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A chemotaxonomic study of essential oils from the leaves of genus *Clerodendrum* (Verbenaceae) native to Taiwan

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Abstract. Fifty-four mature-leaf samples of nine *Clerodendrum* taxa native to Taiwan were collected from various locations on the island. Each sample was steam-distilled, and the resulting essential oil was analyzed by gas chromatography. The relationship among taxa was analyzed by cluster analysis of the gas chromatogram data (463 peaks). We assumed that peaks with the same retention time on different chromatograms were the same compound. The cluster analysis indicated a congruence between morphological and chemical relationships at the intraspecific level. All intraspecific taxa were linked as distinct clusters. At the interspecific level, there was congruence and discrepancy between morphological and chemical relationships. The results of the present study support the taxonomic treatment in which the white-flowered variant of *C. paniculatum* is recognized as a variety of the species. The results also support the existence of *C. trichotomum* var. *fargesii* and *C. trichotomum* var. *ferrugineum* as intraspecific taxa of *C. trichotomum*.

Keywords: Chemotaxonomy; *Clerodendrum*; Essential oils; Taxonomy; Verbenaceae.

Introduction

The genus *Clerodendrum* (Verbenaceae) is widely distributed in the tropics and subtropics, with a few species extending into the temperate regions. The species number has been estimated to be five hundred and sixty (Moldenke, 1971) and five hundred and eighty (Munir, 1989). The genus was first described by Linnaeus in 1753, based on the type species *Clerodendrum infortunatum* from India. In 1763, Adanson changed the Latinized form, *Clerodendrum*, into the Greek form, *Clerodendron*. In 1942, Moldenke re-adopted Linnaeus' original Latinized form, and the practice has been followed by most taxonomists. The genus comprises small trees, shrubs (sometimes climbing), and (rarely) herbs. Morphologically it is characterized by opposite and exstipulate leaves, tetragonal or terete stems, terminal or axillary cymose inflorescence or panicles, hypogynous bisexual flowers, persistent calyx, cylindrical corolla-tube with spreading 5-lobes at the top, epipetalous and exserted stamens, long-exerted style and short-bifided stigma, imperfectly 4-celled ovary, exalbuminous seeds, and endocarp separating into 2 to 4 stony pyrenes.

The species of *Clerodendrum* native in Taiwan are distributed from the coasts to low-to-middle altitudes in the mountains. The treatments by taxonomists are diverse (e. g. Hayata, 1912; Kanehira, 1917; Li, 1963; Moldenke, 1971; Hsiao, 1978; Liu and Liao, 1981; Hue and Chen, 1982; Liu et al., 1988). The indisputable taxa includes *C. canescens* Wall., *C. cyrtophyllum* Turcx., *C. inerme* (L.) Gaertner, and *C. philippinum* Schauer forma *multiplex* (Sweet) Moldenke. Among the more controversial taxa, several authors (Moldenke, 1971; Hsiao, 1978; Liu et al., 1988) treated *C. trichotomum* Thunb. as comprising three varieties, while others (Li, 1963; Liu and Liao, 1981) recognized the existence of only two varieties, with the exclusion of *C. trichotomum* var. *ferrugineum*. Hue and Chen (1982) recognized the species without any intraspecific division. Another controversial taxon is the white-flowered variant of *C. paniculatum* L., which was published as a new variety by Hemsley in 1895 and named *C. paniculatum* var. *albiflorum* Hemsley. Hsieh (1973), however, lowered its rank to a form and named it *C. paniculatum* L. forma *albiflorum* (Hemsl.) Hsieh. The existence of *C. intermedium* Cham. in Taiwan is questionable even though it was treated as a native species by Hsiao (1978) and Liu (1981). In the present study, a plant close to the description of *C. intermedium*, and having leaves without lobes, was collected from Kenting Botanic Garden (*Lin* 1050). This solitary specimen is thought to be a cultivated plant, and this species is not included in the present study.

The information gathered from essential-oil studies has proved to be of value in the taxonomic and evolutionary investigations of plants (Von Rudloff, 1975; Southwell and Stiff, 1990; Brophy and Clarkson, 1992; Brothy et al., 1994). The purpose of the present study is to re-evaluate the relationships among native taxa of *Clerodendrum* in Taiwan, based on their essential-oil compositions. It is expected that these data will shed light on the natural relationships of the taxa.

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Materials and Methods

Materials

Mature-leaf samples and voucher specimens of 54 plants belonging to 9 native taxa of *Clerodendrum* were collected from various locations in Taiwan (Table 1). Voucher specimens are on deposit at TCB (Herbarium, National Chung Hsing University).

