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Essential Oils from Fresh Fruits, Fruit Stalks and Stem Barks of Four *Piper nigrum* Varieties from Sarawak

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ABSTRACT

The variation of chemical compositions in essential oils obtained from fresh fruits, fruit stalks and stem barks of four *Piper nigrum* L. varieties was analysed using capillary gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The essential oils were extracted by hydrodistillation Clevenger-type apparatus. The major oil constituents identified in all samples were β-elemene (1.07-2.93%), (+)-δ-cadinene (0.58-6.20%), α-caryophyllene (1.90-6.21%), δ-elemene (0.38-13.26%), 3-carene (0.07-16.82%) and β-caryophyllene (11.78-38.33%). All oil samples showed high content of sesquiterpenes, whereas fruit oils from Semongok Emas and Semongok Wan yielded mostly monoterpenes. Hierarchical clustering and principal component analysis (PCA) of the oil components revealed significant correlation amongst individual *P. nigrum* oil samples.

Keywords**:** *P. nigrum*, varieties, essential oil, GC-MS, chemometric analysis

INTRODUCTION

Piper nigrum is a perennial woody climber, which grows up to 10 meter (Ravindran & Kallupurackal 2000). Its green tinged young stems becoming dark green upon maturity. The fresh unripe fruits are green in color, while ripe fruits are red and dry fruits are black and wrinkled (Barceloux 2009). The dried ground berries are the most widely traded spices in the world. There are various varieties of black pepper and the most popular cultivar in Malaysia is Kuching variety (Ravindran & Kallupurackal 2000; Janic 2007). Semongok Perak and Semongok Emas varieties of *P. nigrum* were produced from breeding work in the laboratory and were released in 1991 and 1998, respectively (Det 2008). The varieties are known to vary in spike length, berry attributes, quality parameters and yield (Amma *et al.* 2001). The varieties also differ in resistance to pest and disease (Mammootty *et al.* 2008). Various investigations focused mainly on the variability of oil compositions between the plant parts. The aims of this study were to evaluate the variation of chemical compositions in the essentials oil of several *P. nigrum* varieties cultivated in Sarawak and classify theme by using chemometric approach.

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MATERIALS & METHODS

Plant material and hydrodistillation

Fresh fruits, fruit stalks and stem barks of *P. nigrum* varieties (Kuching, Semongok Emas, Semongok Perak and Semongok Wan) were collected from Tarat, Serian in Sarawak. Identification of *P. nigrum* varieties was performed by Tarat Agricultural Seeding Production Centre. Plant materials (100 g) were subjected to hydrodistillation in a Clevenger-type apparatus for 8 hours. The oils collected were dried over sodium sulphate anhydrous. The oil yield was calculated and recorded on the basis of dried weight material.

GC-FID and GC-MS analysis

GC-FID analysis on the essential oils constituents were performed by using a Hewlett Packard HP-6890 gas chromatograph equipped with a flame ionization detector (FID) using a HP-5 fused capillary column (30 m x 0.25 mm i.d. x 0.25 μm of phase thickness). Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. Exactly 1 μL of diluted sample was injected in splitless mode. The oven temperature was initially set at 40 °C for 3 min, and then programmed at rate of 8 °C/min to 160 °C and at a rate of 15 °C/min to 300 °C. The final temperature was held constant for 10 min.

GC-MS analysis of essential oil was performed on a GC-MS model Shimadzu QP-2010 Plus. Prior to GC-MS analysis, exactly 1 μl of diluted essential oil was injected into the column in the splitless mode. The essential oil sample was separated on a capillary BPX-5 column (30 m x 0.25 mm i.d. x 0.25 μm of film thickness). The temperature program used was similar to GC-FID analysis. The injector temperature was set at 25°C. Helium gas was used as carrier gas with a flow rate of 1 mL/min.

Identification of the individual components was made by comparison of their mass spectral with the National Institute for Standard Technology – NIST08 library incorporated in GC-MS data system and reconfirmed by comparing the calculated Kovat's indices with Liu *et al.* (2011). The Kovats index was calculated using n-alkanes homologous series. The semi-quantitative data of the oils were obtained using peak area of each component in the gas chromatogram without applying correction factors.

