Short note

Characterisation of the essential oil components and their multivariate statistical analysis of the genus *Vitex* and *Plectranthus* (Lamiaceae)

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This study investigated the chemical compositions of the essential oils from four species of the Lamiaceae family, *Vitex negundo*, *Vitex trifolia*, *Plectranthus amboinicus*, and *Plectranthus monostachyus*. The essential oils were obtained through hydrodistillation and were fully characterised with gas chromatography with flame ionisation detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The multivariate statistical analysis was determined via the principal component analysis (PCA) and hierarchical clustering analysis (HCA). The study identified 14 and 18 components from the leaf oils from *V. negundo* (92.8%) and *V. trifolia* (91.5%), respectively. The results revealed that the essential oils were made up principally of δ-elemene (43.1%), spathulenol (9.8%), and δ-selinene (7.8%) for *V. negundo*, while viridiflorol (42.3%), β-caryophyllene (21.7%), and β-elemene for *V. trifolia*. For the genus *Plectranthus*, 20 components were found in the essential oils from *P. amboinicus* (91.1%) and 37 components from *P. monostachyus* (98.8%). The major components of *P. amboinicus* oil were carvacrol (54.4%), β-caryophyllene (8.9%), and *α-cis*-bergamotene (7.7%), whereas *P. monostachyus* oil had β-caryophyllene (26.2%), germacrene D (12.5%), δ-cadinene (9.2%), and germacrene B (8.8%). The results from the PCA and HCA analysis showed that the essential oils could be distinctly separated into three clusters; *P. amboinicus* and *V. trifolia* in cluster I, *V. negundo* in cluster II, and *P. monostachyus* grouped in cluster III. The information is vital when selecting the species with economic potentials for the pharmaceutical and cosmetics industries.
Keywords: Essential oil; Lamiaceae; *Vitex*; *Plectranthus*; carvacrol; Multivariate

1. INTRODUCTION

The genus Lamiaceae is known as the mint family and grows particularly in the Mediterranean region. The genus encompasses about 236 genera and is cultivated as an ornamental and herb [1]. Medicinal properties of the Lamiaceae species are often attributed to their high content of volatile compounds. The aromatic volatile oils produced by the species of the genus are widely used in the perfumery, cosmetics, pharmaceutical, and food industries as active ingredients, flavouring agents, or fragrances [2].

The genus *Vitex* is one of the largest genera with approximately 250 species. It is widely distributed worldwide and is mainly found in tropical areas and a few subtropical regions. The plants are mostly shrubs and many of the species proved to have significant medicinal effects. Various parts of the *Vitex* species have been used as traditional medicine since antiquity, particularly in China. The species is commonly used for its analgesic, anti-inflammatory, anti-rheumatism, and insecticidal effects. In the Ayurveda and Unani systems of medicine, the leaves and seeds of the *Vitex* species are widely used to treat rheumatism and joint inflammations [3]. Moreover, several species of the genus have been thoroughly researched such as *V. agnus-castus*, *V. negundo*, *V. limonifolia*, and *V. rotundifolia*. Consequently, different types of

secondary metabolites such as terpenes, flavonoids, lignans, phenolic acids, and anthraquinones were found present in the species [4].

Plectranthus is a large genus that contains about 300 species and is usually located in tropical Africa, Asia, and Australia. It is a horticulturally important genus of predominantly herbaceous plants that are becoming increasingly popular in indigenous landscaping as some species are suitable as shrubs or pruned into hedges. The aromatic nature of the genus is attributed to the essential oil it produces [5]. The phytochemical studies of *Plectranthus* focused mainly on the isolation of diterpenoids with the majority of them were highly modified abietanoids. The chemicals were shown to exhibit pharmacological activities such as antiplasmodial, antibacterial, antifungal, and antitumoral, making *Plectranthus* an important genus in drug development [6].

