.760	0.770	1.000	1.000													
D. pentagyna (1)	0.600	0.600	0.650	0.650	0.580	0.580	0.580	0.580	0.600	0.610	0.650	0.650	0.670	0.680	0.740	0.740	1.000												
D. pentagyna (2)	0.600	0.600	0.650	0.650	0.580	0.580	0.580	0.580	0.600	0.610	0.650	0.650	0.670	0.680	0.740	0.740	1.000	1.000											
D. philippinensis (1)	0.610	0.610	0.620	0.620	0.590	0.590	0.600	0.610	0.590	0.590	0.650	0.650	0.620	0.610	0.680	0.680	0.640	0.640	1.000										
D. philippinensis (2)	0.610	0.610	0.620	0.620	0.590	0.590	0.600	0.610	0.590	0.590	0.650	0.650	0.620	0.610	0.680	0.680	0.640	0.640	1.000	1.000									
D. pulchella (1)	0.630	0.630	0.660	0.660	0.590	0.590	0.600	0.610	0.610	0.610	0.650	0.650	0.600	0.600	0.630	0.630	0.590	0.590	0.730	0.730	1.000								
D. pulchella (2)	0.630	0.630	0.660	0.660	0.590	0.590	0.600	0.610	0.610	0.610	0.650	0.650	0.600	0.600	0.630	0.630	0.590	0.590	0.730	0.730	1.000	1.000							
D. reticulata (1)	0.560	0.560	0.570	0.570	0.600	0.590	0.530	0.530	0.600	0.610	0.650	0.650	0.600	0.610	0.630	0.630	0.620	0.620	0.640	0.640	0.650	0.650	1.000						
D. reticulata (2)	0.560	0.560	0.560	0.560	0.600	0.590	0.530	0.520	0.600	0.600	0.650	0.650	0.600	0.600	0.630	0.630	0.610	0.610	0.630	0.630	0.640	0.640	1.000	1.000					
D. suffruticosa (1)	0.520	0.520	0.570	0.570	0.580	0.580	0.580	0.580	0.590	0.600	0.620	0.620	0.590	0.590	0.600	0.600	0.620	0.620	0.670	0.670	0.630	0.630	0.690	0.690	1.000				
D. suffruticosa (2)	0.520	0.520	0.570	0.570	0.580	0.580	0.590	0.580	0.600	0.600	0.620	0.620	0.600	0.590	0.600	0.600	0.620	0.620	0.680	0.680	0.630	0.630	0.690	0.690	1.000	1.000			
Dillenia sp. (1.1)	0.740	0.740	0.650	0.650	0.680	0.670	0.640	0.630	0.610	0.610	0.610	0.610	0.610	0.580	0.590	0.650	0.650	0.600	0.600	0.540	0.540	0.600	0.600	0.570	0.560	0.530	0.540	1.000	
Dillenia sp. (1.2)	0.740	0.740	0.650	0.650	0.680	0.670	0.640	0.630	0.610	0.610	0.610	0.610	0.610	0.580	0.590	0.650	0.650	0.600	0.600	0.540	0.540	0.600	0.600	0.570	0.560	0.530	0.540	1.000	1.000
Tetracera loureiri	0.550	0.550	0.530	0.530	0.520	0.530	0.520	0.510	0.550	0.550	0.570	0.570	0.570	0.550	0.550	0.550	0.520	0.520	0.540	0.540	0.570	0.570	0.570	0.560	0.530	0.540	0.520	0.520	1.000

The 14 different polymorphic primers used in the RAPD marker analysis produced 2,290 bands for 28 individuals from 14 species with an individual outgroup, *Tetracera loureiroi* (Finet & Gagnep. in Lecomte 1907: 16) Pierre ex Craib in Kerr (1922: 165). RAPD analysis was successfully used to distinctly separate species with the UPGMA method to different dendrogram branches (Figure 2) constructed using the RAPD bands. The dendrogram shows that the high efficiency of the RAPD data used can clearly distinguish each species including the outgroup with the low genetic similarity of interspecific level and high genetic similarity of intraspecific level. The species were separated with the genetic similarity from 0.990 (in *D. grandifolia* and *D. ovata*) to 1.000 (in all of the studied pair species) and interspecific species genetic similarity ranged from 0.520 (*D. suffruticosa* and *D. aurea* 1) to 0.790 (*D. ovata* 1 and *D. obovata* 1, 2) as shown in Table 2 and summarized in Table 3.

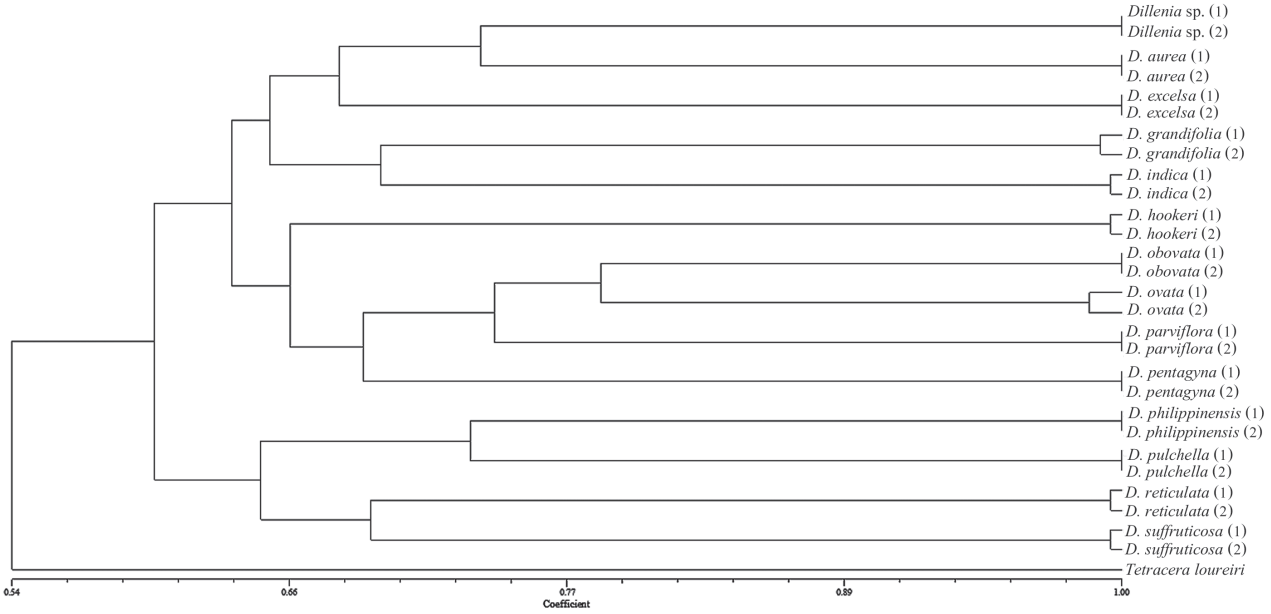

FIGURE 2. The dendrogram constructed from RAPD bands from 14 primers in 28 individuals of 14 species and of the *Dillenia* sp. by NTSYS-pc version 2.1 UPGMA.