Chemometric statistical analysis

Prior to cluster analysis (CA) and principal component analysis (PCA), the set of data was processed using PASW Statistics 18 software. A combination of CA and PCA were used to assess variability existing in composition of essential oils from different varieties of *P. nigrum.*

RESULTS & DISCUSSION

The Percentage Yield of the Essential Oils

The oil yield of samples calculated on dry weight basis showed that the fruit stalk gave highest yield (2.49 – 3.80%, v/w), while stem bark showed the lowest yield of oil (0.33 – 1.13%, v/w). The fruit gave oil yield between 1.05 – 1.49% (v/w) and all the oil was in liquid form. The physical properties of each oil types are summarized in Table 1.

Chemical Composition of the Essential Oils

Figure 1 shows a typical GC-MS chromatogram of essential oils from *P. nigrum*. The identified oil constituents and their Kovat's indices are listed in Table 2. The major components identified in the *P. nigrum* oil were monoterpene (3-carene) and sesquiterpenes (β-elemene, (+)-δ-cadinene, α-caryophyllene, δ-

Table 1. Percentage yield and physical properties of oil isolated from *P. nigrum* varieties.

Plant part	Variety	Oil yield (% v/w)	Oil colour
Fruit	Kuching	1.43	Light yellow
	Semongok Emas	1.05	Light yellow
	Semongok Perak	1.34	Light yellow
	Semongok Wan	1.49	Light yellow
Fruit stalk	Kuching	3.80	Light greenish yellow
	Semongok Emas	3.07	Light yellow
	Semongok Perak	2.49	Dark yellow
	Semongok Wan	3.77	Light yellow
Stem bark	Kuching	0.88	Light yellow
	Semongok Emas	1.04	Light yellow
	Semongok Perak	0.33	Light yellow
	Semongok Wan	1.13	Light yellow

elemene and β- caryophyllene). A total of 19 monoterpenes, 78 sesquiterpenes and 3 non-terpenes compounds were identified in the oil samples. Monoterpenes were detected in the range of 0.07 - 55.50% in oils from different varieties. The presence of monoterpenes in fruit oils were significantly high (31.57 – 55.50%) but very low in stem bark oils (0.07 – 8.39%). Monoterpenes were dominated by hydrocarbon monoterpenes, such as α-pinene (0.56 – 4.58%), β-pinene (1.86 – 7.97%) and limonene (3.24 – 14.47%). 3-Carene was the most abundant monoterpene (0.07 – 16.82%) identified. This finding is different from previous studies (Menon *et al.* 2003; Menon & Padmakumari 2005a) which reported β-pinene as the main constituents in the fruit oils of *P. nigrum*. High content of sabinene was reported in the fruit oil of *P. nigrum* (Menon *et al.* 2003; Tchoumbougnang *et al*. 2009) but sabinene was identified only in fruits oil of Kuching and Semongok Emas varieties. The fruits oil from Semongok Emas has the highest percentage of hydrocarbon monoterpene (53.78%) among varieties, while the stem bark oil—from Semongok Wan showed the lowest content (0.07%). Menon *et al*. (2005b) reported α-pinene (2.3-5.4%) as the principal monoterpene in fruits oil of Kuching variety and similar result was observed in fruits oil where Kuching variety contained 3.33% of α-pinene. Only a few oxygenated monoterpenes were identified and Semongok Emas fruits oil recorded the highest content (1.71%). Oxygenated monoterpenes was not detected in stem bark oil of Kuching and Semongok Wan varieties.