This study is a continuation of our systematic studies on pharmacologically active volatiles from Malaysian plants [7–10]. This report evaluated the chemical compositions of the essential oils from four Lamiaceae plants, *V. negundo*, *V. trifolia*, *P. amboinicus*, and *P. monostachyus*.

2. MATERIALS AND METHODS

2.1 PLANT MATERIALS

The leaves of *V. negundo* (SK01/19), *V. trifolia* (SK02/19), *P. amboinicus* (SK03/19) and *P. monostachyus* (SK04/19) were collected from Tanjung Malim, Perak in October 2019. The species were identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM) and the voucher specimens were deposited at UPSI Herbarium.

2.2 ISOLATION OF ESSENTIAL OILS

The fresh leaves of each sample (200 g each) were weighed and then subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. A hydrodistillation run time of 4 hours was used to obtain optimum yield without drastically affecting the oil components. The obtained oils were then dried using anhydrous magnesium sulphate, weighed, and stored in dry amber vials at 4°C until analysis. The average yield of oil was calculated as a percentage weight by weight (% w/w) of the plant material.

2.3 ANALYSIS OF ESSENTIAL OILS

Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B and an Agilent 7890B FID equipped with HP-5 column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percentages were reported as means ±SD of triplicates. Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped with HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness). Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionisation system, with an ionisation energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu [11].

2.4 IDENTIFICATION OF CHEMICAL COMPONENTS

For identification of essential oil components, co-injection with the standards (major components) were used, together with correspondence of retention indices and mass spectra with respect to those occurring in Adams, Wiley, NIST 08, and FFNSC2 libraries P12]. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components. Quantification was done by means of the external standard method using calibration curves generated by running GC analysis for the representative authentic compounds. Relative percentage values are means of three determinations ±SD.

2.5 MULTIVARIATE STATISTICAL ANALYSIS

Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were performed using Statistica 7.0 software. This multivariate analysis was evaluated the variation in the chemical composition and characterised the most important components which contribute to the difference among samples. HCA used Ward's minimum variance method with a Euclidean distance and PCA used the covariance matrix obtained from the data matrix [13].

3. RESULTS AND DISCUSSION

The chemical compositions of essential oils from two *Vitex* species and two *Plectranthus* species were investigated. The essential oils were extracted from the leaves of the species. The highest percentage yield was obtained from the *V. negundo* (0.028%), followed by *V. trifolia* (0.025%), *P. monostachyus* (0.015%), and *P. amboinicus* (0.012%). Table I shows the chemical components identified in *Vitex* and *Plectranthus* essential oils.

A total of 30 chemical components were identified from the *Vitex* essential oils based on the order of elution on the HP5 column. The essential oil from *V. negundo* was successfully characterised with 14 chemical components that accounted for 92.8% of the

Table I - Chemical components identified in *Vitex* and *Plectranthus* essential oils

