BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Multivariate statistical analysis of the essential oils of five *Beilschmiedia* species from Peninsular Malaysia

[Análisis estadístico multivariado de los aceites esenciales de especies de *Beilschmiedia* de Malasia peninsular]

Wan Mohd Nuzul Hakimi Wan Salleh[1] & Shazlyn Milleana Shaharudin[2]

[1]Department of Chemistry & [2]Department of Mathematics, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, Tanjung Malim, Perak, Malaysia

Reviewed by:
Opeyami Avoseh
Lagos State University
Nigeria

Mamadou Bah
Universidad Autónoma de Querétaro
Mexico

Correspondence:
Wan Mohd Nuzul Hakimi Wan SALLEH:
awmnhakimi@fsmt.upsi.edu.my

Section
Phychemistry

Received: 1 August 2019
Accepted: 14 February 2020
Accepted corrected: 22 April 2020
Published: 30 January 2021

Citation:
Salleh WMNHW, Shaharudin SM. Multivariate statistical analysis of the essential oils of five *Beilschmiedia* species from Peninsular Malaysia
Bol Latinoam Caribe Plant Med Aromat
20 (1): 61 - 70 (2021).
https://doi.org/10.37360/blacpma.21.20.1.5

Abstract: Identification of the chemical composition of essential oils is very important for ensuring the quality of finished herbal products. The objective of the study was to analyze the chemical components present in the essential oils of five *Beilschmiedia* species (i.e. *B. kunstleri*, *B. maingayi*, *B. penangiana*, *B. madang*, and *B. glabra*) by multivariate data analysis using principal component analysis (PCA) and hierarchical clustering analysis (HCA) methods. The essential oils were obtained by hydrodistillation and fully characterized by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). A total of 108 chemical components were successfully identified from the essential oils of five *Beilschmiedia* species. The essential oils were characterized by high proportions of β-caryophyllene (*B. kunstleri*), δ-cadinene (*B. penangiana* and *B. madang*), and β-eudesmol (*B. maingayi* and *B. glabra*). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) revealed that chemical similarity was highest for all samples, except for *B. madang*. The multivariate data analysis may be used for the identification and characterization of essential oils from different *Beilschmiedia* species that are to be used as raw materials of traditional herbal products.

Keywords: Essential oils; *Beilschmiedia*; Principal component analysis; Hierarchical cluster analysis.

Resumen: La identificación de la composición química de los aceites esenciales es muy importante para garantizar la calidad de los productos herbales terminados. El objetivo del estudio fue analizar los componentes químicos presentes en los aceites esenciales de cinco especies de *Beilschmiedia* (*B. kunstleri*, *B. maingayi*, *B. penangiana*, *B. madang* y *B. glabra*) mediante análisis de datos multivariados utilizando los métodos de análisis de componente principal (PCA) y análisis de agrupamiento jerárquico (HCA). Los aceites esenciales se obtuvieron por hidrodestilación y se caracterizaron completamente por cromatografía de gases (GC) y cromatografía de gases-espectrometría de masas (GC-MS). Se identificaron con éxito un total de 108 componentes químicos a partir de los aceites esenciales de las cinco especies de *Beilschmiedia*. Los aceites esenciales se caracterizaron por altas proporciones de β-cariofileno (*B. kunstleri*), δ-cadineno (*B. penangiana* y *B. madang*) y β-eudesmol (*B. maingayi* y *B. glabra*). El análisis de componentes principales (PCA) y el análisis de conglomerados jerárquicos (HCA) revelaron que la similitud química fue más alta para todas las muestras, excepto para *B. madang*. El análisis de datos multivariados puede usarse para la identificación y caracterización de aceites esenciales de diferentes especies de *Beilschmiedia* que se utilizan como materias primas de productos herbales tradicionales.

Palabras clave: Aceites esenciales; *Beilschmiedia*; Análisis de componentes principales; Análisis jerárquico de conglomerados

INTRODUCTION

Chromatography is an important and widely used separation technique of a complex mixture. Essential oils are mainly separated by gas chromatography (GC) combined with usually either flame ionization detector (FID) or mass spectrometry (MS). Recently, the application of chromatographic fingerprint analysis has been accepted for quality control of herbal medicines in order to resolve problems with the identification and authentication of multicomponent materials such as herbal extracts and essential oils (Saraswathy *et al*., 2010; Ruijing *et al*., 2011). Thus, a combination of the chromatographic fingerprint data and multivariate analysis provides comprehensive information on the total chemical composition (Mahdi & Hadi, 2011). 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. Whereas hierarchical clustering analysis (HCA) is used to group things according to their similarities based on specified characteristic variables. This method is now gaining acceptance as one of the approaches for quality control of herbal materials (James & Jane, 2005; Chun *et al*., 2011).

Plants of the genus *Beilschmiedia* belonging to the Lauraceae family comprises about 250 species represented in tropical Asia and Africa (Nishida, 1999; Nishida, 2008). Some species of the genus are used in traditional medicine for the treatment of several ailments. The leaves of *B. tonkinensis* are used to make medicine for easing the pain, inflammation and broken bone (Wiart, 2006). In Cameroon, *B. anacardiodes* stem bark is used to cure uterine tumors, rubella, female genital infections, and rheumatisms (Tchoula, 2001). Besides, the fruits of *B. manii*, *B. gabonensis*, and *B. zenkeri* are used as appetite stimulants and also as spices. In addition, *B. manii* is used in Africa for the treatment of dysentery and headache (Iwu, 1993). In Peninsular Malaysia, a decoction of the bark of *B. pahangensis* is used as a drink after childbirth and also for stomachache and diarrhea (Banfield *et al*., 1994). In addition, the leaves of *B. tonkinensis* are used by Indonesians and Malays to make poultices for application to broken bones (Banfield *et al*., 1994). The leaf of *B. acuta* and *B. obscura* has been used to treat cancer and gastrointestinal infections in Cameroon (Fankam *et al*., 2014). The wood of *B. madang* and the bark of *B. cryptocaryoides* are used traditionally for antimalarial preparation (Kitagawa *et al*., 1993).