Approximately 44.50-98.87% of total content of *P. Nigrum* oil samples were sesquiterpenes, mainly in fruit stalk oils (87.31-90.45%) and stem bark oils (78.01-98.87%). The stem barkoils reported the highest percentage of hydrocarbon sesquiterpenes (36.68-82.84%), while the fruit oils gave the lowest percentage of hydrocarbon sesquiterpenes (39.68-59.10%).The major hydrocarbon sesquiterpene identified in all oil samples was β-caryophyllene (11.78-38.33%). This finding is similar with those previous reports (Pino *et al.* 2003; Martins *et al*. 1998; Sasidharan & Menon 2010). Pino *et al.* (2003) have identified α-terpinene with significant amount in stem bark oil but less than 1% of this compound was detected from the stem bark oil in this study. Hydrocarbon sesquiterpene were abundance in fruit stalk oil of Semongok Perak variety (81.54%), while the fruit oil of Semongok Emas variety contained only 39.68% of hydrocarbon sesquiterpene. Oxygenated sesquiterpenes represent 1.38-16.74% of total oil content. The most abundance oxygenated hydrocarbon compounds identified are caryophyllene oxide (0.30-3.06%) and spathulenol (0.06-5.49%). Oxygenated sesquiterpenes were found mostly in the stem bark oil of Kuching variety (16.74%). Only a few of oxygenated sesquiterpenes were identified in fruit oil of Semongok Perak variety (1.38%).

Statistical Analysis on Essential Oil Constituents

The chemometric analysis of oil components revealed similarity between plant parts among *P. nigrum* varieties. In comparison of similarity level of plant parts from *P. nigrum* varieties, some close relationships were revealed, especially among the fruit oils. Dendrogram

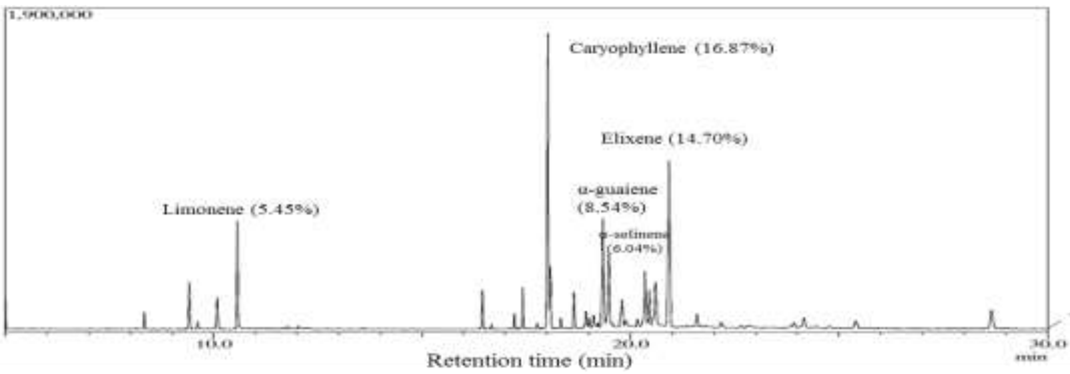

Figure 1. A GC chromatogram of fruit stalk oil from Kuching variety of *P. nigrum.*

(Figure 2) and PCA plot (Figure 3) showed that the fruit oil of Semongok Perak variety has the highest similarity level with that of Semongok Wan variety fruit oils.

The fruit oil of Semongok Perak variety also has some similarity with the fruit oil of Semongok Emas variety. There were also some correlations between the stem bark oils in all samples. The stem bark oil of Kuching variety was clustered next to stem bark oil Semongok Perak variety. The stem bark oil of Kuching variety was also correlated to Semongok Emas variety stem bark oil. There was some similarity between the fruit stalk oils of Kuching and Semongok Perak varieties.

Results obtained from cluster analysis and principal component analysis also exhibited the existence of several variations within the oils according to the plant parts. The stem bark and fruit stalk oils of Semongok Wan were clustered separately from other oil samples as shown in Figures 2 and 3.