N.	Components	KI[a]	KI[b]	Percentage (%)			
				VNOL	VTOL	PAOL	PMOL
1	α-Pinene	932	930	**-**	**-**	**-**	**0.2**
2	Camphene	945	946	-	-	0.2	-
3	α-Terpinene	1014	1016	-	-	-	1.2
4	Limonene	1033	1032	-	-	-	0.7
5	γ-Terpinene	1055	1056	-	-	-	0.4
6	Linalool	1092	1090	-	-	-	0.5
7	Terpinen-4-ol	1175	1174	-	-	0.2	1.0
8	α-Terpineol	1186	1185	-	-	-	1.0
9	Verbenol	1195	1197	-	-	1.6	-
10	Carvacrol	1295	1298	-	-	54.4	-
11	Thymol acetate	1330	1330	-	-	0.2	-
12	δ-Elemene	1335	1335	43.1	-	-	-
13	Carvacrol acetate	1370	1370	-	-	0.2	-
14	Cyclosativene	1370	1369	-	-	-	0.1
15	α-Ylangene	1372	1373	-	-	-	0.1
16	α-Copaene	1375	1374	1.5	-	-	-
17	β-Bourbonene	1387	1388	-	-	-	0.2
18	β-Cubebene	1388	1387	-	-	-	2.5
19	β-Elemene	1390	1389	2.1	10.3	-	2.4
20	α-Gurjunene	1405	1409	2.0	-	-	0.6
21	Longifolene	1407	1407	-	-	-	2.2
22	β-Ionol	1410	1412	-	0.2	-	-
23	α-*cis*-Bergamotene	1410	1411	-	-	7.7	-
24	β-Caryophyllene	1415	1417	2.2	21.7	8.9	26.2
25	β-Copaene	1430	1430	3.0	-	-	-
26	β-Gurjunene	1432	1431	5.2	-	-	-
27	γ-Elemene	1435	1434	3.8	-	-	-
28	β-Humulene	1436	1436	-	1.7	-	0.5
29	α-Guaiene	1436	1437	-	2.0	-	-
30	Aromadendrene	1439	1439	-	-	-	1.2
31	(Z)-α-Farnesene	1440	1440	-	-	1.4	-
32	α-Humulene	1450	1452	-	0.6	3.1	-
33	(E)-β-Farnesene	1455	1454	-	1.1	0.2	-
34	Alloaromadendrene	1458	1458	-	0.7	-	0.2
35	Dehydroaromadendrane	1460	1460	-	0.4	-	-
36	Germacrene B	1460	1559	-	-	-	8.8
37	Amorpha-4,7(11)-diene	1480	1479	3.5	-	-	-
38	α-Amorphene	1482	1483	-	-	-	2.2
39	Germacrene D	1484	1484	-	-	-	12.5
40	β-Selinene	1485	1489	-	-	-	1.2
41	δ-Selinene	1490	1492	7.8	-	1.7	-
42	*cis*-Cadina-1(6),4-diene	1495	1495	-	0.4	-	-
43	Valencene	1495	1496	1.4	-	-	-
44	Cadine-1,4-diene	1495	1495	-	-	-	0.2
45	Bicyclogermacrene	1500	1500	-	-	-	5.5
46	α-Muurolene	1502	1501	-	-	-	3.2
47	β-Bisabolene	1505	1505	-	-	1.8	-
48	δ-Amorphene	1510	1511	1.4	-	-	-
49	β-Sesquiphellandrene	1520	1521	-	-	0.4	-
50	δ-Cadinene	1520	1522	-	-	-	9.2
51	(Z)-Nerolidol	1530	1531	-	-	-	2.5
52	Elemol	1545	1548	-	5.7	-	2.0
53	*epi*-Longipinanol	1565	1562	-	-	1.1	-
54	Palustrol	1565	1567	-	-	-	1.0
55	Germacrene D-4-ol	1574	1574	-	-	-	1.0
56	Spathulenol	1577	1577	9.8	-	-	1.5
57	Caryophyllene oxide	1580	1582	-	0.7	6.0	-
58	Viridiflorol	1592	1592	-	42.3	-	1.2
59	Ledol	1602	1602	-	1.2	-	0.2

Continua Tabella I

N.	Components	KI[a]	KI[b]	Percentage (%)			
				VNOL	VTOL	PAOL	PMOL
60	Humulene epoxide II	1605	1608	-	-	1.1	-
61	β-Cedrene epoxide	1620	1621	-	-	0.3	-
62	Aromadendrene epoxide	1638	1639	-	-	0.3	-
63	γ-Eudesmol	1630	1630	6.0	-	-	-
64	Cubenol	1645	1645	-	-	-	1.0
65	t-Muurolol	1645	1644	-	0.5	-	1.2
66	β-Eudesmol	1648	1649	-	-	-	1.8
67	α-Cadinol	1655	1652	-	0.5	-	2.4
68	(Z)-α-*trans*-Bergamotol	1690	1690	-	-	0.3	-
69	13-*epi*-Manool oxide	2005	2009	-	0.4	-	-
70	Sclareol	2220	2222	-	1.1	-	-
Group components							
	Monoterpene hydrocarbons					0.2	3.0
	Oxygenated monoterpenes					56.6	2.0
	Sesquiterpene hydrocarbons			77.0	39.1	25.2	83.0
	Oxygenated sesquiterpenes			15.8	52.4	9.1	10.8
Identified components (%)				92.8	91.5	91.1	98.8