The genus produces several classes of compounds such as terpenoids, endiandric acid derivatives, essential oils, fatty acids, epoxyfuranoid lignans, flavonoids, and alkaloids. Some of these compounds are reported to exhibit antioxidant, antibacterial, antimalarial and antituberculosis activities (Chen *et al*., 2007; Lenta *et al*., 2009; Chouna *et al*., 2010; Salleh *et al*., 2015b; Salleh *et al*., 2016a; Salleh *et al*., 2016b; Salleh *et al*., 2016c; Salleh *et al*., 2016d). Previous studies on the compositions of the essential oils of *Beilschmiedia* species have been reported on *B. alloiophylla*, *B. brenesii*, *B. 'chancho blanco'*, *B. costaricensis*, *B. erythrophloia*, *B. miersii*, *B. pendula*, *B. tarairie*, and *B. tilaranensis* (Kumamoto & Scora, 1970; Scora & Scora, 2001; Setzer & Haber, 2007; Chaverri & Ciccio, 2010; Su & Ho, 2013).

In Malaysia, *Beilschmiedia* species has been used as traditional medicines, however, information regarding the volatile composition quality of essential oils from these herbal materials is still limited. Five *Beilschmiedia* species have been selected for this study which is *B. kunstleri*, *B. maingayi*, *B. penangiana* (Salleh *et al*., 2015d), *B. madang* (Salleh *et al*., 2015a), and *B. glabra* (Salleh *et al*., 2015c) and their essential oil compositions have been reported by us. Thus, in a continuation of our systematic studies on these species, herein we characterize their essential oils constituents by multivariate data analysis using principal component analysis (PCA) and hierarchical cluster analysis (HCA).

MATERIAL AND METHODS

Plant materials

Fresh leaf and bark of five *Beilschmiedia* species were collected from Johor and Selangor. The authenticity of the plant materials was confirmed by Dr. Shamsul Khamis from the Herbarium of Universiti Kebangsaan Malaysia, at which the voucher specimens were deposited (Table 1).

Extraction and analysis of essential oils

Extraction of essential oils was done by the hydrodistillation method. The fresh leaf and bark of each plant (300 g) were chopped and hydrodistilled using a Dean-stark apparatus for 8 h. The essential oils obtained were dried over anhydrous magnesium sulfate and stored at 4-6°C.

Table No. 1
Information on plant materials used in the study and their percentage yield

Species	Collection site	Date of collection	Voucher specimen	Yield
B. kunstleri	Kluang, Johor	October 2014	SK2573/14	Leaf: 0.20 g, 0.080% Bark: 0.21 g, 0.084%
B. maingayi	Kluang, Johor	October 2014	SK2571/14	Leaf: 0.58 g, 0.193% Bark: 0.49 g, 0.163%
B. penagiana	Kluang, Johor	October 2014	SK2572/14	Leaf: 0.26 g, 0.104% Bark: 0.25 g, 0.108%
B. madang	Bangi, Selangor	September 2012	SK1984/12	Leaf: 2.20 g, 0.450% Bark: 2.05 g, 0.420%
B. glabra	Kluang, Johor	October 2014	SK2570/14	Leaf: 0.38 g, 0.130% Bark: 0.12 g, 0.04%

Gas chromatography (GC) analysis was performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with an Ultra-1 column (100% polymethylsiloxanes). Helium was used as a carrier gas at a flow rate of 0.7 mL min^{-1}. Injector and detector temperature 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^{-1} 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 percents were reported as means ±SD of triplicates. The calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies, USA).

Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatograph and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with Ultra-1 column (25 m long, 0.33 µm thickness and 0.20 mm inner diameter). Helium was used as a carrier gas at a flow rate of 1 mL min^{-1}. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) at 10°C min^{-1} to 250°C and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization 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.

The constituents of the oil were identified by comparison of their mass spectra with reference spectra in the computer library (Wiley) and also by comparing their retention indices with data in the literature (Adams, 2001). The quantitative data were obtained electronically from the FID area percentage without the use of the correction factor.

Multivariate data analysis

The constituents' common to all essential oil samples were used to determine the similarity among species with a CA performed with the software Statistica 7.0. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to cluster groups based on Euclidean distance. The PCA was carried out with the software Statistica 7.0. PCA was used to reveal interrelationships among the ten species of the genus *Beilschmiedia* based on the essential oil common constituents of these species (Wickramagamage, 2010; Shaharudin *et al*., 2013; Shaharudin *et al*., 2018).

RESULTS AND DISCUSSION

The percentage yields (w/w) of the fresh leaf essential oils ranged from 0.12 to 0.58%. The chemical components identified in the essential oils are listed in Table No. 2.

