COMPARATIVE LEAF MORPHOLOGICAL, ANATOMICAL AND PHYTOCHEMICAL STUDY OF SOME TAXA IN LAMIACEAE AND VERBENACEAE

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

The comparative study was conducted to investigate the systematics of six genera of Lamiaceae and two genera of Verbenaceae. Three aspects of the study, which were morphological, anatomical and phytochemical, were carried out to determine significant taxonomic characters. The data obtained were then used to construct taxonomic keys for genera and species involved. A total of 22 species were collected from several forest reserves in Malaysia. Samples were prepared for herbarium vouchers, anatomical study, and phytochemical procedures. Morphological characters were observed and recorded. Anatomical slides of leaf were prepared using standard microtechnique method, while phytochemical screening was conducted using Qualitative Phytochemical Screening and Thin Layer Chromatograph (TLC). All characters obtained were numerically analyzed using the Multivariate Statistical Package (MVSP) and Phylogeny Analysis Using Parsimony (PAUP). The findings showed that some characters have high taxonomic significant value. Leaves margin and venation are diagnostic characters in morphological findings. Petiole and midrib outline, petiole and midrib vascular bundle, type of stomata, type of trichomes and the presence of vascular subsidiaries are the diagnostic characters in anatomical findings. In phytochemical study, terpenoids, flavonoids, and alkaloids are present in almost all species studied, while saponins are only detected in nine species, namely *Congea forbesii, Congea griffithiana, Sphenodesme racemosa, Clerodendrum breviflorum, Clerodendrum disparifolium, Lantana camara, Stachytarpheta indica, Stachytarpheta jamaicensis* and *Stachytarpheta cayennensis*. Numerical analysis showed that two main clades were formed at 0.709 and 0.821 of GGSc value to indicate the delineation of the two families. Morphological and anatomical findings able to contribute solution to taxonomic problem, while phytochemical data provide vital preliminary information of the studied species. Finally, it is concluded that all aspects of studies numerically support the classification of Li et al., therefore implicates that the findings can serve as important taxonomic references to the current classification of the families.

KAJIAN PERBANDINGAN MORFOLOGI, ANATOMI DAN FITOKIMIA DAUN BEBERAPA TAKSON DALAM LAMIACEAE DAN VERBENACEAE

ABSTRAK

Kajian perbandingan ini telah dijalankan bertujuan untuk mengkaji sistematik enam genus daripada famili Lamiaceae dan dua genus daripada famili Verbenaceae. Tiga aspek kajian iaitu morfologi, anatomi dan fitokimia dijalankan bagi menentukan ciri taksonomi yang signifikan. Data yang diperoleh seterusnya digunakan untuk membina kunci taksonomi bagi genus dan spesies yang terlibat. Sejumlah 22 spesies diperolehi daripada beberapa hutan simpan di Malaysia. Sampel disediakan untuk baucar herbarium, kajian anatomi dan prosedur fitokimia. Karakter morfologi diperhatikan dan direkodkan. Slaid anatomi disediakan menggunakan kaedah piawai mikroteknik manakala saringan fitokimia dijalankan menggunakan kaedah *Qualitative Phytochemical Screening* dan *Thin Layer Chromatograph (TLC).* Kesemua karakter dianalisis secara numerik menggunakan *Multivariate Statistical Package (MVSP)* dan *Phylogeny Analysis Using Parsimony (PAUP).* Dapatan kajian menunjukkan beberapa karakter mempunyai nilai taksonomi signifikan yang tinggi. Margin dan peruratan daun adalah karakter diagnostik bagi dapatan morfologi. Garis luar bagi petiol serta tulang daun, bentuk berkas vaskular petiol dan tulang daun, jenis stomata, jenis trikom dan kehadiran vaskular subsidiari adalah karakter diagnostik bagi dapatan anatomi. Kajian fitokimia pula menunjukkan kehadiran terpenoid, flavonoid dan alkaloid dalam hampir kesemua spesies yang dikaji manakala saponin hadir pada sembilan spesies iaitu *Congea forbesii, Congea griffithiana, Sphenodesme racemosa, Clerodendrum breviflorum, Clerodendrum disparifolium, Lantana camara, Stachytarpheta indica, Stachytarpheta jamaicensis* dan *Stachytarpheta cayennensis.* Analisis numerik mempamerkan dua klade utama terbentuk dengan nilai GGSc pada 0.709 dan 0.821 yang menunjukkan persempadan dua famili. Dapatan morfologi dan anatomi mampu menyumbang kepada penyelesaian masalah taksonomi manakala data fitokimia memberikan maklumat awal yang penting bagi spesies yang dikaji. Akhirnya, ia dapat disimpulkan bahawa semua aspek yang dikaji secara numerik menyokong klasifikasi oleh Li et al., oleh itu memberi implikasi bahawa dapatan ini boleh berfungsi sebagai rujukan taksonomi yang penting kepada pengelasan famili yang terkini.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

