SYSTEMATIC MORPHOLOGY, ANATOMY AND NUMERICAL EVALUATION OF *Clerodendrum* L. IN PENINSULAR MALAYSIA

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ABSTRACT

The aim of this study was to investigate the *Clerodendrum* interspecific variations toward morphological and anatomical characters in order to determine their taxonomic significance. Both anatomical and morphological characters of taxonomic significance were then used to construct dichotomous key for species of the genus. The distribution of the genus in designated locations were also recorded and mapped. A total number of 16 *Clerodendrum* species and *Tectona grandis* as an out-group species were collected from several reserved forest in Peninsular Malaysia. The herbarium voucher specimens were carefully examined and the characters were recorded. Anatomical slide of leaf transverse sections was prepared using modification of standard microtechnique method. Observed morphological and anatomical data were numerically analyse using MultiVariate Statistical Package (MVSP) and Phylogeny Analysis Using Parsimony (PAUP). Result shows morphological character are of taxonomic significance at genus level. Leaf shape, inter 2nd vein, leaf venation, petiole length and petiole hair proved to be of diagnostic characters. In contrary, anatomical characters are highly significant at species level. Type of midrib outline, petiole vascular bundle and presence of subsidiary bundles proved to be unique toward almost all members. Multivariate analysis of both morphological and anatomical characters shows an overall high GGSc at 0.66 – 0.97. PAUP analysis however, favour morphological characters over anatomical characters with CI, RI and HI recorded at 0.6121, 0.420 and 0.3879 respectively. Therefore, these findings support the classification of Olmstead in 1995 and partly of Yuan et. al, in 2010 based on molecular classification. It is concluded, both morphological and anatomical characters are of high taxonomic value but at different taxanomic levels. *C. chinense* and three unnamed species are subjected to further study for species confirmation. This study implicates that both morphological and anatomical characters can be used to complements current classification of *Clerodendrum* and serve as important taxonomic references.

PENILAIAN SISTEMATIK MORFOLOGI ANATOMI DAN NUMERIKAL TERHADAP *Clerodendrum* L. DI SEMENANJUNG MALAYSIA

ABSTRAK

Kajian ini bertujuan menentukan variasi interspesifik genus *Clerodendrum* dari segi ciri morfologi dan anatomi dan seterusnya menentukan kepentingan taksonomi dalam membina kedua-dua kunci anatomi dan morfologi terhadap genus tersebut. Taburan genus di lokasi yang ditentukan juga direkodkan dan dipetakan. Sejumlah 16 spesis *Clerodendrum* dan *Tectona grandis* sebagai kumpulan rujukan dikutip dari beberapa hutan simpan di Semenanjung Malaysia. Spesimen baucer herbarium dan daun segar diperiksa secara berhati-hati dan direkodkan cirinya. Sampel herbarium telah diperiksa dengan teliti dengan menggunakan mikroskop pembedahan. Karakter daripada pemerhatian direkodkan. Slaid anatomi disediakan menggunakan kaedah ubahsuaian mikroteknik standard. Ciri morfologi dan anatomi yang diperhatikan dianalisis secara numerikal menggunakan *MultiVariate Statistical Package (MVSP)* dan *Phylogeny Analysis Using Parsimony (PAUP)*. Dapatan kajian menunjukkan kebanyakan karakter morfologi mempunyai kepentingan taksonomi di peringkat genus. Bentuk daun, peruratan *inter 2*nd, peruratan daun, panjang tangkai dan trikom petiole, terbukti menjadi karakter diagnostik. Sebaliknya, ciri anatomi mempunyai nilai signifikan pada spesis. Jenis midrib, jenis berkas vaskular dan kehadiran vaskular subsidiari terbukti unik bagi kebanyakan ahli genus *Clerodendrum*. Analisis *multivariate* bagi morfologi dan anatomi menunjukkan *GGSc* berada pada nilai yang tinggi dicatatkan pada 0.66 – 0.97. Analisis PAUP bagaimanapun memihak kepada kriteria morfologi berbanding anatomi dengan IK, IP dan IH dicatatkan pada 0.6121, 0.520 dan 0.3879. Dapatan kajian juga menunjukkan menyokong kepada klasifikasi Olmstead pada tahun 1995 dan sebahagian daripada Yuan et. al pada tahun 2010. Kesimpulannya, karakter morfologi dan anatomi mempunyai nilai taksonomi yang tinggi pada aras yang berbeza. *C. Chinense* dan tiga spesis yang belum dinamakan disarankan untuk mendapat kajian lanjut. Implikasi kajian menunjukkan karakter yang dikenal pasti boleh digunakan untuk melengkapi pengkelasan semasa *Clerodendrum* dan dijadikan rujukan taksonomi yang penting.