The essential oils of *B. kunstleri* revealed the presence of 48 components with a percentage of 85.5% and 80.3% in the leaf and bark oils. The essential oils were characterized by the presence of a high concentration of sesquiterpene hydrocarbon (leaf oil 50.5%; bark oil 61.1%). The leaf oil was characterized by its richness in β-caryophyllene (12.1%), while δ-cadinene (13.4%) is present in a high amount in the bark oil. In *B. mangayi* oils, a total of 72 components were detected with the constitution of 86.5% and 82.1% in the leaf and bark oil respectively. The oils were made up predominantly of oxygenated sesquiterpene, constituting about 42.2% (leaf oil) and 43.5% (bark oil), while the most abundant component was β-eudesmol (leaf 24.1%; bark 17.5%).

In the case of B. penangiana, the leaf and bark oils consisted of 35 components, representing 98.2% and 97.7% respectively. δ-Cadinene (leaf 28.7%; bark 17.5%) was found to be the main component in these oils. Analysis of B. madang oils led to the identification of 55 and 42 components, representing 89.8 and 81.5% of the total essential oils from leaf and bark, respectively. Sesquiterpene hydrocarbons were the major group components in the leaf (63.8%) and bark (65.3%) oils with δ-cadinene (leaf 17.0%; bark 20.5%) as the major component. Meanwhile, the analysis of the B. glabra oils revealed the presence of 47 components, of which 45 were identified in the leaf oil (86.8%) and 16 in the bark oil (89.7%). Both oils were characterized by the presence of high concentrations of sesquiterpene hydrocarbons (53.1-66.4%) and the most abundant component was β-eudesmol (leaf 15.4%; bark 19.3%).

Table No. 2
Chemical composition of the essential oils of five *Beilschmiedia* species

No	Components	KI	Percentage (%)									
			BK LO	BK BO	BM LO	BM BO	BP LO	BP BO	BD LO	BD BO	BG LO	BG BO
1	α-Pinene	932	-	0.2	-	-	-	-	0.1	-	-	-
2	Camphene	946	0.3	0.3	0.1	-	1.8	-	-	-	0.5	-
3	Sabinene	969	-	-	-	-	-	-	0.1	0.1	-	-
4	β-Pinene	974	-	-	-	-	-	-	0.1	-	-	-
5	*p*-Mentha-1(7),8-dien-2-ol	1003	0.1	-	0.3	0.2	-	-	-	-	-	-
6	δ-3-Carene	1008	-	-	0.1	0.1	-	-	-	-	0.1	-
7	α-Terpinene	1014	-	-	-	0.1	-	-	0.1	-	0.2	-
8	*p*-Cymene	1020	-	-	0.1	-	-	-	-	-	0.1	-
9	*o*-Cymene	1022	-	-	-	-	-	-	0.1	-	-	-
10	Limonene	1024	-	-	2.0	0.3	-	-	0.1	-	-	-
11	β-Phellandrene	1025	-	-	-	-	-	-	0.1	-	-	-
12	1,8-Cineole	1026	-	0.2	-	0.3	-	-	-	-	2.4	-
13	β-(*E*)-Ocimene	1044	-	-	-	-	-	2.2	-	-	-	-
14	γ-Terpinene	1054	-	-	-	-	-	-	0.1	0.1	-	-
15	*p*-Cresol	1071	-	-	-	1.3	-	-	-	-	-	-
16	Terpinolene	1086	-	-	0.2	-	-	-	0.1	-	-	-
17	*p*-Mentha-2-en-1-ol	1118	-	-	-	-	-	-	-	0.1	-	-
18	*allo*-Ocimene	1128	-	-	-	0.1	-	-	-	-	-	-
19	*trans*-Linalool oxide	1137	-	-	0.1	0.1	-	-	-	-	-	-
20	Terpinen-4-ol	1174	-	-	-	0.3	-	-	2.2	1.7	0.2	-
21	α-Terpineol	1186	-	-	-	0.1	-	-	0.3	0.4	0.1	-
22	Myrtenal	1195	-	-	0.1	-	-	-	-	-	-	-
23	Verbenone	1204	-	-	-	-	-	1.7	-	-	-	-
24	*trans*-Carveol	1215	0.2	-	0.7	0.4	-	-	-	-	-	-
25	Carvone	1239	0.3	-	1.9	0.6	-	-	-	-	-	-
26	*cis*-Carvone oxide	1259	-	-	0.1	-	-	-	-	-	-	-
27	Bornyl acetate	1287	-	0.7	0.2	0.1	-	-	0.1	0.1	0.3	-
28	Carvacrol	1298	-	-	-	0.1	-	-	-	-	-	-
29	Bicycloelemene	1313	-	-	-	-	-	2.7	0.2	-	-	-
30	δ-Elemene	1335	-	-	-	-	-	-	0.3	0.2	-	-
31	α-Cubebene	1345	0.9	2.6	0.5	0.3	-	-	11.3	15.6	0.4	0.9
32	Cyclosativene	1369	-	1.6	-	-	-	-	0.1	-	0.1	-
33	α-Ylangene	1373	0.7	1.3	4.3	-	1.3	1.8	1.0	0.2	3.8	-
34	α-Copaene	1374	1.6	2.7	1.2	0.6	7.7	1.2	0.5	1.1	1.7	3.8
35	Isoledene	1374	0.8	-	0.4	0.7	-	1.2	-	-	-	-
36	β-Patchoulene	1379	0.4	-	0.9	-	-	0.8	-	-	0.4	-
37	β-Panasinsene	1381	-	-	10.2	11.6	-	-	-	-	-	-
38	(*E*)-β-Damascenone	1383	0.1	-	-	-	-	-	0.1	-	-	-
39	Calarene	1384	-	-	-	-	-	-	0.3	-	-	-
40	β-Bourbonene	1387	0.1	-	0.1	-	0.6	-	0.7	-	0.2	-
41	β-Cubebene	1387	-	-	-	-	0.7	-	0.5	-	0.2	-
42	β-Elemene	1389	1.1	-	0.2	0.1	3.2	1.5	2.6	1.0	0.4	1.9