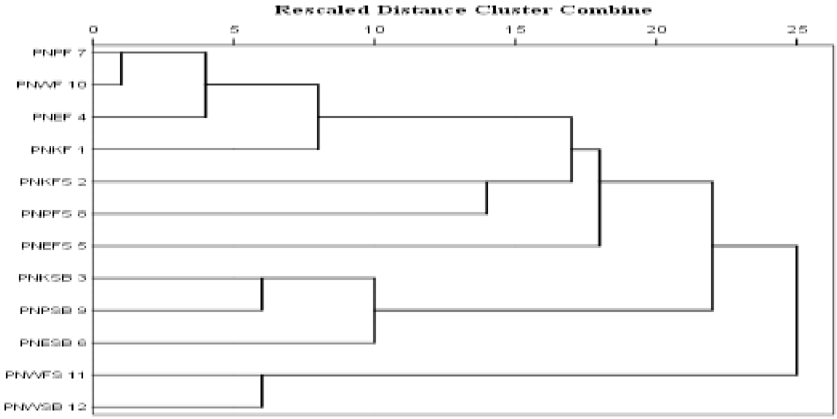

Figure 2. Two-dimensional dendrogram obtained in the hierarchal cluster analysis of fruits, fruit stalks and stem barks oils from four *P. nigrum* varieties based on the GC-MS analysis of essential oils. Vertical: Oil samples analysed; Horizontal: Similarity level between oil samples. Distance cluster is the distance of each oil sample from all the others.

Note: PNKF: Kuching variety (fruit); PNKFS: Kuching variety (fruit stalk); PNKSB: Kuching variety (stem bark); PNEF: Semongok Emas variety (fruit); PNEFS: Semongok Emas variety (fruit stalk); PNESB: Semongok Emas variety (stem bark); PNPF: Semongok Perak variety (fruit); PNPFS: Semongok Perak variety (fruit stalk); PNPSB: Semongok Perak variety (stem bark); PNWF: Semongok Wan variety (fruit); PNWFS: Semongok Wan variety (fruit stalk); PNWSB: Semongok Wan variety (stem bark)

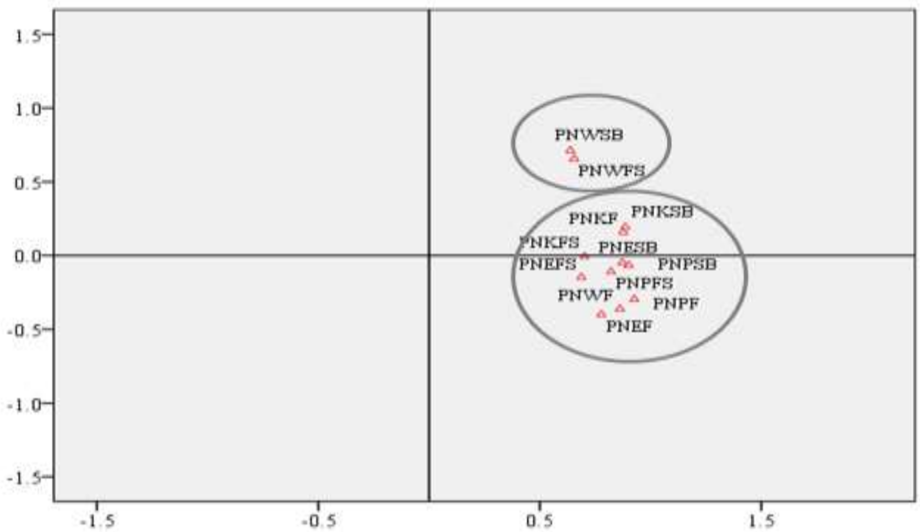

Figure 3. Component plot obtained in PCA of fruit, fruits stalk and stem bark of four *P. nigrum* varieties based on their oil constituents identified by GC-MS

Note: PNKF: Kuching variety (fruit); PNKFS: Kuching variety (fruit stalk); PNKSB: Kuching variety (stem bark); PNEF: Semongok Emas variety (fruit); PNEFS: Semongok Emas variety (fruit stalk); PNESB: Semongok Emas variety (stem bark); PNPF: Semongok Perak variety (fruit); PNPFS: Semongok Perak variety (fruit stalk); PNPSB: Semongok Perak variety (stem bark); PNWF: Semongok Wan variety (fruit); PNWFS: Semongok Wan variety (fruit stalk); PNWSB: Semongok Wan variety (stem bark)