FRIM	Forest Research Institute Malaysia
KEP	Kepong Herbarium
UKMB	Universiti Kebangsaan Malaysia Bangi Herbarium
K	Kew Herbarium
L	Leiden Herbarium
SING	Singapore Botanic Garden Herbarium
CS	Cross-section
SEM	Scanning electron microscope
FEVs	Freely ending ultimate veins
FL	Flexuous
ST	Straight
CV	Convex
C	Concave
HPLC	High-Performance Liquid Chromatography

APPENDIX LIST

CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter discusses the research background, problem statements, study objectives, and research questions underpinning the topic under study. It furthers the significance of this study and study limitations faced in the process. In general, the current study delves into researching two plant families, which are the Lamiaceae and Verbenaceae.

Lamiaceae is a family under the umbrella of the Lamiales order, whereby it is grouped together with Verbenaceae, Acanthaceae, Bignoniaceae, and 21 other families. In English, it is also known as the mint family in which the family follows the name from the genus of European herbs, *Lamium*, meaning throat in Greek (LaFrankie, 2010). The alternative name, Labiatae, meanwhile, is translated into two-

lipped: the corolla or calyx is divided into two differently shaped parts forming the upper and lower lips (Glimn-Lacy & Kaufman, 2006).

Lamiaceae consists of 236 genera with 7200 species (Harley et al., 2004), is common in open areas, and is typically made up of trees, shrubs, lianas, or herbs with simple or compound leaves and lack any stipules (Bramley, Go & de Kok, 2010). It is cosmopolitan in nature, ranging from tropical forests to arctic tundra and from sea level to high altitude. Besides, the leaves are opposite or very rarely alternate, while the stems are characteristically quadrangular in sections in the more herbaceous genera (Gray, 2011).

Traditionally, Lamiaceae are related to the Verbenaceae or *Verbena* family and viewed as a pair family (Walters & Lavelle, 2012). In theory, these two families are naturally related to each other in which the same characteristics are shared, such as quadrangular stems, opposite leaves that lack stipules but display a scar at the node, a lipped flower, and an ovary with two carpels, each equipped with two ovules (Cronquist, 1981).

The Verbena family, in contrast, consists of 34 genera with about 1200 species recorded. Mainly from the New World and with a few groups in Asia, Africa, Europe, and Madagascar, it consists of aromatic herbs, shrubs, sometimes tree, and rarely lianas with opposites and sometimes whorled leaves (Atkins, 2004).

Both families are known for their rich source of biologically active compounds and various medicinal values (Venkateshappa & Sreenath, 2013; Atkins,

2004), rendering a valid revision of its classification and identification necessary. Furthermore, the combination of data identification and distribution will undoubtedly yield robust biodiversity information and contribute to family conservation.

1.2 Research Background

In the 1990s, a series of non-molecular and molecular research on several genera of the family Lamiaceae-Verbenaceae had been undertaken (Ryding, 1995; Cantino, 1992; Cantino, Olmstead, & Wagstaff, 1997; Wagstaff & Olmstead, 1997; Wagstaff, Hickerson, Spangler, Reeves & Olmstead, 1998). Accordingly, some of the Verbenaceae genera were reclassified under the Lamiaceae family based on the molecular findings by Cantino et al. (1997). The study has been further strengthened by a phylogenetic study by Wagstaff et al. (1998), which has displayed that many genera classified in Verbenaceae supposedly belong to Lamiaceae.

As a result of the Verbenaceae genera transfer in the 1990s as mentioned above and a morphological study conducted by Harley et al. (2004), Bramley, Forest, and de Kok (2009) have stated that the Lamiaceae members are to be reorganised across all levels, resulting movement of numerous subfamilies, genera, and species.