43	*iso*-Longifolene	1389	0.1	0.8	0.2	0.1	1.1	-	-	-	0.3	1.1
44	γ-Gurjunene	1401	-	-	-	0.8	-	-	0.2	1.4	5.2	-
45	Longifolene	1407	1.0	-	-	0.4	-	-	-	-	-	-
46	α-Gurjunene	1409	1.1	-	0.2	0.4	-	1.6	0.9	0.9	0.1	-
47	α-Cedrene	1410	0.2	-	1.1	0.5	0.9	-	-	0.1	0.8	-
48	β-Caryophyllene	1417	12.1	10.6	0.5	0.2	10.4	12.6	10.3	6.7	0.5	5.5
49	Isocaryophyllene	1413	-	-	-	-	-	-	0.1	-	-	-
50	α-*trans*-Bergamotene	1432	-	-	-	1.6	-	-	-	-	-	-
51	β-Gurjunene	1431	-	-	-	-	-	-	-	0.3	1.0	-
52	γ-Elemene	1434	-	-	-	-	-	-	2.5	0.4	-	-
53	β-Humulene	1436	-	-	-	0.3	-	-	0.8	-	-	-
54	α-Guaiene	1437	0.8	0.7	-	1.8	-	4.4	-	-	1.1	-
55	Aromadendrene	1439	1.0	1.8	3.7	1.0	0.9	1.1	1.5	1.3	2.3	-
56	α-Humulene	1452	1.8	0.7	0.2	-	3.0	-	4.3	0.3	0.3	1.6
57	α-Patchoulene	1454	-	-	-	1.0	-	-	-	-	-	-
58	*allo*-Aromadendrene	1458	0.7	0.5	0.2	-	-	-	-	0.3	0.4	-
59	Dehyroaromadendrene	1460	1.0	4.4	0.3	-	-	-	-	0.2	1.0	-
60	γ-Muurolene	1478	-	-	-	0.3	-	-	-	-	-	-
61	*ar*-Curcumene	1479	-	2.0	-	0.3	-	-	-	-	-	-
62	α-Amorphene	1483	0.3	-	0.3	0.8	1.8	-	1.8	1.7	2.1	-
63	Germacrene D	1484	3.1	0.3	0.3	-	20.7	14.6	4.7	1.6	-	9.5
64	β-Selinene	1489	-	0.9	0.3	0.2	1.2	-	0.5	-	12.2	16.9
65	*cis*-β-Guaiene	1492	-	-	-	0.1	-	-	-	0.4	-	-
66	δ-Selinene	1492	-	-	-	-	-	-	0.3	0.7	-	-
67	Cadina-1,4-diene	1495	1.6	0.3	0.4	0.4	-	-	0.4	1.7	3.2	-
68	Valencene	1496	0.3	-	0.6	2.2	-	-	0.2	0.5	4.0	4.1
69	α-Selinene	1498	0.2	-	-	-	-	1.6	-	0.1	0.6	0.9
70	Bicyclogermacrene	1500	-	0.7	-	-	-	-	6.7	-	-	-
71	α-Muurolene	1500	-	-	-	-	0.9	-	1.0	-	0.7	-
72	Epizonarene	1501	0.1	-	0.2	0.2	-	0.9	-	0.6	-	-
73	(*E*,*E*)-α-Farnesene	1505	-	3.0	0.4	-	0.8	-	-	-	-	-
74	α-Bisabolene	1506	-	-	-	1.4	-	-	-	-	1.7	-
75	α-Bulnesene	1509	-	-	-	0.9	-	-	-	-	-	-
76	(*E*,*Z*)-α-Farnesene	1508	-	-	-	-	-	-	-	1.0	-	-
77	γ-Cadinene	1513	1.6	2.6	3.7	0.3	-	1.6	-	1.6	1.1	-
78	δ-Cadinene	1522	5.9	13.4	2.0	1.5	28.7	17.5	17.0	20.5	2.7	15.8
79	*cis*-Calamenene	1528	0.6	1.7	3.1	0.7	0.5	0.5	0.4	2.3	0.7	-
80	α-Cadinene	1537	0.2	-	-	-	-	-	-	0.6	-	-
81	α-Calacorene	1544	-	-	0.5	0.6	0.8	-	1.0	0.9	2.3	-
82	Elemol	1548	-	-	-	-	-	-	1.5	0.8	-	-
83	Germacrene B	1559	11.2	8.5	-	0.2	5.9	10.7	1.3	-	1.2	3.8
84	(*E*)-Nerolidol	1562	-	-	-	-	-	-	5.0	0.9	-	-
85	Palustrol	1567	-	-	-	-	-	-	0.5	-	-	-
86	α-Cedrene epoxide	1574	-	-	0.7	-	-	-	-	-	-	-
87	Spathulenol	1577	-	-	0.2	0.1	-	-	1.5	1.0	-	-
88	Caryophyllene oxide	1582	7.0	5.4	11.0	12.8	-	-	-	-	8.1	-
89	Globulol	1590	-	-	-	-	1.5	-	-	-	-	1.7
90	Viridiflorol	1592	3.1	-	0.5	0.7	-	8.0	2.8	-	-	-
91	Ledol	1602	4.9	-	1.0	-	-	-	-	-	-	-
92	Guaiol	1600	-	-	-	-	-	-	2.4	-	-	-
93	5-Cedranone	1628	-	-	-	-	-	2.0	-	-	-	-
94	γ-Eudesmol	1630	-	-	-	-	-	-	1.5	-	-	-
95	Alloaromadendrene epoxide	1639	-	-	-	0.2	-	-	-	-	1.6	-
96	Caryophylle-4(12),8(13)-dien-5β-ol	1639	-	-	-	-	-	-	-	-	2.5	-
97	t-Muurolol	1644	7.2	3.4	-	-	2.7	7.5	-	-	-	-
98	β-Eudesmol	1649	-	-	24.1	17.5	1.1	-	1.0	-	15.4	19.3
99	Vulgarone B	1649	-	-	2.3	-	-	-	-	-	-	-
100	α-Cadinol	1652	10.4	9.0	-	-	-	-	5.8	10.6	2.2	2.3
101	α-Eudesmol	1652	-	-	-	12.2	-	-	-	0.4	-	-
102	Valerianol	1656	-	-	2.4	-	-	-	-	-	-	-