Table 2. Chemical compounds identified in fruit, fruit stalk and stem bark oils for *P. nigrum* samples

	Percentage of compound (%)											
Chemical Composition (KI*)	KF	KFS	KSB	EF	EFS	ESB	PF	PFS	PSB	WF	WFS	WSB
Monoterpene hydrocarbons												
α-Thujene (931)	0.38	-	-	1.05	0.09	-	-	-	-	-	0.31	-
α-Pinene (938)	3.33	0.80	-	4.58	1.12	0.28	4.13	0.56	0.30	3.88	0.78	-
Sabinene (979)	2.82	-	-	6.41	-	0.50	-	-	-	-	-	-
β-Pinene (985)	5.80	2.46	0.10	6.08	1.87	0.23	6.93	1.86	0.38	7.97	1.76	-
β-Myrcene (993)	1.34	0.41	-	2.18	0.37	0.18	1.75	0.38	0.12	2.37	0.30	-
3-Carene (1014)	6.18	1.80	0.58	16.82	3.94	5.17	12.31	2.46	3.83	16.11	0.96	0.07
α-Terpinene (1024)	-	-	-	0.27	0.13	0.05	0.50	-	-	0.28	0.10	-
Limonene (1035)	9.83	5.45	0.29	10.36	3.94	0.90	11.65	5.33	0.94	14.47	3.24	-
β-Phellandrene (1040)	1.22	-	-	2.79	0.24	0.35	0.59	0.07	0.10	1.04	0.16	-
δ-Terpinene (1067)	-	-	-	0.77	-	0.18	-	-	-	0.38	-	-
Terpinolene (1089)	0.38	0.20	-	1.41	0.27	0.12	0.87	0.15	-	1.46	0.15	-
Other monoterpene hydrocarbons				1.06	0.16	0.55				1.36	0.61	
Sub-total	**31.29**	**11.13**	**0.97**	**53.78**	**12.13**	**8.51**	**38.98**	**11.38**	**5.83**	**49.32**	**8.36**	**0.07**
Oxygenated monoterpene derivatives												
6-methyl-2-(oxiran-2- yl)hept-5-en-2-ol (1080)	-	-	-	-	0.17	-	-	-	-	-	-	-
Linalool (1107)	0.28	0.19	-	0.90	0.24	0.07	0.54	0.33	0.62	1.54	0.42	-
1-Terpinen-4-ol (1196)	-	-	-	0.82	-	0.07	-	-	-	-	-	-
Sub-total	**0.28**	**0.19**	**0.00**	**1.71**	**0.41**	**0.13**	**0.54**	**0.33**	**0.62**	**1.54**	**0.42**	**0.00**
Sesquiterpene hydrocarbons												
δ-Eiemene (1345)	2.88	1.88	0.38	5.23	1.62	0.86	8.95	13.26	7.09	4.66	3.44	3.00
α-Cubebene (1357)	-	0.27	-	0.23	1.44	0.92	0.29	1.69	0.50	0.57	0.21	0.20
Ylangene (1383)	-	-	-	0.54	0.54	0.52	-	0.14	-	-	-	-
Copaene (1390)	0.29	0.75	0.13	3.53	7.39	5.27	6.89	8.90	3.03	5.40	-	0.22
β-Elemene (1401)	1.44	2.09	1.87	1.07	2.42	2.07	2.50	2.93	2.38	2.17	1.89	3.04
(+)-α-Longipinene (1421)	-	1.11	-	-	0.67	0.44	-	0.40	-	-	1.12	0.43
Cedr-8-ene (1423)	-	0.40	-	-	0.86	-	-	1.19	-	-	0.52	-
α-Bergamotene (1423)	0.31	0.89	0.25	0.25	0.65	-	0.49	0.15	-	0.55	1.42	-
β-Caryophyllene (1437)	18.90	16.87	38.33	15.29	15.64	36.86	25.87	21.73	33.20	21.82	11.78	16.82
γ-Elemene (1440)	1.77	3.17	1.71	0.29	1.52	-	-	0.53	-	0.28	3.72	4.73
Germacrene D (1446)	2.77	0.13	0.32	4.33	0.19	16.55	0.77	0.29	0.22	0.61	-	2.89
β-Cubebene (1447)	-	-	-	-	0.59	-	-	-	-	-	0.08	0.16
(Z)-β-Farnesene (1455)	0.76	0.73	1.16	0.28	0.74	0.33	-	1.13	-	-	1.03	0.65
α-Caryophyllene (1473)	2.45	2.13	4.43	1.90	1.93	4.21	3.39	3.74	6.21	2.97	2.23	2.32
Isoledene (1479)	-	1.14	-	-	17.08	0.21	-	2.42	1.90	-	2.13	0.15
β-Muurolene (1490)	-	1.29	1.05	-	1.60	1.36	-	0.57	0.37	-	1.30	1.28
Cycloisosativene (1494)	-	0.10	0.36	-	1.42	1.39	-	0.20	3.82	-	0.22	0.89
α-Zingiberene (1505)	0.49	0.50	0.52	0.23	0.75	-	-	0.06	-	-	1.35	0.87
β-Selinene (1509)	-	-	-	-	-	-	-	-	6.04	-	-	-
α-Guaiene (1511)	-	8.54	-	-	1.11	-	2.27	6.12	-	-	7.03	0.34
Elixene (1513)	0.36	14.70	-	-	-	-	-	0.28	0.37	3.12	0.61	-
α-Amorphene (1513)	-	-	-	-	1.80	0.54	-	-	-	-	-	0.55
β-bisabolene (1516)	6.22	-	6.95	3.20	7.66		-	-	-	-	5.57	