VNOL – *V. negundo* oil; VTOL – *V. trifolia* oil; PAOL – *P. amboinicus* oil; PMOL – *P. monostachyus* oil
[a]Linear retention index, experimentally determined using homologous series of C_6-C_{30} alkanes
[b]Linear retention index taken from Adams[12]

total composition of the oil. The essential oil extracted from *V. trifolia* contained 18 chemical components which correspond to 91.5% of the total composition of the oil. The essential oil from *V. negundo* indicated the presence of 12 components of sesquiterpene hydrocarbons (77.0%) and two components of oxygenated sesquiterpenes (15.8%). The oil showed an abundance of δ-elemene (43.1%), spathulenol (9.8%), δ-selinene (7.8%), γ-eudesmol (6.0%), and β-gurjunene (5.2%). Additionally, substantial amounts of compounds that accounted for more than 2% of the total composition were observed namely 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 10 sesquiterpene hydrocarbons and eight oxygenated sesquiterpenes that represented approximately 39.1% and 52.4% of the total composition, respectively. The most abundant components were viridiflorol (42.3%), β-caryophyllene (21.7%), β-elemene (10.3%), and elemol (5.7%). A previous report also observed δ-elemene as the major constituent in the essential oil of *V. megapotamica* (10.65%) [14].

From the analysis of the essential oil of *P. amboinicus*, 20 components (91.1%) were detected. Oxygenated monoterpenes were the most abundant components in the essential oil of *P. amboinicus* which represented 56.6% of the total composition. Additionally, sesquiterpene hydrocarbons which contributed 25.2% of the total composition of essential oil from *P. amboinicus*. The major components identified were carvacrol (54.4%), β-caryophyllene (8.9%), α-*cis*-bergamotene (7.7%), and caryophyllene oxide (6.0%). Monoterpene hydrocarbons made up a minor fraction of the oil with only camphene (0.2%) detected. Carvacrol was reported by previous studies as the main component of several essential oils from *Plectranthus*. Carvacrol was found in the species collected from the Philippines [15], Brazil [16], Morocco [17], Egypt [18], Cuba [19], and Thailand [20]. However, the leaf oil of *P. cylindraceus* collected from Oman also showed the presence of carvacrol [21]. In the essential oil extracted from the leaves of *P. monostachyus*, 37 components were characterised with sesquiterpene hydrocarbons as the major fraction (83.0%). From the hydrocarbons, β-caryophyllene (26.2%) supplied the most substantial amount, followed by germacrene D (12.5%), δ-cadinene (9.2%), germacrene B (8.8%), and bicyclogermacrene (5.5%). Additionally, 10 components of oxygenated sesquiterpenes were observed. β-Caryophyllene was found in *P. ciliates* [22] and *P. grandis* [23].