103	Isoledene	1723	-	-	-	-	-	-	0.3	0.1	-	-
104	Eupatoriochromene	1761	-	-	0.8	0.5	-	-	-	-	-	-
105	Aristolone	1762	0.3	-	1.6	-	-	-	-	-	-	-
106	Guaiazulene	1779	1.1	-	-	2.3	-	-	-	1.0	0.4	0.6
107	Phytol	1942	-	-	-	-	-	-	0.2	-	-	-
108	Hexadecanoic acid	1959	-	-	-	0.2	-	-	0.3	-	-	-
Group components												
Monoterpene hydrocarbons			0.3	1.2	2.5	0.6	1.8	2.2	0.9	0.2	0.9	-
Oxygenated monoterpenes			0.3	0.2	1.4	2.9	-	-	2.6	2.3	3.0	-
Sesquiterpene hydrocarbons			**50.5**	**61.1**	36.8	34.3	**91.1**	**76.3**	**63.8**	**65.3**	**53.1**	**66.4**
Oxygenated sesquiterpenes			33.7	17.8	**42.2**	**43.5**	5.3	15.5	22.0	13.7	29.8	23.3
Others			0.7	-	3.6	0.8	-	3.7	0.5	-	-	-
Identified components (%)			**85.5**	**80.3**	**86.5**	**82.1**	**98.2**	**97.7**	**89.8**	**81.5**	**86.8**	**89.7**

KI – Kovats index; BKLO – *B. kunstleri* leaf oil; BKBO - *B. kunstleri* bark oil; BMLO – *B. maingayi* leaf oil; BMBO – *B. maingayi* bark oil; BPLO – *B. penangiana* leaf oil; BPBO – *B. penangiana* bark oil; BDLO – *B. madang* leaf oil; BDBO - *B. madang* bark oil; BGLO – *B. glabra* leaf oil; BGBO – *B. glabra* bark oil

Furthermore, the chemical components of the essential oils were subjected to PCA. This analysis was employed to provide an overview of the capacity to distinguish essential oil components based on GC-MS data. The CA revealed three distinct groups for each leaf and bark oils, based on the Euclidian distance as illustrated in Figure No. 1.

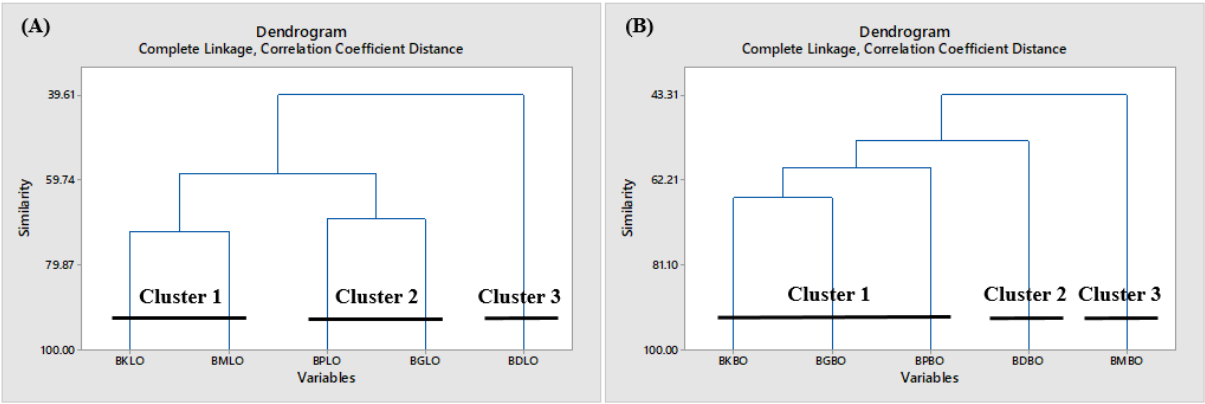

Figure No. 1
UPGMA dendrogram based on the similarity of *Beilschmiedia* leaf (A) and bark (B) oils