In Peninsular Malaysia, 15 genera and 78 species of Verbenaceae reportedly exist according to Turner (1995), some of which include certain Lamiaceae species. They comprise *Callicarpa, Clerodendrum, Congea, Glossocarya, Vitex, Gmelina,*

Premna, *Sphenodesme Petraeovitex, Teijsmanniodendron,* and *Peronema,* as well as *Phyla, Verbena, Lantana,* and *Stachytarpheta.*

Meanwhile, Kiew, Chung, Saw, and Soepadmo (2010) have highlighted that the Verbenaceae genera in Peninsular Malaysia are reduced to a few genera due to the incorporation of some (e.g. *Peronema, Petraeovitex, Premna, Sphenodesme, Symphorema, Callicarpa, Clerodendrum, Congea, Glossocarya, Gmelina, Tectona, Teijsmanniondendron,* and *Vitex*). This leads to the expansion of the Lamiaceae family, except for *Phyla,* and mostly of tropical American origin (*Lantana, Stachytarpheta, Citharexylum,* and *Verbena*).

Regarding the aforementioned genera transfer, previous studies include those undertaken in the context of the Malesian region, namely by de Kok (2008, 2012, 2013). Moreover, research on the Lamiaceae family across a few genera has been conducted in Peninsular Malaysia, which includes that on *Vitex* by Nafizah et al. (2018) and *Clerodendrum* by Mazatul (2018). Meanwhile, researchers such as Go (1999), Phongoudome (2000), Abraham-Oanes (2002) and Bramley et al. (2010), have published their own Sabah and Sarawak-focused research on the topic. Bramley et al. (2010), for example, have recorded 25 genera and the key to the species of seven genera based on their respective morphological characters, while de Kok, Sengun, and Bramley (2016) have underlined 44 species across 21 genera of Lamiaceae and two new recorded species (i.e. *Vitex rotundifolia* and *Callicarpa pentandra)* in Singapore. Alternatively, Bramley (2019) has presented 50 genera of Lamiaceae in the newest Flora Malesiana, with 304 species thereby recognised as native or naturalised. They also include genera formerly recognised under the Verbenaceae.

According to Kiew, Chung, Saw, and Rafidah (2007), morphological and anatomical characteristics are critical in organising different plants for systematic purposes and understanding the relationship between them. Besides, such knowledge provides a mountain of details and accessible information for further research and investigation. This study, in particular, includes both families of Lamiaceae and Verbenaceae, henceforth focusing on the similarities and dissimilarities between them in terms of the morphological, anatomical, and phytochemical aspects. The following six genera of Lamiaceae were formerly grouped under Verbenaceae, namely *Petraeovitex, Peronema, Congea, Sphenodesme, Clerodendrum,* and *Rotheca*, and thus studied with the two genera of Verbenaceae (i.e. *Lantana* and *Stachytarpheta*). Therefore, the results obtained in this work can be very important and deemed helpful in understanding the phylogenetic relationships within these two families.

1.3 Problem Statements

In Peninsular Malaysia, only a few outdated studies of Lamiaceae species have been recorded, whereby they were undertaken by Henderson (1959), Oxon (1978), Corner (1988), and Turner (1995). These studies have recorded the morphological descriptions of vegetative and reproductive characteristics of said species in detail. Nevertheless, they also reflect the limited amount of morphological studies and show that anatomical studies available are not comprehensive, rendering these studies far from complete. To date, an extensive study on Lamiaceae in the context of Peninsular

Malaysia is yet to be recorded, especially one that could provide full morphological, anatomical, micromorphological, and phytochemical descriptions as supporting pieces of evidence.

In this study, leaves denote the part of plant chosen to be thoroughly investigated. According to Kulkarni, Rai, Jahagirdar, and Upparamani (2013), plant classification based on leaf characteristics is the foremost choice compared to other methods like cell and molecular characteristics.

Taxonomic classification requires the incorporation of several research fields, such as plant morphology, anatomy, and phytochemicals. Previous molecular and non-molecular studies on these families were performed in the 1990s and the results obtained led to this study, albeit prioritising other aspects, namely their morphological, anatomical, and phytochemical elements. Turrill (1936) and Cutler, Botha, and Stevenson (2008) have further added that the judgement of taxonomic classification can only present a final value when all methods or fields are utilised to undertake the revisions of plant classification towards the production of a more natural system. Here, they encompass the morphology, pollen, physiology, cytology, chemistry, or molecular biology studies accordingly.

Moreover, prior works have underlined the prevailing confusion on classifying Lamiaceae members, of which some have remained unplaced while others are deemed problematic (Harley et al., 2004; Wagstaff et al., 2008). Here, LaFrankie (2010) has indicated that a large number of species are closely related to each other and possess morphological complexity that leads to many taxonomic problems. The researcher is

then of the opinion that carrying out additional detailed studies to handle such problematic genera, such as *Peronema, Petraeovitex, Clerodendrum,* and *Rotheca,* is a requirement. This will allow one to address any taxonomic issues faced and improve their knowledge of the family classification. For example, Steane, de Kok, and Olmstead (2004) and Yuan, Mabberley, Steanes, and Olmstead (2010) have identified the genus *Clerodendrum* as problematic, wherein their investigations did not yield any satisfactory resolution pertaining to the relationships between said species and other genera.