TABLE 3. Summary of intraspecific species and interspecific species genetic similarity (S) values of *Dillenia* species analyzed from RAPD fingerprint data of 14 RAPD profiles.

Comparison level	**genetic similarity**		
	Minimum	**Maximum**	**Mean±S.D.**
intraspecific species	0.99	1.00	0.999±0.004
interspecific species	0.52	0.79	0.632±0.051

Successful amplifications of DNA bands from the *rbcL* gene and *psbA-trnH* spacer produced approximately 600 base pair and 400 base pair products, respectively. Table 4 showed the GenBank accession numbers corresponding to the 48 sequences from the *rbcL* gene and *psbA-trnH* spacer regions for all 14 samples studied. The *rbcL* sequence was rather ineffective, as none of the following 13 species pairs displayed genetic distance values (Table 5): *D. obovata* and *D. excelsa*, *D. parviflora* and *D. excelsa*, *D. pentagyna* and *D. excelsa*, *D. reticulata* and *D. excelsa*, *D. indica* and *D. hookeri*, *D. ovata* and *D. hookeri*, *D. ovata* and *D. indica*, *D. parviflora* and *D. obovata*, *D. pentagyna* and *D. obovata*, *D. reticulata* and *D. obovata*, *D. pentagyna* and *D. parviflora*, *D. reticulata* and *D. parviflora*, and *D. reticulata* and *D. pentagyna*. The following 15 species pairs showed rather low values (0.002) to rather high values (0.152): *D. hookeri* and *D. excelsa*, *D. indica* and *D. excelsa*, *D. ovata* and *D. excelsa*, *D. obovata* and *D. hookeri*, *D. parviflora* and *D. hookeri*, *D. pentagyna* and *D. hookeri*, *D. reticulata* and *D. hookeri*, *D. obovata* and *D. indica*, *D. parviflora* and *D. indica*, *D. pentagyna* and *D. indica*, *D. reticulata* and *D. indica*, *D. ovata* and *D. obovata*, *D. parviflora* and *D. ovata*, *D. pentagyna* and *D. ovata*, and *D. reticulata* and *D. ovata*. The nucleotide variations for the *psbA-trnH* spacer were rather effective, with only one pair (*D. reticulata* and *D. parviflora*) showed no distance values. The remaining pairs indicated high nucleotide variations, ranged from 0.006 (*D. parviflora* and *D. grandifolia*) to 0.376 (*Dillenia* sp. and *D. indica*) as shown in Table 6. The intraspecific and interspecific genetic distance values were summarized in Table 7. Phylogenetic tree with bootstrap supported constructed from sequences of each region are shown in Figures 3 and 4.

TABLE 4. GenBank accession numbers of DNA barcoding from two regions of *Dillenia* species. The sequence data were deposited at www.ncbi.nlm.nih.gov/Genbank.

Name	*rbcL*	*psbA-trnH*
Dillenia sp.	KR857436, KR857437	KT452058
D. aurea	KR857438, KR857439	KT452059, KT452060
D. excelsa	KR857440, KR857441	KT452061
D. grandifolia	KT343251	KT452062
D. hookeri	KR857442, KR857443	KT452063
D. indica	KR857444, KR857445	KT452064
D. obovata	KR857446, KR857447	KT452065, KT452066
D. ovata	KR857448, KR857449	KT452067, KT452068
D. parviflora	KR857450, KR857451	KT452069, KT452070
D. pentagyna	KR857452, KR857453	KT452071
D. philippinensis	KR857454, KR857455	KT452072, KT452073
D. pulchella	KR857456, KR857457	KT452074, KT452075
D. reticulata	KR857458, KR857459	KT452076
D. suffruticosa	KR857460, KR857461	KT452077, KT452078