Table 1. Sources of the samples studied.

Taxa	Collector's field No.	Locality (County)
C. canescens	Lin 916	Longtang (Ilan)
	Lin 921	Hoping (Taichung)
	Lin 933	Chungho (Taichung)
C. cyrtophyllum	Lin 226	Longtang (Ilan)
	Lin 228	Longtang (Ilan)
	Lin 231	Chunghsingling (Taichung)
	Lin 232	Chunghsingling (Taichung)
	Lin 236	Dahan Forest Road (Pintung)
C. inerme	Lin 704	Dadu (Taichung)
	Lin 714	Sutiping (Taitung)
	Lin 723	Baisawuan (Pintung)
	Lin 724	Baisawuan (Pintung)
	Lin 726	Wuanlitung (Pintung)
	Lin 727	Wuanlitung (Pintung)
C. paniculatum	Lin 136	Tienbien (Taichung)
	Lin 140	Chuchi (Chiayi)
	Lin 145	Chuchi (Chiayi)
	Lin 148	Chuchi (Chiayi)
	Lin 1001	Kantou (Yunling)
	Lin 1011	Dahan Forest Road (Pintung)
	Lin 1020	Tienbien (Taichung)
	Lin 1051	Kanting (Pintung)
C. paniculatum var. *albiflorum*	Lin 157	Hsinhua (Tainang)
	Lin 187	Chushan (Nantou)
	Lin 1501	Kanting (Pintung)
	Lin 1521	Tienbien (Taichung)
C. philippinum f. *multiplex*	Lin 305	Danshue (Taipei)
	Lin 306	Danshue (Taipei)
	Lin 309	Danshue (Taipei)
	Lin 316	Tienli (Taichung)
	Lin 332	Chushan (Nantou)
	Lin 333	Tienli (Taichung)
	Lin 336	Kantou (Yunling)
	Lin 337	Kantou (Yunling)
	Lin 342	Yangmingshan (Taipei)
C. trichotomum	Lin 820	Ginshan (Taipei)
	Lin 822	Ginshan (Taipei)
	Lin 830	Ginshan (Taipei)
	Lin 851	Ginshan (Taipei)
	Lin 852	Ginshan (Taipei)
	Lin 853	Ginshan (Taipei)
C. trichotomum var. *fargesii*	Lin 862	Kanting (Pintung)
	Lin 863	Kanting (Pintung)
	Lin 864	Liduo (Pintung)
	Lin 866	Liduo (Pintung)
	Lin 867	Gankuo (Pintung)
	Lin 868	Dahan Forest Road (Pintung)
	Lin 894	Yuoshu (Nantou)
	Lin 895	Yuoshu (Nantou)
C. trichotomum var. *ferrugineum*	Lin 827	Wushuo (Nantou)
	Lin 828	Wushuo (Nantou)
	Lin 844	Ali (Taitung)
	Lin 845	Ali (Taitung)
	Lin 893	Zunchuguan (Nantou)

Capillary Gas Chromatography

For each sample, 250 grams of fresh mature leaves were steam-distilled to obtain the essential oil. The essential oils were analyzed by capillary gas chromatography with a Hitachi G3000 gas chromatograph equipped with a flame ionization detector (FID) and a Hitachi D2000 recorder. A 60-meter DB-5 fused silica capillary column 0.25 mm in diameter (J & W, USA.) was used for the separation of essential oil components. We injected 0.2 μl of each essential oil sample. The GC separating conditions were as follows: (N_2 carrier-gas flow rate) make-up gas, 30 ml min^{-1}, capillary column, 0.76 ml min^{-1}; H_2 flow rate, 30 ml min^{-1}; air-flow rate, 300 ml min^{-1}; column pressure, 1 kg cm^{-2}; split ratio, 1/10; injection-port temperature, 260°C; detector temperature, 260°C; oven temperature, 60°C to 230°C at 2°C per min, then 230°C for 50 min.

Numerical Taxonomic Analysis

Nine taxa were treated as OTUs (operational taxonomic units). The values of gas chromatogram peaks were recorded as integrated-area percentages and were used as taxonomic characters. The peak values were averaged over the samples used, and this value was used in subsequent cluster analysis. The average taxonomic distances between each OTU-pair was calculated, and the resulting distance matrix was used in a UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis (Sneath and Sokal, 1973). The formula for average taxonomic distance is

$$\sqrt{\Sigma_n \frac{1}{n}(Xi - Xj)^2}$$

where n represents character number, and Xi and Xj represent the average percentage data of OTU i and OTU j, respectively. The numerical taxonomic analysis was performed using NTSYS-pc (version 1.80) software (Rohlf, 1993).