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No	Abbreviation	Full name
1	CI	Consistency Index
2	GGSc	Gower General Similarity Coeefficient
3	HI	Homoplasy Index
4	IH	Indeks Homoplasi
5	IK	Indeks Konsistensi
6	IP	Indeks Pengekalan
7	MVSP	MultiVariate Statistical Package
8	PAUP	Phylogeny Analysis Using Parsimony
9	RI	Retention Index

CHAPTER 1

INTRODUCTION

1.0 Lamiaceae

Lamiaceae or formerly known as labiate are a family of flowering plant. The family Lamiaceae (Labiate) is the largest of the order Lamiales consist between 20 and 30 families' worldwide order including nor depending on family boundaries. Harley et. al., (2004) stated that, Lamiaceae family are composed of trees, shrubs, sub shrubs or perennial or annual herbs, rarely climbers, aromatic or not. The family is widely known for its fine ornamental or culinary herbs such as thyme, sage, rosemary and it is also rich source of essential oil for flavorings and perfume industry. Lamiaceae family are

widely cultivated not only for their aromatic qualities but these family is easily cultivated. The plant in these family is grown either for food purposes or for decorative foliage.

Morphology, chemistry and molecular phylogeny that were recently studied, have caused major changes in the family classification, causing in the addition of a large number of genera, which are traditionally placed in Verbenaceae (Harley et. al., 2004). This has caused an increasing number of genera and species into the family Lamiaceae (Labiate). Harley et. al., (2004) also state that the order is currently has an outstanding 240 genera and 7200 species, which are occurring in tropical to temperate areas worldwide. In tropical Asia, Lamiaceae compose of about 13 genera with an outstanding 120 species.

1.1 Introduction to the genus *Clerodendrum*

The genus *Clerodendrum* was first mention by Linnaeus in 1753 consist of only one species that is *C. infortunatum* L. The genus was first placed in Verbenaceae before it was placed in the family Lamiaceae accordance to resircumscription of family boundaries determined based on molecular and morphological phylogenic evidence (Harley et. al., 2004).

The genus *Clerodendrum* composed of shrubs or small trees which have large inflorescences of white or brightly-colored zygomorphic flowers. Most of *Clerodendrum* are important ornamentals or have medicinal properties. Some are pernicious weeds (Shrivastava & Patel 2007)

Harley et. al., (2004) stated that *Clerodendrum* is a genus of flowering plant which is also known as Glory bower, Bag flower and Bleeding-heart. Currently it is classified in the subfamily of *Ajugoidae* as one of the genera that has been transferred from the family Verbenaceae into Lamiaceae based on phylogenetic analysis of morphological and molecular data conduct by Wagstaff et. al., (1998).

The number of species in the genus varies widely. Yuan et. al., (2010) state of about 150 species while Hartley et. al., (2004) state of about 450 species. The number varies as a result of some of the species formerly placed in the genus being transferred into *Rotheca*, *Volkameria* and *Ovieda* (Yuan et. al., 2010). Shrivastava & Patel (2007) state that the genus *Clerodendrum* composed of more than 500 species in which are richly important with ethno-medicinal properties.