For *Beilschmiedia* leaf oil, the first group, Cluster I consisted of *B. kunstleri* and *B. maingayi*. This cluster was characterized by the presence of camphene, *p*-mentha-1(7),8-dien-2-ol, *trans*-carveol, carvone, α-cubebene, α-ylangene, α-copaene, isoledene, β-patchoulene, β-bourbonene, β-elemene, *iso*-longifolene, α-gurjunene, α-cedrene, β-caryophyllene, aromadendrene, α-humulene, *allo*-aromadendrene, dehyroaromadendrene, α-amorphene, germacrene D, cadina-1,4-diene, valencene, epizonarene, γ-cadinene, δ-cadinene, *cis*-calamenene, caryophyllene oxide, viridiflorol, ledol, and aristolone. Cluster II included *B. penangiana* and *B. glabra* with camphene, α-ylangene, α-copaene, β-bourbonene, β-cubebene, β-elemene, *iso*-longifolene, α-cedrene, β-caryophyllene, aromadendrene, α-humulene, α-amorphene, β-selinene, α-muurolene, δ-cadinene, *cis*-calamenene, α-calacorene, germacrene B, and β-eudesmol. Meanwhile, Cluster III consisted of *B. madang*. The element in this cluster was characterized by the components α-pinene, sabinene, β-pinene, *o*-cymene, β-phellandrene, γ-terpinene,

bicycloelemene, δ-elemene, calarene, isocaryophyllene, γ-elemene, β-humulene, bicyclogermacrene, elemol, (*E*)-nerolidol, Palustrol, guaiol, γ-eudesmol, isoledene, phytol, and hexadecanoic acid.

For *Beilschmiedia* bark oil, the first group, Cluster I consisted of *B. kunstleri*, *B. glabra*, and *B. penangiana*. This cluster was characterized by the presence of α-copaene, β-caryophyllene, germacrene D, δ-cadinene, and germacrene B. Cluster II included *B. madang* with sabinene, γ-terpinene, *p*-mentha-2-en-1-ol, δ-elemene, β-gurjunene, γ-elemene, (*E*,*Z*)-α-farnesene, (*E*)-nerolidol, and isoledene. In addition, Cluster III consisted of *B. maingayi*. The element in this cluster was characterized by the components *p*-mentha-1(7),8-dien-2-ol, δ-3-carene, α-terpinene, limonene, *p*-cresol, *allo*-ocimene, *trans*-linalool oxide, *trans*-carveol, carvone, carvacrol, β-panasinsene, longifolene, α-*trans*-bergamotene, β-humulene, γ-muurolene, α-bisabolene, α-bulnesene, alloaromadendrene epoxide, eupatoriochromene, and hexadecanoic acid.

Furthermore, to evaluate the accuracy of this classification, the cluster obtained was confirmed by PCA as illustrated in Figure No. 2. Similarly, the species of *Beilschmiedia* were divided into three groups: for leaf oil, group I consisted of the species *B. maingayi* and *B. kunstleri*, group II comprised *B. glabra* and *B. penangiana*; while and group III included *B. madang*. On the other hand, for bark oil, group I consisted of the species *B. penangiana*, *B. glabra*, and *B. kunstleri*, group II comprised *B. madang*; while and group III included *B. maingayi*. The results were obtained by PCA based on seventeen (leaf oil) and fifteen (bark oil) chemical components as shown in Table No. 3. Three factors explained 79.43% (leaf oil) and 60.0% (bark oil) of accumulated variation of the data analyzed. The first three are considered the most important as they represent ≥60% of the accumulated variation.

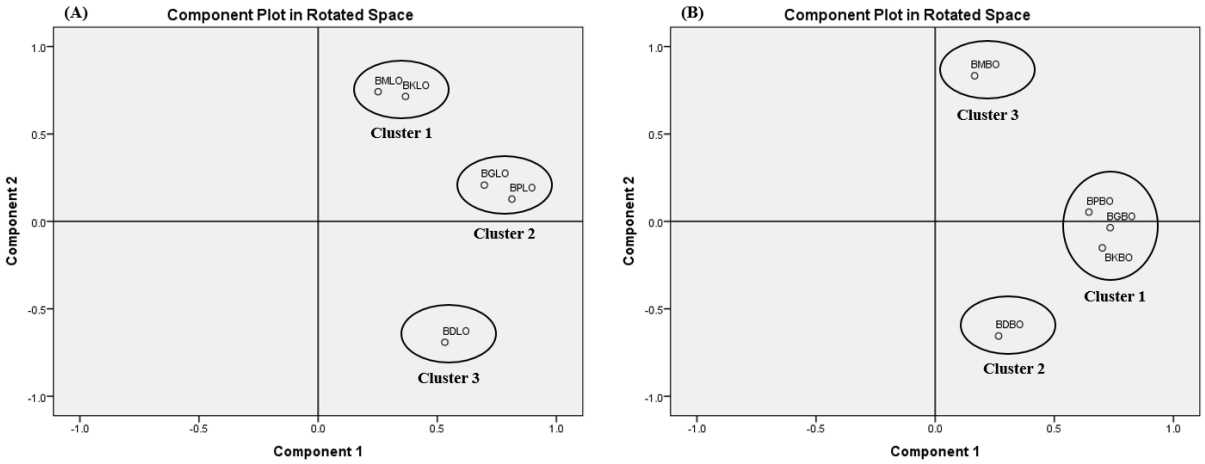

Figure No. 2
Biplot of the first three factors of *Beilschmiedia* leaf (A) and bark (B) oils

The PCA revealed a weaker inter-relationship in the composition of essential oils of *B. madang* and *B. maingayi*. This might be a result of the high production of δ-cadinene (leaf oil 17.0%; bark oil 20.5%) in *B. madang*, and β-eudesmol (leaf oil 24.1%; bark oil 17.5%) in *B. maingayi*. These results may be correlated with other factors involving a genetic determination that could also be modulated by biotic pressures, volatile constituents during flowering influenced by pollinators and during the vegetative phase by pathogens and herbivores, or differences in environmental conditions (Silva *et al*., 2007). Thus, the variation pattern in essential oil composition may reflect selective pressures in different ecological and geographical environments (ecotypes).

