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Essential oils composition of Vitex negundo and Vitex trifolia and its anatomical study

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Abstract

Chemical compositions of V. negundo and V. trifolia essential oils and their anatomical study were determined. The GC-FID and GC–MS analysis of essential oils resulted in the identification of 14 and 18 components were identified from the leaf oils of V. negundo (92.8%) and V. trifolia (91.5%), respectively. The results revealed that the essential oils are made up principally of δ-elemene (43.1%), spathulenol (9.8%) for V. negundo, while viridiflorol (42.3%) and β-caryophyllene (21.7%) for V. trifolia. Meanwhile, for anatomical part, the oil gland has been found in the lamina, midrib and petiole of all essential oils.

Keywords: Verbenaceae, Vitex negundo, Vitec trifolia, essential oil, δ-elemene, viridiflorol, anatomy

INTRODUCTION

The Lamiaceae is known as the mint family which is also the one among of flowering plants family. It is a cosmopolitan distribution, growing over the entire planet and particularly high dispersion in the Mediterranean region. The Lamiaceae is containing about 236 genera and has been stated to 7,534 species [1]. For their habitat, they prefer the open fields. Lamiaceae are cultivated as ornamental and herbs. Some are shrubs, trees or, rarely in form of vines. Due to their aromatic odour and nice flavour, several species of this family are used in the culinary to come out the satisfaction of the gourmets. There are acquainted by people with the merits of basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and rosemary (*Salvia rosmarinus*) as smell and taste garnishes in the numerous dishes [2].

The genus *Vitex* is one of the largest genera in the Verbenaceae family, with roughly 250 species. The plants are shrubs, extensively disseminated, but mainly found in the tropical areas with a few in subtropical regions. Many species in the *Vitex* genus have important medicinal properties [3]. Numerous parts of *Vitex* species, counting the leaves, roots and seeds, have been locally used as traditional folk medicines since ancient times, predominantly in China. It is generally used for its analgesic, anti-rheumatism, and

insecticidal properties. In the Ayurveda and Unani systems of medicine, the leaves and seeds of *Vitex* species are extensively used for the management of rheumatism and inflammatory joint circumstances [4,5].

V. negundo are large and erect aromatic shrubs, which cultivate to a height of 2-5 m. The leaves have five leaflets in a palmately arrangement, an acute terminal leaflets (16-32 mm) with petiolate having 1.0-1.3 cm long, lanceolate, 4-10 cm long, hairy beneath and pointed at both ends. The fruit is succulent, black when ripe, rounded and about 4 mm in diameter [6]. Meanwhile, *V. trifolia* is a shrub and pretty blue flower and grows to a height of 10-12 feet. These soft leaves have greyish pubescence on their underside and smell pungent when crushed. Attractive blue or lavender flowers with white spots appear in terminal clusters during the summertime [7]. Abroad research on the *Vitex* genus is explored on the essential oil. From the reviews on the medicinal purposes, the *Vitex* genus plants were largely applied for curing and preventing female hormonal disorders [8].

Despite the conservative uses of various species in the Lamiaceae family, lots of the plants that come in with the same species whether in the same family such as *Vitex* has not been researched properly especially in Malaysia. In addition, many studies pointed out the importance of morphological characters in delimitation and identification in some Lamiaceae species. The anatomical characters are important for the characterization of Lamiaceae taxa. Besides, these features play an important role in elucidating phylogenetic relationships in many taxa. The most current review of the family in the Flora Malesiana [9] were not much discussed on the anatomical characters and phytochemical constituents including essential oil. Therefore, the study that is involving the extraction and analysis of the essential oils as well as the relationship of anatomical characteristics of the leaves and their essential oils were studied.

MATERIALS AND METHODS

Plant Material

The leaves of *V. negundo* (SK01/19) and *V. trifolia* (SK02/19) were collected from Tanjung Malim, Perak in June 2020. This species was identified by Shamsul Khamis and the voucher specimens deposited at UPSI.

Isolation of Essential Oil

The fresh samples (250 g) of the leaf part were cut into small pieces and loaded in a round bottom flask (5 L). Distilled water was added until the water covered the entire samples. The flask was equipped with a Dean-stark apparatus and water condenser and the mixture was hydrodistilled for 5 h. The mixture of oil and water was extracted with Et_2O (3×10 mL), dried over anhydrous $MgSO_4$ and filtered. The filtrate was vaporized at room temperature to afford the essential oils. The essential oil had a spicy odor and yielded 0.03% (*V. negundo*) and 0.025% (*V. trifolia*) calculated from the fresh weight of the leaves.