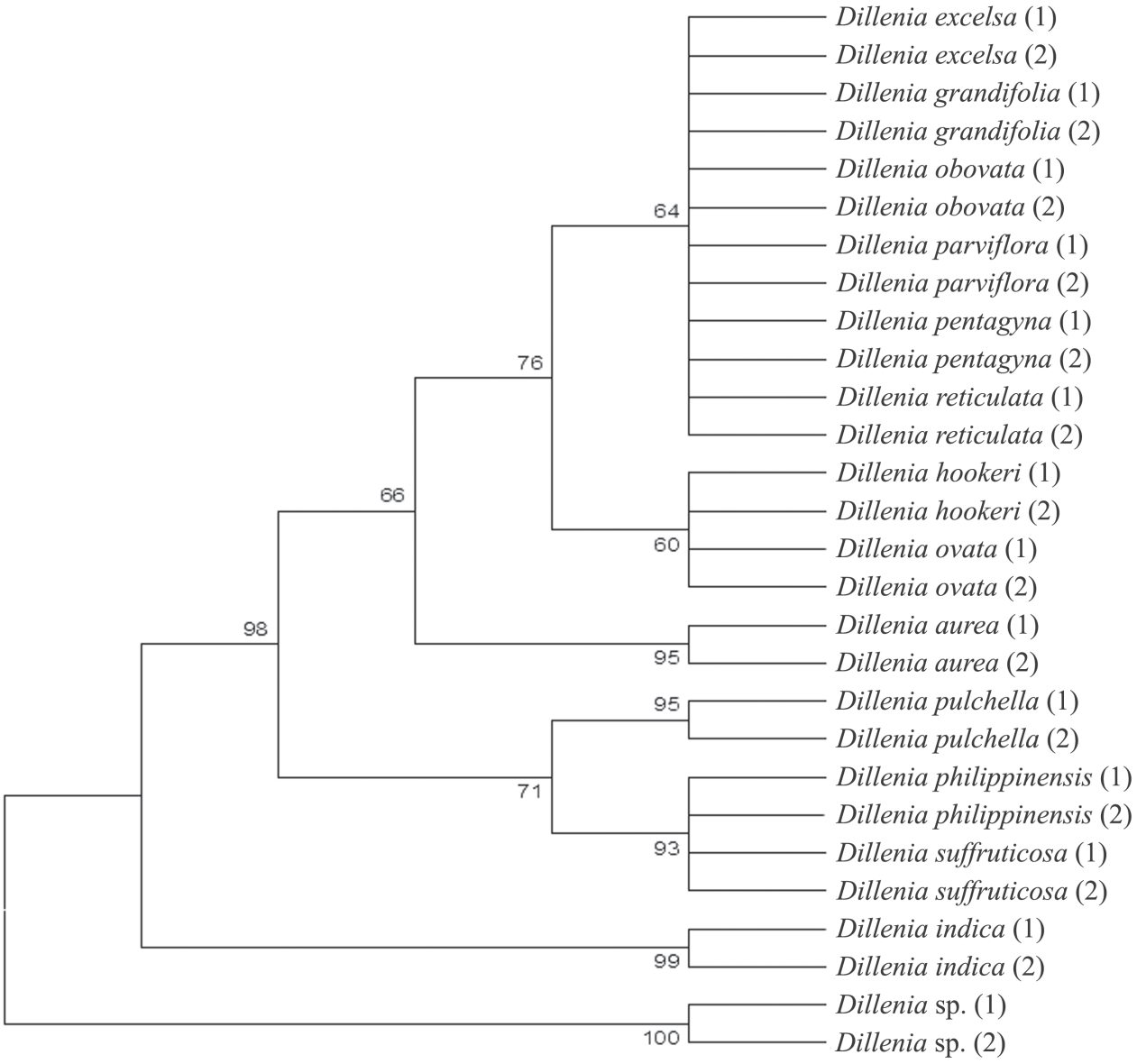

FIGURE 3. The phylogenetic tree with bootstrap support levels from *rbcL* gene sequences of 13 species and of the *Dillenia* sp. by MEGA6.

TABLE 5. Genetic distance matrix based on nucleotide variations from sequence alignment of *rbcL* region of the *Dillenia* species by the MEGA6 program.

	D. aurea (1)	*D. aurea*(2)	*D. excelsa*(1)	*D. excelsa* (2)	*D. grandifolia*(1)	*D. grandifolia* (2)	*D. hookeri* (1)	*D. hookeri* (2)	*D. indica* (1)	*D. indica* (2)	*D. obovata* (1)	*D. obovata*(2)	*D. ovata* (1)	*D. ovata* (2)	*D. parviflora* (1)	*D. parviflora* (2)	*D. pentagyna* (1)	*D. pentagyna* (2)	*D. philippinensis*(1)	*D. philippinensis*(2)	*D. pulchella* (1)	*D. pulchella* (2)	*D. reticulata* (1)	*D. reticulata* (2)	*D. suffruticosa* (1)	*D. suffruticosa* (2)	*Dillenia* sp. (1.1)	*Dillenia* sp. (1.2)
D. aurea (1)	0.000																											
D. aurea(2)	0.000	0.000																										
D. excelsa (1)	0.010	0.010	0.000																									
D. excelsa (2)	0.010	0.010	0.000	0.000																								
D. grandifolia (1)	0.010	0.010	0.002	0.002	0.000																							
D. grandifolia (2)	0.007	0.007	0.002	0.002	0.002	0.000																						
D. hookeri (1)	0.007	0.007	0.002	0.002	0.002	0.002	0.000																					
D. hookeri (2)	0.005	0.005	0.005	0.005	0.005	0.002	0.002	0.000																				
D. indica (1)	0.007	0.007	0.002	0.002	0.002	0.002	0.002	0.002	0.000																			
D. indica (2)	0.010	0.010	0.005	0.005	0.005	0.002	0.002	0.005	0.002	0.000																		
D. obovata(1)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.000																	
D. obovata (2)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.000	0.000																
D. ovata (1)	0.007	0.007	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000															
D. ovata (2)	0.007	0.007	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000														
D. parviflora (1)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.000													
D. parviflora (2)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.000	0.000												
D. pentagyna (1)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.000											
D. pentagyna (2)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.000	0.000										
D. philippinensis (1)	0.017	0.017	0.012	0.012	0.012	0.010	0.010	0.012	0.010	0.012	0.012	0.012	0.010	0.010	0.012	0.012	0.012	0.012	0.000									
D. philippinensis (2)	0.017	0.017	0.012	0.012	0.012	0.010	0.010	0.012	0.010	0.012	0.012	0.012	0.010	0.010	0.012	0.012	0.012	0.012	0.000	0.000								
D. pulchella (1)	0.022	0.022	0.017	0.017	0.017	0.014	0.014	0.017	0.014	0.017	0.017	0.017	0.014	0.014	0.017	0.017	0.017	0.017	0.005	0.003	0.000							
D. pulchella (2)	0.022	0.022	0.017	0.017	0.017	0.014	0.014	0.017	0.014	0.017	0.017	0.017	0.014	0.014	0.017	0.017	0.017	0.017	0.005	0.005	0.005	0.000						
D. reticulata (1)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.012	0.012	0.017	0.017	0.000					
D. reticulata (2)	0.010	0.010	0.002	0.002	0.002	0.002	0.002	0.005	0.002	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.012	0.012	0.017	0.017	0.000	0.000				
D. suffruticosa(1)	0.017	0.017	0.012	0.012	0.012	0.010	0.010	0.012	0.010	0.012	0.012	0.012	0.010	0.010	0.012	0.012	0.012	0.012	0.002	0.002	0.005	0.005	0.012	0.012	0.000			
D. suffruticosa (2)	0.017	0.017	0.012	0.012	0.012	0.010	0.010	0.012	0.010	0.012	0.012	0.012	0.010	0.010	0.012	0.012	0.012	0.012	0.002	0.002	0.005	0.005	0.012	0.012	0.000	0.000		
Dillenia sp. (1.1)	0.140	0.140	0.152	0.152	0.152	0.149	0.149	0.146	0.149	0.152	0.152	0.152	0.149	0.149	0.152	0.152	0.152	0.152	0.140	0.140	0.140	0.143	0.152	0.152	0.140	0.140	0.000	
Dillenia sp. (1.2)	0.140	0.140	0.152	0.152	0.152	0.149	0.149	0.146	0.149	0.152	0.152	0.152	0.149	0.149	0.152	0.152	0.152	0.152	0.140	0.140	0.140	0.143	0.152	0.152	0.140	0.140	0.000	0.000