α-Selinene (1517)	-	6.04	-	-	-	-	2.36	6.08	3.76	-	-	-
δ-Cadinene (1530)	0.58	2.82	2.27	2.36	6.20	4.52	3.82	4.88	2.52	2.96	2.15	2.62
α-Farnesene (1534)	0.92	-	-	0.38	-	-	-	-	-	-	-	-
α-Patchoulene(1534)	-	-	0.84	-	1.19	-	-	-	-	-	0.28	1.56
cis-α-Bisabolene (1546)	0.74	-	-	-	-	-	0.21	-	-	-	-	-
α-Gurjunene (1546)	-	0.68	1.04	-	0.51	-	-	3.39	0.81	-	2.39	1.30
α-Muurolene (1551)	-	-	-	-	0.41	0.32	0.52	-	0.11	-	-	-
1,1,3a-trimethyl-7- methylene decahydro-1H-cyclopropa[a]- naphthalene (1554)	-	-	7.83	-	-	-	-	-	0.20	-	-	3.64
Valencene (1555)	-	4.96	-	-	-	-	-	-	-	-	4.85	-
Eudesma-3,7(11)- diene (1559)	-	3.10	4.95	-	0.23	-	-	0.07	-	-	3.03	-
Germacrene B (1579)	10.68	-	7.61	0.29	-	1.21	-	0.04	-	-	17.11	25.59
β-Cadinene (1658)	-	-	-	-	0.29	0.20	0.34	1.00	0.92	-	0.33	-
γ-Gurjunene (1679)	-	0.36	-	-	0.39	0.21	-	0.53	0.72	-	0.26	0.28
Other Sesquiterpene hydrocarbons	0.75	0.19		0.28	0.83	0.87		1.10	4.62		0.37	0.41
Sub-total	**52.29**	**74.84**	**82.12**	**39.68**	**77.86**	**79.42**	**59.10**	**82.84**	**78.81**	**45.12**	**76.40**	**73.91**
Oxygenated sesquiterpenes derivatives												
*Iso*furanogermacren e (1510)	5.28	1.23	5.75	-	-	-	-	-	-	2.46	0.68	-
α-*trans*-Bergamotol (1519)	0.63	-	-	-	-	-	-	-	-	-	0.56	0.58
Elemol (1562)	0.40		-	-	0.28	-	-	-	-	-	-	-
trans-Nerolidol (1565)	3.49	4.69	7.59	-	0.21	-	-	-	-	0.63	4.13	-
Germacrene D-4-ol (1599)	-	0.36	-	-	0.22	-	-	0.24	-	-	0.27	0.10
Caryophyllene oxide (1606)	2.49	1.49	3.06	-	0.33	1.17	0.24	0.30	2.58	0.38	1.20	1.53
Globulol (1609)	-	0.33	-	-	-	-	-	0.42	1.06	-	0.24	-
T-Cadinol (1639)	-	-	-	-	-	-	-	-	-	-	0.61	-
Eudesm-7(11)-en-4- ol (1644)	-	0.59	-	-	0.43	-	-	1.79	-	-	0.16	-
T-Muurolol (1660)	-	-	-	-	0.85	0.49	-	0.15	-	-	-	-
α-Cadinol (1673)	-	-	-	0.39	0.47	0.71	-	0.11	0.93	-	-	0.21
Eudesm-4(14)-en-11-ol (1676)	-	0.87	-	-	-	-	-	-	-	-	1.14	-
Viridiflorol (1691)	-	-	0.36	-	-	-	-	-	1.17	-	-	-
6-Isopropenyl-4,8a- dimethyl-1,2,3,5,6,7,8,8a-octahydro-2- naphthalenol(1690)	-	-	-	0.64	0.68	0.62	-	0.59	1.98	-	0.43	-
Spathulenol (1705)	1.35	0.06	-	3.80	5.49	2.43	1.14	1.15	-	0.53	1.74	0.50
Alloaromadendrene oxide(1788)	-	2.34	-	-	-	-	-	-	1.08	-	2.15	1.18
Other oxygenated sesquiterpenes derivatives	-	0.49	-	-	0.57	-	-	0.23	-	-	0.74	-
Sub-total	**13.64**	**12.47**	**16.74**	**4.83**	**9.54**	**5.42**	**1.38**	**4.98**	**8.80**	**4.01**	**14.05**	**4.10**
Miscellaneous compounds												
trans-3,6-diethyl-3,6-dimethyl- tricyclo[3.1.0.0(2,4)] hexane (1534)	-	-	-	-	-	0.39	-	-	-	-	-	-
6,7-dimethyl-1,2,3,5,8,8a- hexahydronaphthalene (1604)	-	0.15	-	-	0.07	-	-	-	-	-	0.41	-
Andrographolide (1612)	0.44	-	-	-	-	-	-	-	-	-	0.36	-
Sub-total	**0.44**	**0.15**	**-**	**-**	**0.07**	**0.39**	**0.00**	**0.00**	**0.00**	**0.00**	**0.77**	**0.00**