Table No. 3
Eigenvalues and cumulative variance of factors obtained from PCA analysis based on the composition of leaf (A) and bark (B) oils of *Beilschmiedia*

Composition	(A)			Composition	(B)		
	F1	F2	F3		F1	F2	F3
α-Ylangene	1.72			α-Cubebene		1.59	
α-Copaene	1.72			α-Copaene	1.84		
β-Elemene	1.76			β-Caryophyllene	1.91		
β-Caryophyllene	1.81			α-Guaiene	1.83		
Aromadendrene	1.87			Dehyroaromadendrene			-1.64
α-Humulene	1.90			Germacrene D	2.20		
α-Amorphene	1.97			Valencene		1.95	
Germacrene D	1.11			γ-Cadinene	1.53		
β-Selinene	1.70		1.75	δ-Cadinene	2.10		
γ-Cadinene		1.66		*cis*-Calamenene	1.55		
δ-Cadinene	2.06			Germacrene B	2.60		
cis-Calamenene	2.07			t-Muurolol	2.35		
α-Calacorene	1.81		1.85	β-Eudesmol			1.95
Germacrene B	2.01			α-Cadinol		2.16	
Caryophyllene oxide		1.74		Guaiazulene		2.49	
Ledol		1.67					
β-Eudesmol	2.03		2.04				
Eigenvalue	1.94	1.28	0.74	Eigenvalue	1.00	1.00	1.00
% of Variance	38.88	25.67	14.89	% of Variance	20.0	20.0	0.20
Cumulative (%)	38.88	64.54	79.43	Cumulative (%)	20.0	40.0	0.60

Significant ≥60

CONCLUSION

In conclusion, our study reports the chemical variability of the essential oils of five species of the genus *Beilschmiedia*. This information is critical when selecting species with economic potential for the pharmaceutical and cosmetics industry. In addition, the multivariate data analysis may be used as quality control tools for the identification and characterization of essential oils from different *Beilschmiedia* species that are to be utilized as raw materials in traditional herbal products. Further studies need to be carried out to determine fingerprints and chemical compositions of other *Beilschmiedia* species and those collected from different origins.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for research facilities.

REFERENCES

Adams RP. 2001. **Identification of Essential oil by gas Chromatography-Quadrupole/Mass Spectroscopy**. Carol, Allured Publishing Corporation, Illinois, USA.

Banfield JE, Black DC, Collins DJ, Hyland BPM, Lee JJ, Pranowo RS. 1994. Constituents of some species of *Beilschmiedia* and *Endiandra* (Lauraceae): New endiandric acid and benzopyran derivatives isolated from *Beilschmiedia oligandra*. **Aust J Chem** 47: 587 - 607. **https://doi.org/10.1071/CH9940587**

Chaverri C, Ciccio JF. 2010. Essential oils from *Beilschmiedia pendula* (Sw.) Hemsl. (Lauraceae) from Costa Rica. **J Essent Oil Res** 2: 259 - 262. **https://doi.org/10.1080/10412905.2010.9700320**

Chen JJ, Chou ET, Peng CF, Chen IS, Yang SZ, Huang HY. 2007. Novel epoxyfuranoid lignans and antitubercular constituents from the leaves of *Beilschmiedia tsangii*. **Planta Med** 73: 567 - 571. **https://doi.org/10.1055/s-2007-967195**

Chouna JR, Nkeng-Efouet PA, Lenta BN, Wansi JD, Kimbu SF, Sewald N. 2010. Endiandric acid derivatives from the stem bark of *Beilschmiedia anacardioides*. **Phytochem Lett** 3: 13 - 16. **https://doi.org/10.1016/j.phytol.2009.10.005**

Chun MH, Kim EK, Yu SM, Oh MS, Moon KY, Jung JH, Hong J. 2011. GC/MS combined with chemometrics methods for quality control of *Schizonepeta tenuifolia* Briq: determination of essential oils. **Microchemical J** 97: 274e281. **https://doi.org/10.1016/j.microc.2010.09.015**

Fankam AG, Kuiate JR, Kuete V. 2014. Antibacterial activities of *Beilschmiedia obscura* and six other Cameroonian medicinal plants against multi-drug resistant Gram-negative phenotypes. **BMC Complement Altern Med** 14: 241 - 250. **https://doi.org/10.1186/1472-6882-14-241**

Iwu MM. 1993. **Handbook of African medicinal plants**. CRC Press, Boca Raton, USA.

James NM, Jane CM. 2005. **Statistic and chemometrics for analytical chemistry**. England: Pearson.

Kitagawa I, Minagawa K, Zhang RS, Hori K, Doi M, Inoue M, Ishida T, Kimura M, Uji T, Shibuya H. 1993. Dehatrine, an antimalarial bisbenzylisoquinoline alkaloid from the Indonesian medicinal plant *Beilschmiedia madang*, isolated as a mixture of two rotational isomers. **Chem Pharm Bull** 41: 997 - 999.