TABLE 6. Genetic distance matrix based on nucleotide variations from sequence alignment of *psbA-trnH*spacer region of the *Dillenia* species by the MEGA6 program.

	D. aurea (1)	*D. aurea*(2)	*D. excelsa*(1)	*D. excelsa* (2)	*D. grandifolia*(1)	*D. grandifolia* (2)	*D. hookeri* (1)	*D. hookeri* (2)	*D. indica* (1)	*D. indica* (2)	*D. obovata* (1)	*D. obovata*(2)	*D. ovata* (1)	*D. ovata* (2)	*D. parviflora* (1)	*D. parviflora* (2)	*D. pentagyna* (1)	*D. pentagyna* (2)	*D. philippinensis*(1)	*D. philippinensis*(2)	*D. pulchella* (1)	*D. pulchella* (2)	*D. reticulata* (1)	*D. reticulata* (2)	*D. suffruticosa* (1)	*D. suffruticosa* (2)	*Dillenia* sp. (1.1)	*Dillenia* sp. (1.2)
D. aurea (1)	0.000																											
D. aurea(2)	0.000	0.000																										
D. excelsa (1)	0.033	0.033	0.000																									
D. excelsa (2)	0.033	0.033	0.000	0.000																								
D. grandifolia (1)	0.026	0.026	0.019	0.019	0.000																							
D. grandifolia (2)	0.026	0.026	0.019	0.019	0.000	0.000																						
D. hookeri (1)	0.117	0.117	0.133	0.133	0.125	0.125	0.000																					
D. hookeri (2)	0.117	0.117	0.133	0.133	0.125	0.125	0.000	0.000																				
D. indica (1)	0.141	0.141	0.157	0.157	0.133	0.133	0.033	0.033	0.000																			
D. indica (2)	0.141	0.141	0.157	0.157	0.133	0.133	0.033	0.033	0.000	0.000																		
D. obovata(1)	0.039	0.039	0.019	0.019	0.039	0.039	0.148	0.148	0.173	0.173	0.000																	
D. obovata (2)	0.039	0.039	0.019	0.019	0.039	0.039	0.148	0.148	0.173	0.173	0.000	0.000																
D. ovata (1)	0.033	0.033	0.008	0.008	0.019	0.019	0.133	0.133	0.157	0.157	0.019	0.019	0.000															
D. ovata (2)	0.033	0.033	0.008	0.008	0.019	0.019	0.133	0.133	0.157	0.157	0.019	0.019	0.000	0.000														
D. parviflora (1)	0.026	0.026	0.019	0.019	0.006	0.006	0.125	0.125	0.133	0.133	0.039	0.039	0.019	0.019	0.000													
D. parviflora (2)	0.026	0.026	0.019	0.019	0.006	0.006	0.125	0.125	0.133	0.133	0.039	0.039	0.019	0.019	0.000	0.000												
D. pentagyna (1)	0.135	0.135	0.097	0.097	0.120	0.120	0.256	0.256	0.286	0.286	0.119	0.119	0.097	0.097	0.120	0.120	0.000											
D. pentagyna (2)	0.135	0.135	0.097	0.097	0.120	0.120	0.256	0.256	0.286	0.286	0.119	0.119	0.097	0.097	0.120	0.120	0.000	0.000										
D. philippinensis (1)	0.144	0.144	0.119	0.119	0.143	0.143	0.283	0.283	0.315	0.315	0.142	0.142	0.119	0.119	0.143	0.143	0.026	0.026	0.000									
D. philippinensis (2)	0.160	0.160	0.135	0.135	0.160	0.160	0.295	0.295	0.328	0.328	0.159	0.159	0.135	0.135	0.160	0.160	0.039	0.039	0.013	0.000								
D. pulchella (1)	0.067	0.067	0.067	0.067	0.067	0.067	0.188	0.188	0.215	0.215	0.088	0.088	0.067	0.067	0.067	0.067	0.097	0.097	0.090	0.105	0.000							
D. pulchella (2)	0.067	0.067	0.067	0.067	0.067	0.067	0.188	0.188	0.215	0.215	0.088	0.088	0.067	0.067	0.067	0.067	0.097	0.097	0.090	0.105	0.000	0.000						
D. reticulata (1)	0.026	0.026	0.019	0.019	0.006	0.006	0.125	0.125	0.133	0.133	0.039	0.039	0.019	0.019	0.000	0.000	0.120	0.120	0.143	0.160	0.067	0.067	0.000					
D. reticulata (2)	0.026	0.026	0.019	0.019	0.006	0.006	0.125	0.125	0.133	0.133	0.039	0.039	0.019	0.019	0.000	0.000	0.120	0.120	0.143	0.160	0.067	0.067	0.000	0.000				
D. suffruticosa(1)	0.081	0.081	0.060	0.060	0.081	0.081	0.190	0.190	0.217	0.217	0.081	0.081	0.060	0.060	0.081	0.081	0.074	0.074	0.067	0.081	0.033	0.033	0.081	0.081	0.000			
D. suffruticosa (2)	0.082	0.082	0.060	0.060	0.081	0.081	0.207	0.207	0.235	0.235	0.081	0.081	0.060	0.060	0.081	0.081	0.075	0.075	0.053	0.067	0.033	0.033	0.081	0.081	0.013	0.000		
Dillenia sp. (1.1)	0.179	0.179	0.152	0.152	0.179	0.179	0.328	0.328	0.364	0.364	0.169	0.169	0.152	0.152	0.179	0.179	0.046	0.046	0.046	0.061	0.121	0.121	0.179	0.179	0.097	0.098	0.000	
Dillenia sp. (1.2)	0.188	0.188	0.161	0.161	0.188	0.188	0.340	0.340	0.376	0.376	0.178	0.178	0.161	0.161	0.188	0.188	0.053	0.053	0.053	0.068	0.130	0.130	0.188	0.188	0.105	0.105	0.006	0.000