Analysis of Essential Oil

Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B equipped with HP-5MS capillary column (30 m long, 0.25 μm thickness and 0.25 mm inner diameter). Gas chromatography-mass spectrometry (GC-MS) analysis was recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with an HP-5 column [10].

Identification of Components

For the identification of essential oil components, co-injections with the standards (major components) were used, together with the correspondence of retention indices and mass spectra with respect to those reported in Adams [11].

Anatomical Study

Fresh samples underwent several processes prior to sectioning and anatomical slide preparation. Microtechnique procedure adopted is according to the modification of Johansen [12-13].

RESULTS AND DISCUSSION

A total of 30 chemical components were recognized from the *Vitex* essential oils based on the order of elution on the HP5 column as shown in Table 1. The *V. negundo* essential oil has successfully characterized 14 chemical components, accounting for 92.8% of the total composition, meanwhile the *V. trifolia* essential oil gave 18 chemical components correspond to 91.5% of the total composition.

The essential oils of *V. negundo* indicated the presence of 12 components of sesquiterpene hydrocarbons (77.0%) and two components for oxygenated sesquiterpenes (15.8%). The oil was characterized by the abundance of δ-elemene (43.1%), spathulenol (9.8%), δ-selinene (7.8%), γ-eudesmol (6.0%) and β-gurjunene (5.2%). There were also a substantial amount of components that displayed more than 2% which were β-elemene (2.1%), α-gurjunene (2.0%), β-caryophyllene (2.2%), β-copaene (3.0%), γ-elemene (3.8%) and amorpha-4,7(11)-diene (3.5%). The essential oil of *V. trifolia* consisted of ten sesquiterpene hydrocarbons and eight oxygenated sesquiterpenes, representing approximately 39.1% and 52.4%, respectively. The most abundant components were viridiflorol (42.3%), β-caryophyllene (21.7%), β-elemene (10.3%) and elemol (134) (5.7%). Similar to these results, δ-elemene has been reported to be the major constituent in the oil of *V. megapotamica* (10.65%) [14]. Meanwhile, viridiflorol was also reported as the most abundant component from the leaves oil of *Plectranthus barbatus* (14.13%) [15].

This study focuses on glandular trichomes, which are frequently present in Lamiaceae. Previous research has linked the existence of glandular trichomes to each species' ability to produce essential oils. The function of glandular trichomes vary depending on their location, the substances they secrete, and the timing of secretion. Structurally, similar trichomes can produce different materials, but even when producing similar materials, when the trichomes exist at different locations to suit their different function. For example, the functions of trichomes in seed, the mucilage are significantly different from those of leaves. The different types of glandular trichomes can vary depending on the stage of development of the organ [16].

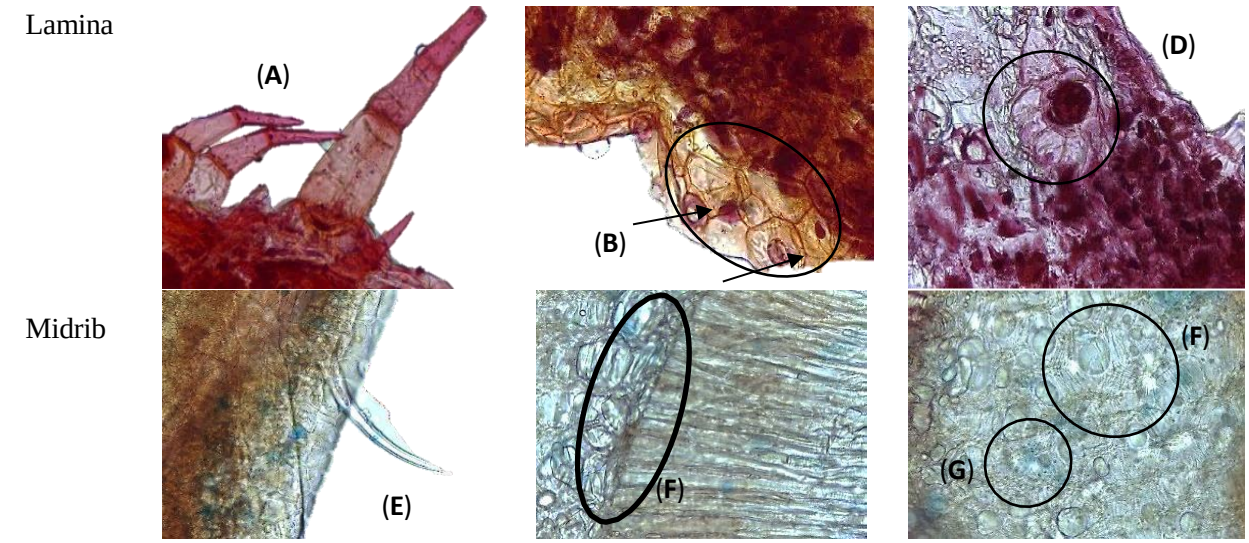
Table 1. Chemical components identified in *Vitex* essential oils