Kumamoto J, Scora RW. 1970. Structure of sarisan, an isomer of myristicin, isolated from the leaf oil of *Beilschmiedia miersii*. **J Agric Food Chem** 18: 544 - 545. **https://doi.org/10.1021/jf60169a006**

Lenta BN, Tantangmo F, Devkota KP, Wansi JD, Chouna JR, Soh RC, Neumann B, Stammler HG, Tsamo E, Sewald N. 2009. Bioactive constituents of the stem bark of *Beilschmiedia zenkeri*. **J Nat Prod** 72: 2130 - 2134. **https://doi.org/10.1021/np900341f**

Mahdi H, Hadi P. 2011. Recent trends in application of multivariate curve resolution approaches for improving gas chromatography mass spectrometry analysis of essential oil. **Talanta** 85: 835e849. **https://doi.org/10.1016/j.talanta.2011.05.045**

Nishida S. 1999. Revision of *Beilschmiedia* (Lauraceae) in the neotropics. **Ann Missouri Bot Gard** 86: 657 - 701. **https://doi.org/10.2307/2666150**

Nishida S. 2008. Taxonomic revision of *Beilschmiedia* (Lauraceae) in Borneo. **Blumea** 53: 345 - 383. **https://doi.org/10.3767/000651908X608007**

Ruijing P, Fangqiu G, Hongmei L, Wei-Wei F, Yi-Zeng L. 2011. Development of the chromatographic fingerprint of *Scutellaria barbata* D.Don by GC-MS combined with chemometrics methods. **J Pharm Biomed Anal** 55: 391e396. **https://doi.org/10.1016/j.jpba.2011.01.016**

Salleh WMNHW, Ahmad F, Khong HY. 2015a. Chemical compositions and biological activities of the essential oils of *Beilschmiedia madang* Blume (Lauraceae). **Arch Pharm Res** 38: 485 - 493. **https://doi.org/10.1007/s12272-014-0460-z**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. 2015b. Chemical composition and biological activities of essential oil of *Beilschmiedia pulverulenta*. **Pharm Biol** 54: 322 - 330. **https://doi.org/10.3109/13880209.2015.1037003**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. 2015c. Chemical compositions and biological activities of essential oils of *Beilschmiedia glabra*. **Nat Prod Commun** 10: 1297 - 1300. **https://doi.org/10.1177/1934578X1501000740**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. 2015d. Comparative study of the essential oils of three *Beilschmiedia* species and their biological activities. **Int J Food Sci Tech** 51: 240 - 249. **https://doi.org/10.1111/ijfs.12962**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. 2016a. Anticholinesterase and anti-inflammatory constituents from *Beilschmiedia pulverulenta* Kosterm. **Nat Prod Sci** 22: 225 - 230. **https://doi.org/10.20307/nps.2016.22.4.225**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. 2016b. Chemical constituents and bioactivities from the leaves of *Beilschmiedia glabra* Kosterm (Lauraceae). **Marmara Pharm J** 20: 400 - 406. **https://doi.org/10.12991/mpj.20162079371**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM, Chen JJ, Nahar L, Wansi JD, Sarker SD. 2016c. Beilschglabrines A and B: Two new bioactive phenanthrene alkaloids from the stem bark of *Beilschmiedia glabra*. **Phytochem Lett** 16: 192 - 196. **https://doi.org/10.1016/j.phytol.2016.04.013**

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM, Sarker SD. 2016d. Madangones A and B: Two new neolignans from the stem bark of *Beilschmiedia madang* and their bioactivities. **Phytochem Lett** 15: 168 - 173. **https://doi.org/10.1016/j.phytol.2016.01.004**

Saraswathy A, Shakila R, Sunilkumar KN. 2010. HPTLC fingerprint profile of some *Cinnamomum* species. **Phcog J** 2: 211e215. **https://doi.org/10.1016/S0975-3575(10)80095-1**

Scora RW, Scora PE. 2001. Essential leaf oil of *Persea subgenus*, *Eriodaphne* and closely related *Perseoid* genera. **J Essent Oil Res** 13: 37 - 42. **https://doi.org/10.1080/10412905.2001.9699599**

Setzer WN, Haber WA. 2007. Leaf essential oil composition of five species of *Beilschmiedia* from Monteverde, Costa Rica. **Nat Prod Commun** 2: 79 - 83. **https://doi.org/10.1177/1934578X0700200116**

Shaharudin SM, Ahmad N, Yusof F. 2013. Improved cluster partition in principal component analysis guided clustering. **Int J Comput Appl** 75: 22 - 25. **https://doi.org/10.5120/13156-0839**

Shaharudin SM, Ahmad N, Zainuddin NH, Mohamed NS. 2018. Identification of rainfall patterns on hydrological simulation using robust principal component analysis. **Indo J Elect Eng Comp Sci** 11: 1162 - 1167. **https://doi.org/10.11591/ijeecs.v11.i3.pp1162-1167**

Silva FG, Oliveira CBA, Pinto JEBP, Nascimento VE, Santos SC, Seraphin JC, Ferri PH. 2007. Seasonal variability in the essential oils of wild and cultivated *Baccharis trimera*. **J Braz Chem Soc** 18: 990 - 997. **https://doi.org/10.1590/S0103-50532007000500017**

Su YC, Ho CL. 2013. Composition and in vitro cytotoxic activities of the leaf essential oil of *Beilschmiedia erythrophloia* from Taiwan. **Nat Prod Commun** 8: 143 - 144. **https://doi.org/10.1177/1934578X1300800135**

Tchoula JM. 2001. **Inventaire ethnobotanique des plantes medicinales du Noun (Ouest-Cameroun) utilisees pour soigner les infections fongiques et bacteriennes**. Memoire, Universite de Dschang, Cameron.

Wiart C. 2006. **Medicinal plants of Asia and the Pacific**. Taylor & Francis, Boca Raton, USA.

Wickramagamage P. 2010. Seasonality and spatial pattern of rainfall of Sri Lanka: Exploratory factor analysis. **Int J Climatol** 30: 1235 - 1245. **https://doi.org/10.1002/joc.1977**