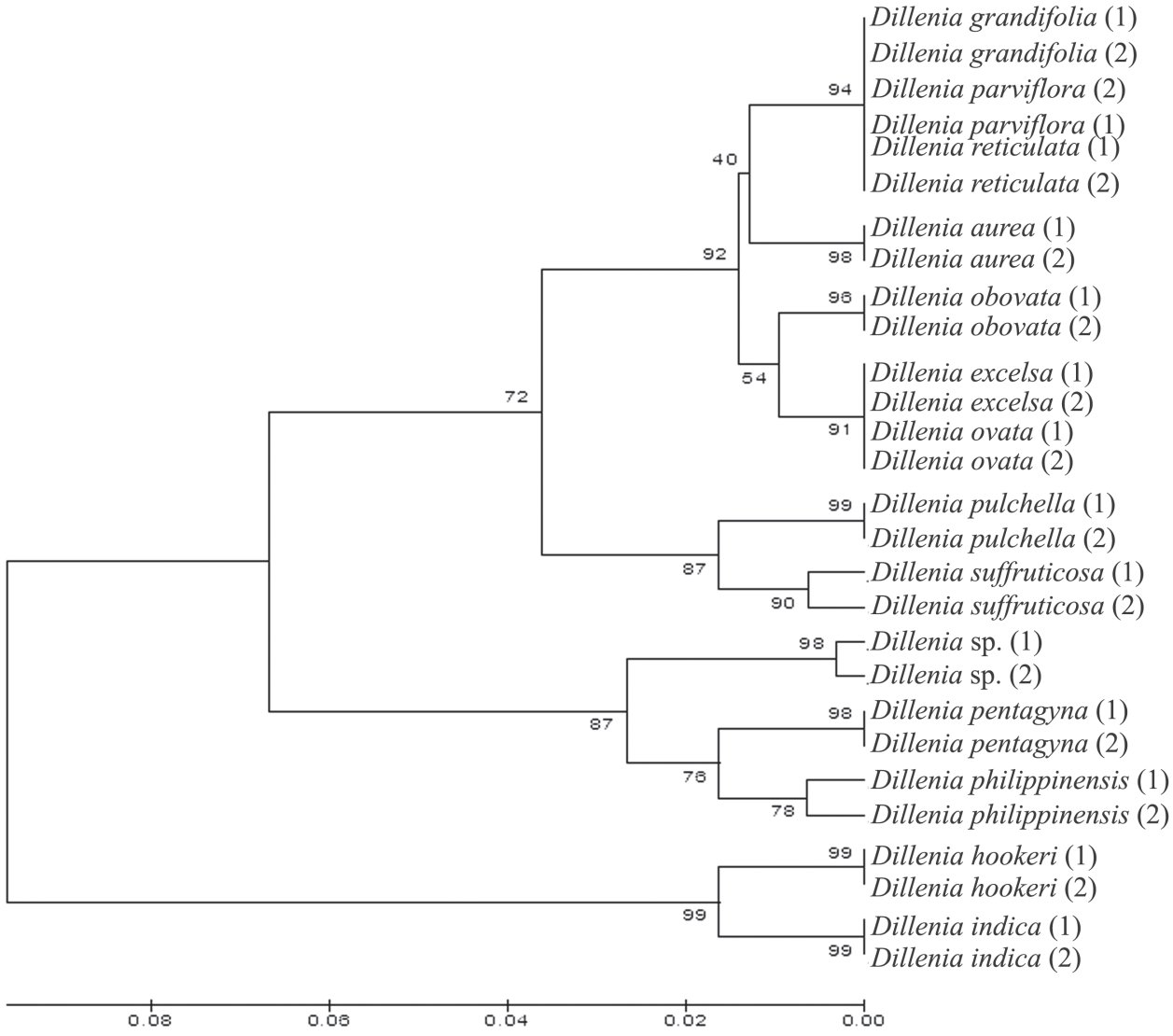

FIGURE 4. The phylogenetic tree with bootstrap support levels from *psbA-trnH* spacer sequences of 13 species and the *Dillenia* sp. by MEGA6.

TABLE 7. Summary of intraspecific and interspecific genetic distance (D) values of *Dillenia* species based on nucleotide variations from sequence alignment of *rbcL* and *psbA-trnH*spacer regions by the MEGA6 program.

Comparison level	**genetic distance (D)**		
	Minimum	**Maximum**	**Mean±S.D.**
rbcL region			
intraspecific species	0.000	0.003	0.000±0.001
interspecific species	0.002	0.152	0.028±0.049
*psbA-trnH*region			
intraspecific species	0.000	0.013	0.002±0.005
interspecific species	0.000	0.376	0.109±0.078

Discussion

Because these plants have been widely used in several forms, which include fresh plant parts, capsule formulations and other preparations, their authenticities should be verified using nucleotide variations in DNA barcodes to determine the exact effects of these products for medicinal and house-hold uses. DNA barcoding has been employed to overcome the

problems associated with identifying species based on morphological characteristics. The *psbA-trnH* spacer region was suggested as an efficient DNA barcode marker for flowering plants in Thailand, including species of the *Senna* Miller (1753: 128) (Monkheang *et al*. 2011), *Smilax* Linnaeus (1753: 1028) and *Cissus* Linnaeus (1753: 117) (Kritpetcharat *et al*. 2011). The *rbcL* gene has also been suggested as a marker for parasitic plants, including *Scurrula* Linnaeus (1753: 110), *Dendrophthoe* Martius (1830: 109), *Helixanthera* Loureiro (1790: 142), *Macrosolen* (Blume in Blume & Fischer 1830: 16) Blume in Roemer & Schultes (1830: 1731) and *Viscum* Linnaeus (1753: 1023) (Kwanda *et al*. 2013). Additionally, the *matK* gene marker has been identified in some medicinal *Piper* species (Sudmoon *et al*. 2012).