No	Components	KI[a]	KI[b]	Percentage (%)	
				VNOL	VTOL
1	**δ-Elemene**	1335	1335	**43.1**	-
2	α-Copaene	1375	1374	1.5	-
3	β-Elemene	1390	1389	2.1	**10.3**
4	α-Gurjunene	1405	1409	2.0	-
5	β-Ionol	1410	1412	-	0.2
6	β-Caryophyllene	1415	1417	2.2	**21.7**
7	β-Copaene	1430	1430	3.0	-
8	β-Gurjunene	1432	1431	5.2	-
9	γ-Elemene	1435	1434	3.8	-
10	β-Humulene	1436	1436	-	1.7
11	α-Guaiene	1436	1437	-	2.0
12	α-Humulene	1450	1452	-	0.6
13	(E)-β-Farnesene	1455	1454	-	1.1
14	Alloaromadendrene	1458	1458	-	0.7
15	Dehydroaromadendrane	1460	1460	-	0.4

16	Amorpha-4,7(11)-diene	1480	1479	3.5	-
17	δ-Selinene	1490	1492	**7.8**	-
18	*cis*-Cadina-1(6),4-diene	1495	1495	-	0.4
19	Valencene	1495	1496	1.4	-
20	δ-Amorphene	1510	1511	1.4	-
21	Elemol	1545	1548	-	5.7
22	Spathulenol	1577	1577	**9.8**	-
23	Caryophyllene oxide	1580	1582	-	0.7
24	**Viridiflorol**	1592	1592	-	**42.3**
25	Ledol	1602	1602	-	1.2
26	γ-Eudesmol	1630	1630	6.0	-
27	t-Muurolol	1645	1644	-	0.5
28	α-Cadinol	1655	1652	-	0.5
29	13-*epi*-Manool oxide	2005	2009	-	0.4
30	Sclareol	2220	2222	-	1.1
	Sesquiterpene hydrocarbons			**77.0**	**39.1**
	Oxygenated sesquiterpenes			**15.8**	**52.4**
	Identified components (%)			**92.8**	**91.5**

[a] Linear retention index, experimentally determined using homologous series of C6-C30 alkanes
[b] Linear retention index taken from Adams [11] and literature

The microscope investigation result showed the three terms of unicellular trichomes in Lamiaceae species. The presence of essential oil was suspected as cell inclusions as shown in Figure 1. Furthermore, Figure 2 was suspected the presence of oils in parenchyma cells of midrib *V. trifolia*. Besides, instead of simple glandular trichomes, there are three terms of non-glandular trichomes on four Lamiaceae plant species understudies. One of the simple nonglandular trichome which have multicellular stalk were mostly found in *V. negundo* the simple multicellular trichomes as shown as Figure 1A on lamina while for the *V. trifolia* were found in lamina, midrib and petiole (Figure 2A-C) were confirmed their morphological characteristics by Simpson [17]. Second, the only one simple non-glandular trichome was found between entire of research species was in midrib of *V. negundo* leaves and was called as cylindrical nonglandular hair (Figure 1C) [18]. The third simple non-glandular trichome that had been found are extended stalk trichome. In most trichomes, a short trichome with extended stalk composed of three cells was closely similar as *Salvia officinalis* [19].