Principle component analysis (PCA) is a multivariate exploratory data analysis tool that is used to determine similarities and differences among samples, identify groups of samples, and study correlations among variables. However, hierarchical clustering analysis (HCA) groups things according to their similarities based on specified characteristic variables. This method is being acknowledged as one of the approaches for quality control of herbal materials. The results for PCA and HCA analysis are shown in Figure 1. As many as 30 data sets showing 2.0% or more composition were selected for the multivariate analysis done in this study. The results from the PCA analysis showed that the essential oils were distinctly separated into three clusters, with *P. amboinicus* and *V. trifolia* grouped in cluster I, *V. negundo* in cluster II, while *P. monostachyus* grouped in cluster III. Likewise,

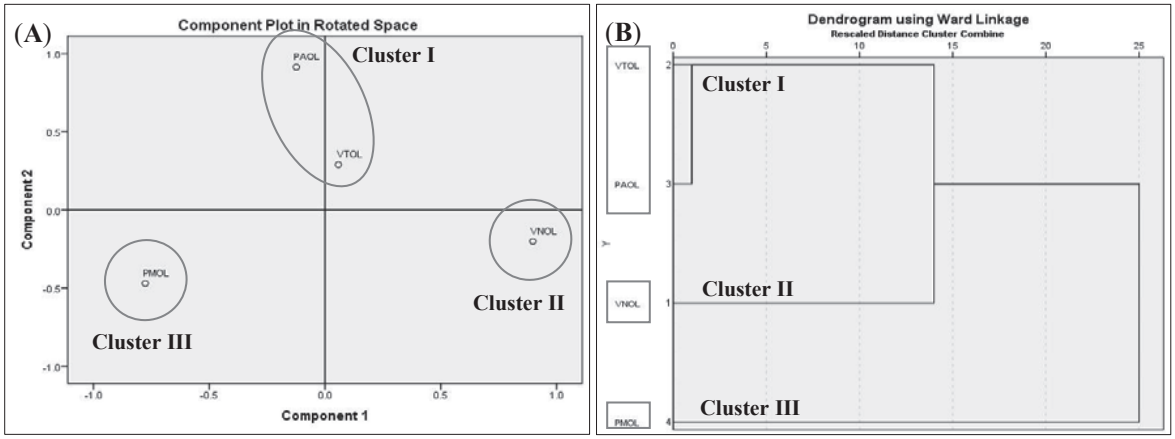

Figure 1 - PCA (A) and HCA (B) analysis of *Vitex* and *Plectranthus* essential oils

the *Vitex* and *Plectranthus* species were also classified into three groups. The PCA score and loading plots comparison enabled the relationships between the oil samples and the variables of chemical composition to be identified. The loading plot indicated that *P. monostachyus* oil (cluster III) could be distinguished by the presence of eight components, α-*cis*-bergamotene, α-amorphene, germacrene D, bicyclogermacrene, α-muurolene, δ-cadinene, (*Z*)-nerolidol, and α-cadinol. The essential oil from *V. negundo* (cluster II) could be characterised by the occurrence of four components which were β-elemene, α-guaiene, elemol, and viridiflorol. Therefore, the PCA results illustrated that *P. monostachyus* and *V. negundo* oils were unique compared to other species. The essential oils from *P. amboinicus* and *V. trifolia* (cluster I) could be categorised by the existence of carvacrol, α-gurjunene, β-caryophyllene, β-copaene, β-gurjunene, γ-elemene, α-humulene, amorpha-4,7(11)-diene, δ-selinene, β-bisabolene, spathulenol, caryophyllene oxide, and γ-eudesmol. The clusters obtained were verified using HCA to evaluate the precision of the classification.

4. CONCLUSION

The results showed that the essential oil of *V. negundo* consisted mainly of δ-elemene, *V. trifolia* mainly consisted of viridiflorol, *P. amboinicus* was predominantly carvacrol, and *P. monostachyus* was chiefly β-caryophyllene. Multivariate statistical analysis showed that the *Vitex* and *Plectranthus* essential oils could be divided into three clusters, cluster I (*V. trifolia* and *P. amboinicus*), cluster II (*V. negundo*), and cluster III (*P. monostachyus*). The information is critical when selecting species with economic potential for the pharmaceutical and cosmetics industries. Furthermore, the multivariate data analysis may be used as a quality control tool for the identification and characterisation of essential oils from different species that intended to be used as raw materials in traditional herbal products.

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