According to the results from DNA barcoding, the nucleotide variations of the *psbA-trnH* spacer were rather effective, as only one pair (*D. reticulata* and *D. parviflora*) showed similar distance values of 0. The other pairs indicated rather high nucleotide variations, ranged from 0.006 (*D. parviflora* and *D. grandifolia*) to 0.376 (*Dillenia* sp. and *D. indica*). In contrast, the nucleotide variations for the *rbcL* gene region were inefficient, displaying low values for many of the species pairs (Table 5). However, the values for *Dillenia* sp. were different when compared with other studied species (Tables 5 and 6) indicating a distinguish species from the 14 studied species. Additionally, the suggested synonym species, *D. grandifolia* and *D. ovata*, displayed a different species nucleotide variations levels of 0.002 and 0.019 for the *rbcL* gene and *psbA-trnH* spacer regions, respectively. Therefore, nucleotide variations within the species showed genetic distances of 0–0.003 in *D. pulchella* and 0–0.013 in *D. philippinensis* and *D. suffruticosa* for the *rbcL* gene and *psbA-trnH* spacer regions. The *psbA-trnH* spacer region can distinguish 92.86% (13 out of 14) of the studied species. The nucleotide variations of these two barcoding regions supported the finding that the two species, *D. grandifolia* and *D. ovata* were distinguished species based on the genetic similarity value and the dendrogram separation analyzed from DNA fingerprinting data. These plants were in the range of values for a different pair of species (0.600–0.610) and were supported by their location on different dendrogram branches. The DNA fingerprinting data showed intraspecific genetic similarity between 0.990 for *D. grandifolia* and 1.000 for all of the other paired species, whereas interspecific genetic similarity ranged between 0.520 for *D. suffruticosa* and *D. aurea* and 0.790 for *D. ovata* and *D. obovata* (Figure 2 and Table 6). Thus, based on the results from both nucleotide variations and DNA fingerprinting, *D. grandifolia* and *D. ovata* species were distinct *Dillenia* species. However, to contribute a taxonomic discretion, more individuals of the plants should be included.

RAPD is the effective method for studying on genetic diversities, similarities and distances due to the fact that it is the study throughout genome and has a large number of characters enough, here it was 226 characters which were reliable as described by Weier *et al*. (1982) and Simpson (2006). Accordingly, there have been many publications that used RAPD in different fields as molecular systematics and environmental toxicology (for examples: Sudmoon *et al*. 2011, 2014, Tanee *et al*. 2012, 2016, Noikotr *et al*. 2013, Neeratanaphan *et al*. 2014, Sanubol *et al*. 2014, Suwannakud *et al*. 2014). In this study the RAPD method can effectively distinguish all *Dillenia* species (Figure 3). However, its quality of unreproducible method, it cannot be reasonable used in any study on specific genes or areas.

This research is important for identifying plant species in Thailand. It uses nucleotide variations in barcoding markers to determine the specific species, especially the medicinal plants, which will allow for the sustainable and worldwide use of these species for the preparation of medicinal natural products.

Acknowledgments

The authors are thankful for the financial support provided by the Thailand Research Fund through the Royal Golden Jubilee Ph.D. program (Grant No. PHD /00138/2554) that was awarded to Tikumporn Thooptianrat and Arunrat Chaveerach.

References

Alam, B.M., Rahman, S.M., Hasan, M., Khan, M.M., Nahar, K. & Sultana S. (2012) Antinociceptive and antioxidant activities of the *Dillenia indica* bark. *International Journal of Pharmacology* 8: 243–251. http://dx.doi.org/10.3923/ijp.2012.243.251

Blume, C.L. (1825) *Bijdragen tot de flora van Nederlandsch Indië*1. Lands Drukkerij, Batavia, pp. 1–42.

Blume, C.L. & Fischer, J.B. (1830) *Flora Javae* 34–35. J. Frank, Bruxelles, 24 pp.

CBOL Plant Working Group. (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United State*

of America 106: 12794–12797.

Chaveerach, A., Aungkapattamagul, S., Tanee, T., Noikotr, K. & Sudmoon, R. (2014) Genetic verification and chemical contents identification of *Allamanda* species (Apocynaceae). *Pakistan Journal of Pharmaceutical Sciences* 27: 417–424.

Chaveerach, A., Tanee, T. & Sudmoon, R. (2011) Molecular identification and barcodes for the genus *Nymphaea*. *Acta Biologica Hungarica* 62: 328–340.
http://dx.doi.org/10.1556/ABiol.62.2011.3.11

Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté, J., Pieters, L. & Vlietinck, A.J. (2002) Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal of Ethnopharmacology* 79: 213–220.
http://dx.doi.org/10.1016/S0378-8741(01)00384-1

Kerr, A.F.G. (1911) Contributions to the Flora of Siam II: List of Siamese plants with descriptions of new species. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* 1911: 7–60.

Craib, W.G. (1922) Contributions to the Flora of Siam. Additamentum XII. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)*. 1922: 161–174.

Dubey, C.P., Sikarwar, S.L.R., Khanna, K.K. & Tiwari, P.A. (2009) Ethnobotany of *Dillenia pentagyna* Roxb. in Vindhya region of Madhya Pradesh, India. *Indian Journal of Natural Products and Resources* 8: 546–548.

Finet, A. & Gngnepain, F. (1907) Dilléniacées. In: Lecomte, M. H. (ed.), *Flore générale de l'Indo-Chine* 1(1). Masson et Cie., Paris, pp. 12–28.

Gabay, O., Sanchez, C., Salvat, C., Chevy, F., Breton, M., Nourissat, G., Wolf, C., Jacques, C. & Berenbaum, F. (2010) Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. *Osteoarthritis and Cartilage* 18: 106–116.
http://dx.doi.org/10.1016/j.joca.2009.08.019

Gilg, E.F. (1893) *Naturlichen Pflanzenfamilien Nachträge* III (6). W. Engelmann, Leipzig, 180 pp.

