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Essential Oils of Three Conifer Species from FRIM's Main Campus

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Abstract

A study was performed to investigate the volatile constituents of three conifer species (*Podocarpus neriifolius*, *Nageia motleyi* and *Dacrydium elatum*) which were planted in the main campus of Forest Research Institute Malaysia (FRIM). The fresh leaves were extracted by *Clavenger* hydrodistillation apparatus and the essential oils were analysed by GC and GC/MS analysis. Analyses revealed that the *P. neriifolius* and *N. motleyi* oils were rich with sesquiterpenes and have monoterpenes in very trace amount. The major sesquiterpenes of *P. neriifolius* oil were phyllocladene (26.37%), kaurene (13.75%), bicyclogermacrene (12.14%) and followed by sandaracopimara-8(14), 15-diene (7.89%) and β-caryophyllene (6.25%). Meanwhile, the major components of *N. motleyi* oil were bicyclogermacrene (45.85%), viridiflorene (12.36%), globulol (9.48%) and germacrene D (6.15%). *D. elatum* oil has mono- and sesquiterpenes as its main components in which β-caryophyllene (29.03%), α-pinene (28.41%), phyllocladene (22.89%), α-humulene (4.27%) and pimaradiene (2.61%) were detected in this oil. The chemical compounds identified are responsible for the aroma of these selected conifer species and showed the potential of conifer plants as a source of aroma chemicals.

Keywords: Podocarpaceae, conifer, essential oil, GC and GC/MS analysis

INTRODUCTION

Forest Research Institute Malaysia (FRIM) has established several arboretums for the conservation and research purposes. One of the arboretum namely Coniferatum, established since 1949 was mainly planted conifer species from various resources. Some of the species planted were from the gymnospermous family, Podocarpaceae. This family is made up of 180 species from 18 genera [1].

Podocarpus is the largest of genera containing about 100 species that are mainly distributed in the tropics and subtropics of the southern hemisphere [1, 2]. *Podocarpus* species were used in traditional medicine for human and animal healthcare [3]. Taxol, a significant anticancer agent was isolated from *P. gracilior*. Other uses of *Podocarpus* species are timber, food, wax, tannin and as ornamental trees [4]. *P. neriifolius* or locally known as Podo bukit are used as ornamental trees, mostly for landscaping. Nagilactone C [5], amentoflavone, podocarpusflavone A, podocarpusflavone B and isoginkgetin [6] 7,4'-dimethylaromadendrin and 7,4'-dimethylaromadendrin 5-glucoside [7] were isolated from *P. neriifolius*.

Nageia motleyi or podo kebal musang is a big tree up to 50m tall. This species can be found in Thailand, Sumatra, Peninsular Malaysia and Borneo. It is important timber trees as valuable construction wood [2].

Dacrydium elatum is an evergreen coniferous tree. The distribution is in China, Thailand, Indo-China, Malaysia and Philippines. In Malaysia, the vernacular name for this species is ekor kuda. It can be found in Kedah, Penang, Perak, Selangor, Terengganu, Pahang and Johor. It is an ornamental plant and recorded as the main source of sempilor wood in Southeast Asia [2].

To the best of our knowledge, there is no previous report on chemical constituents of the oils from these selected three species.

EXPERIMENTAL

Plant materials and essential oil preparation

The fresh leaf samples of *Podocarpus neriifolius*, *Nageia motleyi* and *Dacrydium elatum* were collected at Field 13 (Coniferatum) in FRIM's main campus. All the samples were processed immediately the moment it arrives at the lab. They were cut into small pieces before weighed and subjected to hydrodistillation technique for six hours using *Clavenger*-type apparatus. The oils were collected and isolated from their hydrosol using anhydrous sodium sulphate. The pure oils were kept in fridge prior further analysis.

GC analysis

Analyses of the oils were conducted by Gas Chromatography (GC) using model Shimadzu GC-2010 Plus capillary chromatograph which was equipped with a flame ionization detector (FID) and using split/splitless mode injection technique, under the following conditions: carrier gas helium; similar temperature for injector and detector at 250^{o}C. This chromatograph is using a non-polar capillary column BP-5 (30m by 0.25mm, film thickness 0.25μm). Operating conditions are as follows; initial oven temperature, 60^{o}C for 10min, up to 230^{o}C at 3^{o}C/min and then 230^{o}C for 10min.