Key: “-“ indicates not detected.

Note: KI*: Kovat’s indices on BPX-5 column compared with Liu *et al*. (2011). Unidentified components which are less than 0.5% are not reported. KF: Kuching variety (fruit); KFS: Kuching variety (fruit stalk); KSB variety (stem bark); EF: Semongok Emas variety (fruit); EFS: Semongok Emas variety (fruit stalk); ESB: Semongok Emas variety (stem bark); PF: Semongok Perak variety (fruit); PFS: Semongok Perak variety (fruit sta Semongok Perak variety (stem bark); WF: Semongok Wan variety (fruit); WFS: Semongok Wan variety (fruit stalk); WSB: Semongok Wan variety (stem

CONCLUSIONS

The oil constituents of four *P. nigrum* varieties showed the predominance of sesquiterpenes, except for Semongok Emas and Semongok Wan fruit oils. Hydrocarbon sesquiterpenes were identified as the major components in all oil samples studied. The chemometric analysis based on the GC-MS data of oil illustrated that the oil compositions among the fruit, fruit stalk and stem bark in four different *P. nigrum* varieties are quite similar. A considerable level of similarity between the varieties was also distinguished. Further study on other plant parts of various *P. nigrum* varieties may provide valuable information for current chemotaxonomic investigation based on essential oil composition.

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