Griffith, W. (1854) *Notulae ad Plantas Asiaticas* 4. Charles A. Serrao, Calcutta, 764 pp.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & De Waard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London Series B* 270: 313–321.
http://dx.doi.org/10.1098/rspb.2002.2218

Hollingsworth, P.M., Graham, S.W. & Little, D.P. (2011) Choosing and using a plant DNA barcode. *PLoS One* 6: e19254.
http://dx.doi.org/10.1371/journal.pone.0019254

Hoogland, R.D. (1951) Dilleniaceae. *In:* van Steenis, C.G.G.J. (Ed.) *Flora Malesiana. Series 1. Spermatophyta* 4(3). Noordhofi-Kolff N.V., Djakarta, pp. 141–174.

Hoogland, R.D. (1972) *Dillenia*. *In:* Smitinand, T. & Larsen, K. (Eds.) *Flora of Thailand* 2 (2). ASRCT Press, Bangkok, pp. 95–103.

Hooker, J.D. & Thomson, T. (1855) *Flora Indica* 2(1). W. Pamplin, London, 280 pp.

Hussain, Z., Amresh, G., Singh, S. & Rao, V.C. (2009) Antidiarrheal and antiulcer activity of *Amaranthus spinosus* in experimental animals. *Pharmaceutical Biology* 47: 932–939.
http://dx.doi.org/10.1080/13880200902950769

Jegadeeswari, P., Nishanthini, A., Muthukumarasamy, S. & Mohan, V.R. (2012) GC-MS analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). *Journal of Current Chemical & Pharmaceutical Sciences* 2: 226–232.

King, G. (1889) Materials for a Flora of the Malayan Peninsula. *The Journal of the Asiatic Society of Bengal* 58(2): 359–416.

Kritpetcharat, O., Kritpetcharat, P., Daduang, J., Daduang, S., Suwanrungruang, K., Khemtonglang, N., Bletter, N., Sudmoon, R.& Chaveerach, A. (2011) Using DNA markers and barcoding to solve the common problem of identifying dried medicinal plants with the examples of *Smilax* and *Cissus* in Thailand. *Journal of Medicinal Plants Research* 5: 3480–3487.

Kwanda, N., Noikotr, K., Sudmoon, R., Tanee, T. & Chaveerach, A. (2013) Medicinal parasitic plants on diverse hosts with their usages and barcodes. *Journal of Natural Medicines* 67: 438–445.
http://dx.doi.org/10.1007/s11418-012-0695-2

Li, X., Li, C.W., Cui, C.B., Li, M.M. & Fan, M. (2009) Chemical constituents of *Dillenia kerrii* and their activities on antitumor and anti-hypoxia *in vitro*. *Chinese Journal of Medicinal Chemistry* 19: 130–134.

Linnaeus, C. (1753) *Species Plantarum*. Impensis Laurentii Salvii, Stockholm, 1200 pp.

Loizou, S., Lekakis, I., Chrousos, P.G. & Moutsatsou, P. (2010) Beta-sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. *Molecular Nutrition & Food Research* 54: 551–558.
http://dx.doi.org/10.1002/mnfr.200900012

Loureiro, J. (1790) *Flora Cochinchinensis 1*, Ulyssipone, Lisbon, 749 pp.

Lu, H.M., Liang, Y.Z. & Chen, S. (2006) Identification and quality assessment of *Houttuynia cordata* injection using GC-MS fingerprint: A standardization approach. *Journal of Ethnopharmacology* 105: 436–440.
http://dx.doi.org/10.1016/j.jep.2005.11.018.

Macahig, R.A.S, Matsunami, K. & Otsuka, H. (2011) Chemical studies on an endemic Philippine plant: sulfated glucoside and seco-A-ring triterpenoids from *Dillenia philippinensis*. C*hemical and Pharmaceutical Bulletin* 59: 397–401. http://doi.org/10.1248/cpb.59.397

Mariajancyrani, J., Chandramohan, G., Brindha, P. & Saravanan, P. (2014) GC-MS analysis of terpenes from hexane extract of *Lantana camara* leaves. *International Journal of Advances in Pharmacy*, *Biology and Chemistry* 3: 37–41.

Martelli, U. (1886) *Malesia Raccolta 3*. Tip. del R. Instituto sordo-muti, Genova, 305 pp.

Martius, C.F.P. (1830) *Flora oder Allgemeine Botanischer Zeitung* 13. Die Gesellschaft, Regensburg, 144 pp.

Miller, P. (1753) *The Gardeners Dictionary Abridged fourth edition.* John and James Rivington, London, 505 pp.

Mitova, M., Taskova, R., Popov, S., Berger, R.G., Krings, U. & Handjieva, N. (2003) GC/MS analysis of some bioactive constituents from *Carthamus lanatus* L. Zeitschrift für Naturforschung C. *Journal of Biosciences* 58: 697–703. http://dx.doi.org/10.1515/znc-2003-9-1018

Monkheang, P., Sudmoon, R., Tanee, T., Noikotr, K., Bletter, N. & Chaveerach, A. (2011) Species diversity, usages, molecular markers and barcode of medicinal *Senna* species (Fabaceae, Caesalpinioideae) in Thailand. *Journal of Medicinal Plants Research* 5: 6173–6181. http://dx.doi.org/10.5897/JMPR11.1075

Muhit, A., Tareq, M.S., Apu, S.A., Basak, D. & Lslam, S.M. (2010) Isolation and identification of compounds from the leaf extract of *Dillenia indica* Linn. *Bangladesh Pharmaceutical Journal* 13: 49–53.

Neeratanaphan, L., Sudmoon, R. & Chaveerach, A. (2014). Genetic erosion in the freshwater snail *Filopaludina martensi* is affected by lead and cadmium. *Applied Ecology and Environmental Research* 12: 991–1001. http://dx.doi.org/10.15666/aeer/1204_9911001

Noikotr, K., Pinthong, K., Tanomtong, A., Sudmoon, R., Chaveerach, A. &Tanee, T. (2013) RAPD and barcode analyses of groupers species of the genus *Epinephelus*. *Genetics and Molecular Research* 12: 5721–5732. http://dx.doi.org/10.4238/2013.November.18.21

Office of the Forest Herbarium. (2013) *The encyclopedia of plants in Thailand*. Available from: http://web3.dnp.go.th/botany/detail_wordsci.aspx?groupchar=D (accessed: 11 November 2013)

Parvin, S., Kader, M.A., Muhit, M.A., Haque, M.E., Mosaddik, M.A. & Wahed, M.I.I. (2011) Triterpenoids and phytosteroids from stem bark of *Crataeva nurvala* Buch. Ham. *Journal of Applied Pharmaceutical Science* 1: 47–50.