In another study, Harley et al. (2004) have documented ten genera as unplaced, whereby a subsequent phylogenetic study by Li et al. (2016) has initiated the transfer of certain problematic genera to three new subfamilies. This indicates that the morphological and molecular data yield contradictory results, proving that further extensive studies for the Lamiaceae family as a whole are needed.

Similar to Lamiaceae, the Verbenaceae species also have their own taxonomic issues, including *Lantana* and *Stachytarpheta*. Members of *Lantana,* for instance, are closely allied and difficult to be differentiated (Howard, 1969), while those of *Stachytarpheta* present their own taxonomic uncertainty within the species. The study have identified four problematic species, specifically *S. jamaicensis, S. cayennensis, S. indica,* and *S. urticifolia*, which pose difficult identification processes due to minor differences in morphological characteristics (Chandler, Westaway, & Conn, 2014).

Thus, this study was mainly geared towards addressing the problems highlighted earlier, particularly by obtaining significant data on the studied species in

both Lamiaceae and Verbenaceae families across the morphological, anatomical and phytochemical aspects.

1.4 Research Objectives

The objectives of this study were:

i. To investigate the taxonomic values of leaf morphological, anatomical, and phytochemical characteristics of selected Lamiaceae and Verbenaceae species
ii. To construct the morphological and anatomical keys based on the leaf characteristics of selected Lamiaceae and Verbenaceae members
iii. To evaluate the relationship and dissociation between the Lamiaceae and Verbenaceae species through numerical analysis
iv. To map the distribution of selected Lamiaceae and Verbenaceae genera in Peninsular Malaysia

1.5 Research Questions

In reference to the research objectives stated above, the research questions devised for this study are as follows:

i. Are there any taxonomic values of leaf morphological, anatomical, and phytochemical characteristics for the selected Lamiaceae and Verbenaceae species to revise the placement?

ii. Could the morphological and anatomical keys based on the leaf of selected Lamiaceae and Verbenaceae members be constructed?

iii. What is the relationship and dissociation between Lamiaceae and Verbenaceae members through numerical analysis?

iv. What is the distribution of the selected Lamiaceae and Verbenaceae families in Peninsular Malaysia?

1.6 Research Significance

This research is critical in clearing any confusion that is arising regarding the problematic genera and correctly identifying the species of Lamiaceae. Furthermore, the family members are well-known for their rich ethnomedicinal properties, whereby the importance of phytochemical constituent data is important in classifying the species and identifying the presence or absence of any inherent medicinal value, as well as their possible economical utilisation in the future. Accordingly, the findings are aimed towards aiding the process of Lamiaceae identification and resolving the systematic conflicts and classification of the plant family, which is to complement the classification of Lamiaceae itself. Here, the significance of the data obtained from the morphological, anatomical, and phytochemical studies is discussed in relation to current taxonomic opinions on Lamiaceae.

Henceforth, this study is a fundamental stepping stone in providing value to Malaysian biodiversity, namely via an exploration of diversity possessed by Lamiaceae in Peninsular Malaysia. It will also help in supporting the Economic Transformation Programme 2010 through the change of National Key Economic Areas (NKEA) while concurrently reinforcing the vision of National Biodiversity 1998 for the purpose of presenting Malaysia as a centre of excellence for biodiversity in 2020.

1.7 Study Limitation

This study focused on the Lamiaceae species from Peninsular Malaysia. Therefore, study samples were collected in several localities in the region, whereas only one species was collected in Mulu, Sarawak of the East Malaysia region. The observation of species of both plant families in Peninsular Malaysia was made based on field collections and herbarium sample availability, with a total of 22 species successfully collected. Despite the small number, each species was a representative of the genus available in Peninsular Malaysia, except for the *Clerodendrum* species. For Verbenaceae, however, only a few species could be found in Peninsular Malaysia. Thus, the results obtained in this research were yielded from leaf observations, while other plant parts such as flowers were not included in the process. The focus placed on the leaf was underpinned by its critical role in plant identification and the fact that flowers would not be obtainable throughout the whole year, whereby some flowers could only be found at specific times in a year.