Shrivastava & Patel (2007) also state that *Clerodendrum* are widely distributed in tropical and subtropical region of the world. As most of the genus *Clerodendrum* species are important in term of ethno-medicinal, it is highly important to properly identify these species.

The genus is native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia, but with a few in the tropical Americas and northern Australia, and a few extending north into the temperate zone in eastern Asia (Harley et. al., 2004).

1.2 Research Background

This study focuses on *Clerodendrum* genera in Peninsular Malaysia. The sample were collected from different geographical areas and its distribution mapped to determine its diversity in Peninsular Malaysia. This study is designated to clarify hence valued its anatomical and morphological taxonomic significance that might help in determining interspecific and intraspecific relationship between each member of the genus.

Based from past research, only molecular analysis has been done toward the genus comprehensively. A very limited, non-comprehensive numbers of species have undergone anatomical and morphological analysis hence the genus are due to monographic revision. *Clerodendrum* are known to have taxonomic problem due to multiple molecular studies that had been carried out removing some of the members of the genus. The diversity of the species can be found in Peninsular Malaysia are also in question due to finding from Wearn & Mabberly (2011) indicate that some of the recorded species may have been extinct from the region hence conflicting the data made

by Turner (1995). Therefore, these studies will include diversity, morphological and anatomical analysis characteristic of the genus *Clerodendrum*.

1.3 Problem statement

Lamiaceae (Labiate) and Verbenaceae have long been recognized having a close relationship (Cronquist, 1981). The two families shared zygomorphic flower, opposite leaves and a bicarpellate gynoecium. Labiate members are generally distinguished from Verbenaceae by having a deep four-lobed ovary with a gynobasic style. However, Verbenaceae has an unlobed ovary with terminal style (Wagstaff et. al., 1995). *Clerodendrum* is one of the main genera of Lamiaceae. It was first defined to belong to Verbenaceae but was corrected into Lamiaceae accordance to resircumscription of family boundaries determined based on molecular based study perform by Olmstead et. al., (1993), Wagstaff et. al., (1998), Cantino et. al., (1992) and Harley et. al., (2004).

Clerodendrum is an old-world genus. Most upon which are native to subtropical and tropical regions. Recently, the species has gone under some taxonomic changes. Over the last two centuries the circumscription of the large, pan-tropical genus *Clerodendrum* has changed frequently, as different authorities have added or removed taxa on the basis of various morphological characters (Steane et al., 2004).

Studies carried out by Steane et. al., (1997) shows *Clerodendrum* was first understood as a polyphyletic group. In order to create monophyletic *Clerodendrum*, Steane carried out 3 consecutive studies based on restriction site analysis, ndhF, chloroplast DNA, ITS sequence and nrDNA in 1998, 1999 and 2004. In the process two subgenera, subgenus *Clerodendrum* – section *Konocalyx* and subgenus *Cyclonema* and group centred on the African *C. Myricoides* complex was removed resulting in the revival of genus *Rotheca*. The studies first conclude that there are offending genus namely *Tetracleae* but then dismiss as another finding shows an unresolve relationship between Asian and African *Clerodendrum*, Pan Tropical Coastal *Clerodendrum* and a clade comprising *Aegiphilia*, *Amasonia* and *Tetracleae*. Yuan et. al., (2010) deliminate *Clerodendrum* into monophyletic group by removing Pan Tropical Coastal clade resulting in the revival of *Volkameria* and *Ovieda*.

Wearn & Mabberly (2011) in other studies stated that several of Clerodendrum species has not been found in Malesiana for more than 50 years, for example *C. umbratile* King & Gamble and presumably distinct from the region. They further claimed aside from molecular work done by Steane (2004), there were no morphological revision for nearly a century as its still relies upon Schauer (1847) and Lam (1919). From their studies, they concluded that it is fundamental that Flora Malesiana require a revision of monographic intensity as some of the species' mention were incorrectly understand of its distribution as only a few species are mention but not specifically revised on its location or character.