Roemer, J.J. & Schultes, J.A. (1830) *Systema Vegetabilium* 7(2). J.G. Cottae, Stuttgardtiae, pp. 755–1815.

Rohlf, F.J. (1998) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System, Version 2.1. Applied Biostatics, New York, USA, 31 pp.

Rolfe, R.A. (1884) On the flora of Philippine Islands, and its probable derivation. *Journal of the Linnean Society, Botany* 21: 283–316.

Roxburgh, W. (1795) *Plants of the Coast of Coromandel 1*. W. Bulmer and Co., London, 68 pp.

Sanubol, A., Chaveerach, A., Sudmoon, R., Tanee, T. & Liehr, T. (2014) Verification of selected *Piper* species (Piperaceae) using morphological characters, molecular data, and chemical constituents. *Malayan Nature Journal* 66: 60–81.

Simpson, M.G. (2006) *Plant Systematics*. Elsevier Academic Press, California, 590 pp.

Siripiyasing, P., Kaenratana, K., Mokkamul, P. & Chaveerach, A. (2013) Molecular assessment for genetic identification and stability of *Cymbidium sanderae* (Orchidaceae). *Pakistan Journal of Botany* 45: 519–523.

Siripiyasing, P., Kaenratana, K., Mokkamul, P., Tanee, T., Sudmoon, R. & Chaveerach, A. (2012) DNA barcoding of *Cymbidium* species (Orchidaceae) in Thailand. *African Journal of Agricultural Research* 7: 393–404. http://dx.doi.org/10.5897/AJAR11.1434

Smith, J.E. (1806) *Exotic Botany* 2. R. Taylor and Co., London, 122 pp.

Sudmoon, R., Chaveerach, A. & Tanee, T. (2016) Analysis of genetics and chemical contents relation compared to commonly used *Cissus quadrangularis* and barcode markers of some Thailand *Cissus* species. *Pakistan Journal of Pharmaceutical Sciences* 29: 65–75.

Sudmoon, R., Chaveerach, A., Sanubol, A., Monkheang, P., Kwanda, N., Aungkapattamagul, S., Tanee, T., Noikotr, K., Chuachan, C. & Kaewdoungdee, N. (2014) Identifying efficiency in herbal medicine *Cinnamomum* species (Lauraceae) using banding patterns and sequence alignments of *rpoB*, *rbcL* and *matK* regions. *Chiang Mai Journal of Science* 41: 1094–1108.

Sudmoon, R., Tanee, T. & Chaveerach, A. (2011) *Piper protrusum* (Piperaceae), a new species from southern Thailand based on morphological and molecular evidence. *Journal of Systematics and Evolution* 49: 468–475.

Sudmoon, R., Tanee, T., Wongpanich, V., Bletter, N. & Chaveerach, A. (2012) Ethnobotany and species specific molecular markers of some medicinal *sakhan* (*Piper*, Piperaceae). *Journal of Medicinal Plants Research* 6: 1168–1175.

Sunder, S., Rai, A.K., Praveen, S. & Singh, P.A. (2011) Antidiarrhoeal activity of *Aerva lanata* in experimentally induced diarrhea in rats. *Pharmacology Online* 2: 921–928.

Suwannakud, K.S., Sudmoon, R., Tanee, T. & Chaveerach, A. (2014) Genetic relations related to chemical containing and the efficient barcodes by *psbA-trnH* spacer and its combinations with *rbcL* and *matK* on *Gardenia* species. *Journal of Applied Biological Science*

8: 65–78.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
http://dx.doi.org/10.1093/molbev/mst197

Tanee, T., Chadmuk, P., Sudmoon, R., Chaveerach, A. & Noikotr, K. (2012) Genetic analysis for identification, genomic template stability in hybrids, and barcodes of the *Vanda* species (Orchidaceae) of Thailand. *African Journal of Biotechnology* 11: 11772–11781.
http://dx.doi.org/10.5897/AJB11.3992

Tanee, T., Sudmoon, R., Thamsenanupap, P. & Chaveerach, A. (2016) Effect of cadmium on DNA changes in *Ipomoea aquatica* Forssk. *Polish Journal of Environmental Studies* 25: 303–307.
http://dx.doi.org/10.15244/pjoes/60726

Tate, J.A. & Simpson, B.B. (2003) Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. *Systematic Botany* 28(4):723–737.
http://dx.doi.org/10.1043/02-64.1

Wang, W., Wu, Y., Yan, Y., Ermakova, M., Kerstetter, R. & Messing, J. (2010) DNA barcoding of the Lemnaceae, a family of aquatic monocots. *BMC Plant Biology* 10: 205
http://dx.doi.org/10.1186/1471-2229-10-205

Weier, T.E., Stocking, C.R., Barbour, M.G. & Rost, T.L. (1982) *Botany*. John Wiley and Sons, New York, 720 pp.

Yap, P.S.X., Chong, Y.T.E., Wong, Y.Y., Lim, X.Y., Ang, J.H., Goh, H.S.L., Lai, M.X.S., Mai, C.W., Buru, A.S., Pichika, M.R. & Lim, S.H.E. (2013) Antibacterial and antifungal testing of the different extract of *Dillenia obovata* (Blume) Hoogl. *World Journal of Pharmacy and Pharmaceutical Sciences* 2: 3946–3962.

Yang, J.Y., Wu, C.F., Wang, F., Song, H.R., Pan, W.J. & Wang, Y.L. (2003) The serotonergic system may be involved in the sleep-inducing action of oleamide in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 368: 457–462.
http://dx.doi.org/10.1007/s00210-003-0843-z

Yeshwante, S.B., Juvekar, A.R., Nagmoti, D.M., Wankhede, S.S., Shah, A.S., Pimprikar, R.B. & Saindane, D. (2009) Anti-inflammatory activity of methanolic extracts of *Dillenia indica* L. leaves. *Journal of Young Pharmacists* 1: 63–66.
http://dx.doi.org/10.4103/0975-1483.51885