Results and Discussion

We identified 463 peaks, with the assumption that peaks with the same retention time on different chromatograms were the same compound. The data on these 463 peaks for each of the 54 samples can be found in Lin (1992). The first 5 major peaks and their percentages for each taxon are listed in Table 2. Based on the composition of the 5 major peaks, *C. canescens*, with peaks 379 and 455 as the first and second major peaks, is a distinct taxon. All other taxa have peaks 79 and 126 as their first and second major peaks, except for *C. paniculatum*, in which the rank order is reversed. The unusually high percentage of peak 455 in *C. canescens* is unique among the studied taxa. *Clerodendrum inerme* is distinct from the other taxa, in having peak 434 as its 5th major peak. *Clerodendrum cyrtophyllum* has a very high percentage (35.21%) of peak 79, and peak 47 is the 5th major peak.

Table 2. Five major peaks and their percentages in the taxa studied.

Taxon	1	2	3	4	5
C. canescens	379[a] (9.70%)[b]	455 (7.62%)	79 (6.40%)	126 (5.37%)	43 (4.85%)
C. inerme	79 (14.91%)	126 (5.34%)	379 (3.62%)	32 (2.47%)	434 (1.94%)
C. cyrtophyllum	79 (35.21%)	126 (6.57%)	379 (4.49%)	43 (2.81%)	47 (2.13%)
C. philippinum multiplex	79 (40.60%)	126 (13.52%)	379 (7.37%)	43 (2.77%)	84 (2.60%)
C. paniculatum	126 (14.50%)	79 (14.07%)	379 (4.00%)	46 (3.98%)	113 (2.89%)
C. paniculatum albiflorum	79 (22.82%)	126 (14.56%)	379 (5.82%)	113 (5.24%)	44 (2.73%)
C. trichotomum	79 (14.40%)	126 (12.38%)	409 (11.36%)	379 (5.88%)	43 (3.17%)
C. trichotomum fargesii	79 (15.24%)	126 (8.49%)	379 (6.83%)	409 (6.01%)	215 (4.47%)
C. trichotomum ferrugineum	79 (14.72%)	126 (9.37%)	379 (8.35%)	409 (7.98%)	32 (6.10%)

[a]Peak number.
[b]Percentage of total peak area.

Table 3. Average taxonomic distances of the OTU-pairs.

C. canescens	0.0000								
C. cyrtophyllum	1.4127	0.0000							
C. inerme	0.6664	0.9909	0.0000						
C. paniculatum	0.7530	1.0822	0.5254	0.0000					
C. paniculatum var. *albiflorum*	1.0010	0.7502	0.6694	0.4567	0.0000				
C. philippinum f. *multiplex*	1.7044	0.5034	1.2958	1.2781	0.8920	0.0000			
C. trichotomum	0.8272	1.1405	0.6449	0.5731	0.7175	1.3302	0.0000		
C. trichotomum var. *fargesii*	0.6799	1.0205	0.4162	0.5216	0.6407	1.2618	0.4149	0.0000	
C. trichotomum var. *ferrugineum*	0.7198	1.0830	0.5230	0.5579	0.6913	1.3089	0.3461	0.3548	0.0000

This is unique for this species. *Clerodendrum philippinum* forma *multiplex*, similar to *C. cyrtophyllum*, also has an exceptionally high percentage of peak 79 (40.6%). Its 5th major peak (peak 84), however, is not in the list of major peaks of any other taxa. *Clerodendrum paniculatum* has roughly the same percentages of peaks 126 and 79, while its white-flowered variant has higher percentage of peak 79. The 4th and 5th major peaks of these two taxa are also different. The first 4 major peaks of *C. trichotomum* and its two variants are similar, except that the order of the 3rd and 4th major peaks is different in *C. trichotomum*. The 5th major peak of each of these three taxa are also different from each other.