GC/MS analysis

Gas Chromatography/Mass Spectrometry (GC/MS) analyses were conducted on Agilent Technologies GCMS 7890A/5975C Series MSD with similar condition as described in GC programs using HP-5MS column (30m by 0.25mm, film thickness 0.25μm). The chemical constituents were identified by comparison of retention times and calculated kovat's indices with reference [8] and literature values and matching their mass spectra with database library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L).

RESULTS AND DISCUSSION

The leaf essential oils of *Podocarpus neriifolius*, *Nageia motleyi* and *Dacrydium elatum* were extracted separately by hydrodistillation *Clavenger*-apparatus for 6 hours. The oil yield of *D. elatum* gave higher percentage at 0.39% v/w and followed by *P. neriifolius* (0.02% v/w) and *N. motleyi* (0.01% v/w). All three samples were subjected to GC and GC/MS analysis, which allowed of 20 (88.58%), 15 (84.95%) and 29 (98.71%) compounds identified in *P. neriifolius, N. motleyi* and *D. elatum*, respectively (Table 1).

Table 1 The chemical constituents of *Podocarpus neriifolius, Nageia motleyi* and *Dacrydium elatum* leaf oils

No	Chemical name	Retention index	% Area		
			PN	NM	DE
1	α-Pinene	934	t	t	28.41
2	Sabinene	970	-	-	1.00
3	β-Pinene	976	-	-	1.77
4	Myrcene	989	-	-	1.37
5	γ-Terpinene	1063	-	-	t
6	Terpinolene	1087	-	-	0.08
7	Terpinen-4-ol	1172	-	-	0.05

continue…

No	Chemical name	Retention index	PN	NM	DE
8	α-Terpineol	1187	-	-	0.23
9	α-Cubebene	1351	1.80	-	0.06
10	α-Copaene	1377	1.40	-	0.08

11	β-Cubebene	1390	0.97	-	-
12	β-Elemene	1393	-	1.46	1.01
13	Siberene	1400	t	-	-
14	(*Z*)-Caryophyllene	1405	-	-	0.38
15	α-Gurjunene	1410	-	t	-
16	β-Caryophyllene	1419	6.25	0.98	29.03
17	Aromadendrene	1441	0.76	1.06	t
18	α-Humulene	1455	1.02	-	4.27
19	*trans*-Cadina-1(6),4-diene	1476	-	1.89	-
20	Germacrene D	1480	4.54	6.15	0.34
21	β-Selinene	1483	-	-	0.25
22	Viridiflorene	1495	1.60	12.36	-
23	Bicyclogermacrene	1500	12.14	45.85	1.56
24	Premnaspirodiene	1506	-	-	0.12
25	δ-Amorphene	1511	2.24	-	0.10
26	γ-Cadinene	1513	-	0.65	-
27	Spathulenol	1577	2.10	-	-
28	Caryophyllene oxide	1582	-	-	0.54
29	Globulol	1583	-	9.48	0.05
30	Pogostol	1651	-	2.07	-
31	Selin-11-en-4-alpha-ol	1652	-	-	0.10
32	α-Cadinol	1653	t	1.91	0.14
33	Rimuene	1896	-	-	0.46
34	Beyerene	1925	2.79	-	-
35	Isohibaene	1928	-	-	0.46
36	Pimaradiene	1943	1.80	1.09	2.61
37	Sandaracopimara-8(14),15-diene	1962	7.89	-	1.17
38	13-*epi*-Manool oxide	2010	1.16	-	-
39	Phyllocladene	2013	26.37	-	22.89
40	Kaurene	2035	13.75	-	-
41	Abietatriene	2055	-	t	0.01
42	Abietadiene	2083	-	-	T
43	Sandaracopimarinal	2186	-	-	
			88.58	84.95	98.71

Note: t=trace; PN=*Podocarpus neriifolius*; NM=*Nageia motleyi*; *Dacrydium elatum*

P. neriifolius and *N. motleyi* gave leaf oils in which the majority of the oils were accounted by sesquiterpene and have less amount of monoterpene. Meanwhile, the *D. elatum* produced a leaf oil that contained mixtures of monoterpene and sesquiterpene as the main components (Figure 1). There are seven compounds detected in all of the three oils which were α-pinene, β-caryophyllene, aromadendrene, germacrene D, bicyclogermacrene, α-cadinol and pimaradiene.