Turner (1995), revies a total of 16 *Clerodendrum* in his studies on Vascular Plant of Malaya but this was already denied by the work done by Wearn & Mabberly (2011) which indicate *C. umbratile* King & Gamble was presumably extinct from the region.

Constant confusion on the members of the genus (Wearn & Mabberly, 2011) and long unrevised of the systematics status as mention earlier made in this study an urgency to be conducted. Furthermore, large number of *Clerodendrum* are used as medicine worldwide (Shrivastava & Patel(a), 2007), therefore a current valid revised classification and key to the species identification is also very much needed. The data of *Clerodendrum* distribution will then contribute in understanding biodiversity and contribute to conservation of the species from extinction, especially in Peninsular Malaysia.

1.4 Research objective

a) To identify the diversity of the species in the genus *Clerodendrum* in the Peninsular Malaysia.

b) To determine the interspecific variation towards the morphological and anatomical characteristics of the species in the genus *Clerodendrum*.

c) To determine the taxonomic values of the anatomical, and morphological characteristics of the *Clerodendrum*.

d) To build anatomical and morphological key based on leaf and stem of Clerodendrum in Peninsular Malaysia

1.5 Research Question

a) What is the diversity of the species in the genus *Clerodendrum* in the Peninsular Malaysia?

b) Is there any interspecific variation towards the morphological and anatomical characteristics of the species in the genus *Clerodendrum*?

c) What are taxonomic values of the anatomical, and morphological characteristics of the *Clerodendrum*?

d) Are anatomical and morphological key based on leaf and stem of *Clerodendrum* in Peninsular Malaysia can be built?

1.6 Research Significant

Clerodendrum is a large genus which comprise of about 150 species (Yuan et. al., 2010). A major taxonomic conflict faced by this genus in the last two centuries has made it rather confusing. *Clerodendrum* was first placed in Verbenaceae before it is

removed and placed into Lamiaceae family. Constants adding and removing species of the genus add confusion toward the genus (i.e., *Volkamrie*, *Rotheca* and *Ovieda*) (Yuan et. al., 2010).

A number of researchers also claimed that the genus is not monophyletic but it is rather separated into three major clades related to its geographical distribution that is an Asian clade, an African clade and Pan-tropical coastal clade. It is compulsory to resolve the problem regarding systematic classification arise within the genus. Finding for both anatomy and morphology does not fit any known past research studies.

Clerodendrum is a genus that are rich with ethno-medicinal properties. This research is important in order to clear the confusion aroused on the genus and to correctly identify the species. A lack of study on the genus carried out in Malaysia also contribute to the importance of this research.

1.7 Operational Definition

1.7.1 Plant Morphology

Kaplan (2001) defined plant morphology as the studies of external appearances and structure of plant that are not limited only to leaves, shoots, flower and stem. Inherently he deduced that plant morphology are the studies that defined and comprehensively

describe the whole structure of plant that in turn would be use to correlate and group plant into its respective places

1.7.2 Plant Anatomy

Simpson (2001), defined plant anatomy as an extensive study focusing on the cell structure and tissues. Generally, plant anatomy deals with structure that needed to be observe under a microscope. Simpson added that through plant anatomy, valuable distinctive and comparative structure could be identified and comprehensively analyze to determined it importance toward phylogenetic analyses.

1.8 Conclusion

Current classification of *Clerodendrum* L. is quite confusing as several authors have remove and added new taxa or species into the genus. Problem faced by the genus long have been existed since reclassification of the genus from Verbenaceae family to Lamiaceae family. Both of the family itself are quite hard to be identified morphologically as only a few characters are used to differentiate from another. As reported by several authors, *Clerodendrum* does have medicinal properties and are widely used around the globe whether as an ornamental plant or as a medicinal plant. This research aims to add a few criteria or characters morphologically or anatomically as to assist in ease the identification process of the genus members. It is also aim to locate and plot the diversity of the genus throughout Peninsular Malaysia.