Petiole

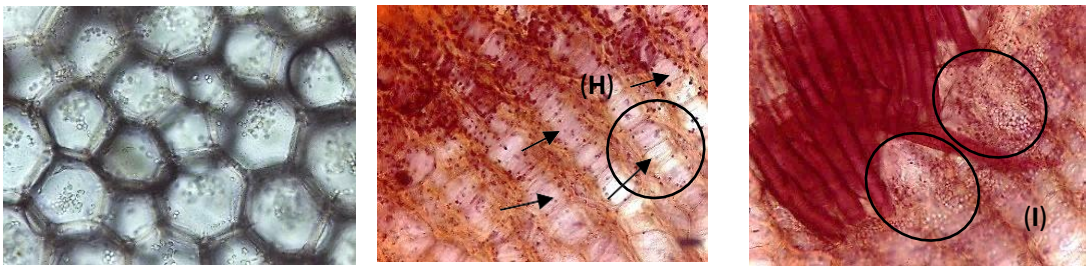


Figure 1. Anatomical characteristics of *Vitex negundo*

Lamina

Midrib

Petiole

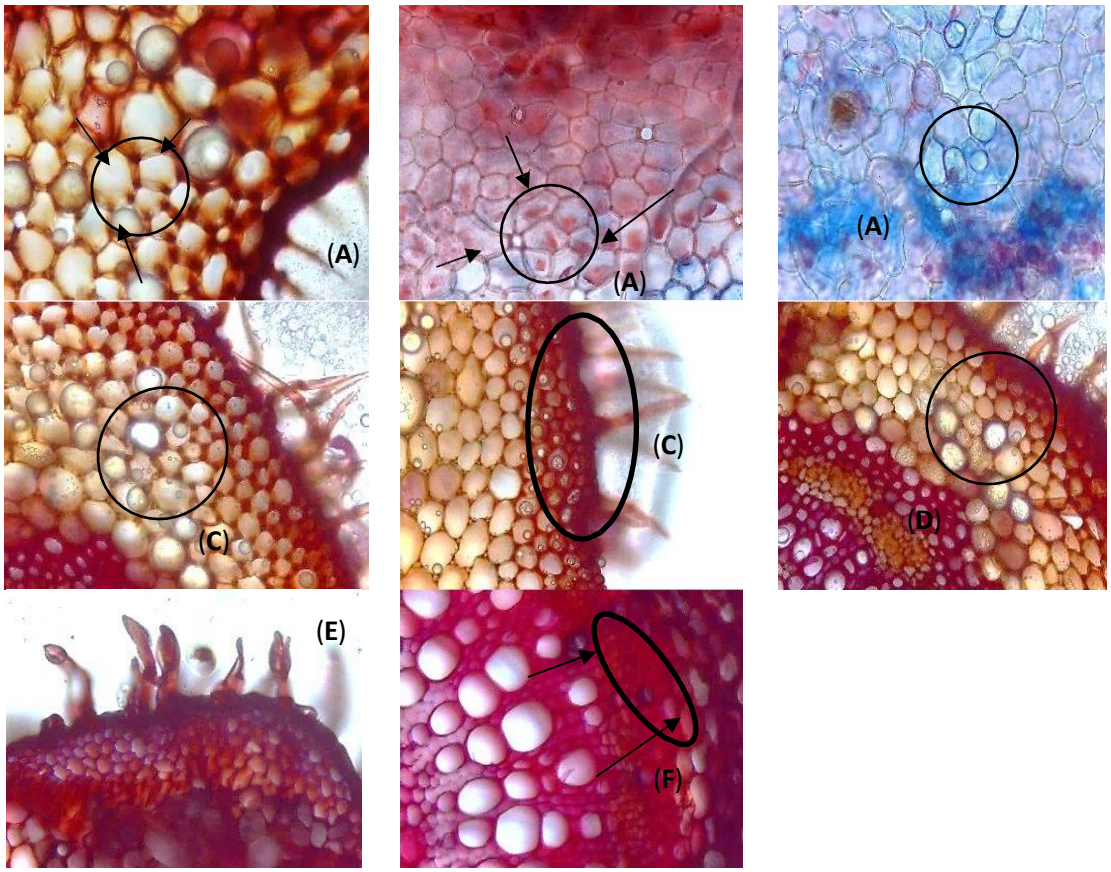


Figure 2. Anatomical characteristics of *Vitex trifolia*

However, microscopic observation concluded that unicellular trichomes did not found in both *Vitex* species, except for the multicellular trichomes present in both. Hence, the trichomes within two *Vitex* species mostly presence on lamina. The findings on the oil residue in the cells and the glandular trichomes supported the result of the analysis of the essential oils which contributed in the yield.

CONCLUSION

The results revealed that the essential oils are made up principally of δ-elemene (43.1%), spathulenol (9.8%) for V. negundo, while viridiflorol (42.3%) and β-caryophyllene (21.7%) for V. trifolia. Beisides, V. negundo the only one epidermis cell was located unicellular non-glandular trichomes, while the other species were found abundant in pilate glandular trichomes. The presence of cell structures accumulated essential oil in plants was noted in lamina, midrib and petiole of leaves epidermis were undoubtedly all of the research plant species were observed. The implication of this study demonstrates the importance of the characterization of Lamiaceae taxa in elucidating phylogenetic relationships.

DECLARATION OF INTEREST

There is no conflict of interest with this study.

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