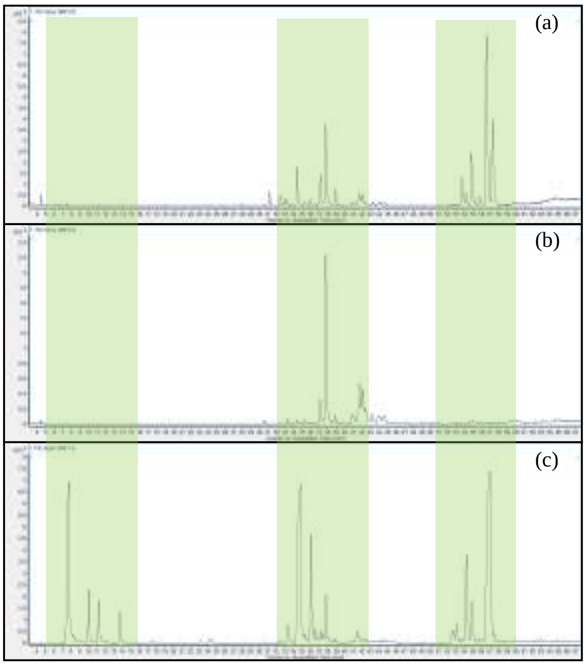

Figure 1 The total ion chromatogram for *P. neriifolius* (a), *N. motleyi* (b) and *D. elatum* (c) leaf oil

The major components of *P. neriifolius* were phyllocladene (26.37%), kaurene (13.75%) and bicyclogermacrene (12.14%). The other appreciable components were sandaracopimara-8(14), 15-diene (7.89%) and β-caryophyllene (6.25%). The remaining components detected were present in lower percentage (below than 5%). Previously, no report on the present of phyllocladene in other *Podocarpus* oils [1]. α-Pinene (9.3%) was detected as the major components of *P. totara* [9]. Bicyclogermacrene (45.85%), viridiflorene (12.36%), globulol (9.48%), germacrene D (6.15%) were identified as the major components in *N. motleyi* oil. Only one monoterpene compound detected which was α-pinene and it was presented in trace amount. The *D. elatum* leaf oil contained approximately 30% monoterpenes in which α-pinene (28.41%) was identified as one of the major components among them. The other monoterpenes were β-pinene, myrcene, sabinene, terpinolene, terpinen-4-ol and α-terpineol in appreciable percentage. The principal sesquiterpenes were β-caryophyllene (29.03%) and phyllocladene (22.89%).

CONCLUSION

The chemical constituents from these coniferous plants represent a valuable chemotaxonomic tool for the identification of species. The oils as well as extracts could further be studied for investigation of their biological potential.

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REFERENCES

[1] Farjon, A. (2010). Podocarpaceae. Flora of Peninsular Malaysia, Malayan Forest Records 49, Series II: Seed Plant 1: 171-195.

[2] Joseph, J.B. and Robert, J.G. (2004). Chemistry of the Australian Gymnosperms. Part 6. Leaf Oils of the Australian Species of Genus *Podocarpus. Journal of Essential Oil Research* 16: 342-346.

[3] Halima S.A., Luc V., Jeffrey F.F & Johannes V.S. (2012). Mutagenicity, antimutagenicity and cytotoxicity evaluation of South African *Podocarpus* species. *Journal of Ethnopharmacology* 139: 728-738.

[4] Abdillahi H.S. Stafford G.I., Finnie J.F. & Van Staden J. (2010). Ethnobotany, phytochemistry and pharmacology of *Podocarpus sensu latissimo* (s.l.). *South African Journal of Botany* 76:1-24.

[5] Shrestha K., Banskota A.H., Kodata S., Shrivastava S.P., Strobel G. & Gewali M.B. (2001). An antiproliferative norditerpene dilactone, Nagilactone C, from *Podocarpus neriifolius*. *Phytomedicine* 8(6): 489-491.

[6] Syed Haider M.R., Wasiur R., Masayoshi O. & Nobusuke K. (1974). Biflavones from *Podocarpus neriifolius. Phytochemistry* 13: 1990.

[7] Syed Haider M.R. & Wasiur R. (1974). 7, 4’-Dimethylaromadendrin and Its 5-Glucoside from *Podocarpus neriifolius*. *Phytochemistry* 13: 2879.

[8] Adam, R.P. (2012). Identification of Essential oil Components by Gas Chromatography/Mass spectrometry, 4th Edition. Allured Business Media: Carol Stream, Illinois.

[9] Franich, R.A. and Cambie R.C. (1999). Essential Oil Constituents of *Podocarpus totara* Leaves. *Journal Of Essential Oil Research* 11: 507-510.