The average taxonomic distances of the OTU-pairs are listed in Table 3. The phenogram from the cluster analysis is shown in Figure 1. In this phenogram, *C. cyrtophyllum* and *C. philippinum* forma *multiplex* form a distinct group, while the rest of the taxa form another group. The similarity of the total composition of the essential oil of *C. cyrtophyllum* and *C. philippinum* forma *multiplex* is also reflected in their five major components—the first 4 major peaks of these two taxa are similar (Table 2). In the other group, *C. canescens* is the most distinct taxon. This is not unexpected, because it possesses distinct major components. The essential oil composition of *C. inerme* is close to that of *C. trichotomum* and its two varieties. As expected, *C. trichotomum* and its 2 varieties link together to form a cluster. Along with their morphological relationship, the essential oil composition of *C. trichotomum* is closer to that of *C. trichotomum* var. *ferrugineum* than to that of *C. trichotomum* var. *fargesii*. *Clerodendrum trichotomum* var. *fargesii* is the most isolated taxon among these three taxa, but the linkage level of this cluster indicates that these three taxa have the most-

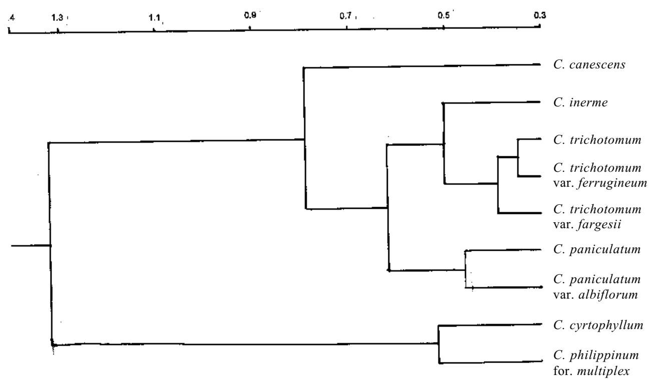

Figure 1. Phenogram based on 463 essential oil characters.

similar essential oil compositions among the 9 taxa studied. As expected, *C. paniculatum* and its white-flowered variant also form a cluster, but the linkage level of this cluster is higher than those of *C. trichotomum* and its two varieties. In a previous study of *C. paniculatum* and its white-flowered variant (Hsiao and Lin, 1990) we concluded that the morphological and chemical evidence supported the taxonomic treatment in which the white-flowered variant is treated as a variety of *C. paniculatum* instead of as a form. The results of the present study reinforce this conclusion, and we suggest that the scientific name *C. paniculatum* var. *albiflorum* Hemsl. be adopted.

The results of the present study show that there is a strong congruence between morphological and chemical relationships at the intraspecific level. In the essential oil phenogram, three intraspecific taxa of *C. trichotomum* and two intraspecific taxa of *C. paniculatum* are clustered as distinct groups. At the interspecific level, there is congruence and discrepancy between morphological and chemical relationships. Stenzel et al. (1988) studied the phenetic relationships of 129 *Clerodendrum* taxa, based on 52 morphological characters, using the methods of cluster analysis and minimum spanning tree. They concluded that these taxa could be divided into two subgenera and seven groups. With the exception of *C. paniculatum* var. *albiflorum* and *C. trichotomum* var. *ferrugineum*, the subjects of the present study were included in their study. In their results, *C. cyrtophyllum* and *C. inerme* belonged to group 1; *Clerodendrum philippinum*, *C. trichotomum*, and *C. trichotomum* var. *fargesii* belonged to group 2; *Clerodendrum paniculatum* belonged to group 3; *Clerodendrum canescens* belonged to group 4. All these taxa are members of the so-called 'subgenera B'. In the present study, we reveal that *C. cyrtophyllum* is closer to *C. philippinum* forma *multiplex* than to *C. inerme*, and *C. inerme* is closer to the members of *C. trichotomum* than to *C. cyrtophyllum*. The members of *C. paniculatum* link to the group that comprises *C. inerme* and members of *C. trichotomum*. The essential oil composition of *C. canescens* is distinct, though it has relationships with members of *C. paniculatum*, *C. trichotomum*, and *C. inerme*. Several observed discrepancies between morphological and chemical relationships in the studied taxa may be the result of the difference in evolutionary directions of these two categories of characters.

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台灣產馬鞭草科海州常山屬植物精油之化學分類學研究

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自台灣各地採得海州常山屬 9 個分類群之 54 成熟葉樣本。各樣本以蒸氣蒸餾法蒸得之精油用毛細管氣相層析法加以分析。由氣相層析圖所得波峰數據經由歸群分析顯示出各分類群在精油資料方面之關係。假設各層析圖上具有相同滯留時間的波峰爲相同波峰，本研究共得 463 個波峰。根據此 463 個化學性狀所做之歸群分析結果顯示種內分類群在形態關係和化學關係上相當一致，各種內分類群皆連結成各自之群團。在種間的層次上，形態關係和化學關係有若干吻合，但也有一些不一致。本研究結果支持白龍船花應被當做是龍船花的一個變種。同時也支持海州常山具有法氏海州常山和鏽毛海州常山兩變種。

關鍵詞：海州常山屬；馬鞭草科；化學分類學；分類學；精油。