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Chemical Composition of Leaf and Seed Oils of *Dryobalanops aromatica* Gaertn. (Dipterocarpaceae)

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The essential oils of the leaves and seed of *Dryobalanops aromatica* Gaertn. obtained by hydro-distillation resulted in 0.07% and 1.89% yield, respectively. These oils were then examined by GC-MS. Eighty-three components (plus an unknown) were identified from the leaf oil, representing 92% of the oil. Oxygenated monocyclic monoterpenes (terpinen-4-ol 15%, α-terpineol 16%), bicyclic monoterpene (α-pinene 7%) and oxygenated bicyclic sesquiterpene (globulol 8%) were the major constituents. In the case of the seed oil, 31 components were identified, representing 100% of the oil, while acyclic monoterpene (myrcene 5%), monocyclic monoterpene (limonene 6%), bicyclic monoterpenes (α-pinene 41%, α-thujene and β-pinene 13% each, sabinene 6%), and bicyclic sesquiterpene (bicyclogermacrene 6%) made up the major components. The remaining constituents of each oil (54% and 10%, respectively) were found to be minor (≤4% each). The chemical compositions of both oils differed quantitatively but showed important qualitative similarities and differences. The results of this study serve as the first report of complete chemical profiles of both oils.

Key words: *Dryobalanops aromatica*; essential oils; leaf; seed; hydro-distillation; GC-MS; oxygenated monocyclic monoterpenes; α-terpineol; bicyclic monoterpene; oxygenated bicyclic sesquiterpene; acyclic monoterpene

Dryobalanops, locally known as *kapur*, is a genus of large and tall trees from the Family Dipterocarpaceae. The genus consists of seven species which are widely distributed in Sumatra, Peninsular Malaysia and Borneo (Corner 1981; Ashton 2004). The four species found in Brunei Darussalam are *Dryobalanops aromatica* Gaertn, *D. beccari* Dyeri, *D. lanceolata* Burck and *D. rappa* Becc.

D. aromatica (*Figure 1*), commonly known as the Bornean Camphor-Tree, and locally known as *kapur peringgi*, is a large and lofty tree, reaching up to 65 m in height and 7 m in girth. The trunk is usually a straight, cylindrical and clear bole of 30 m–40 m. This species is a well-known and valuable timber tree. The timber has been described as being moderately hard, heavy and durable (Ashton 1964). It is used as an internal wood and resembles mahogany when given a good polish. It has a camphor odour, and the camphor in the wood was sought after and sold as medicine in the past (Burkill 1966). The camphor produced by the tree is less important today than its timber, but in the earlier days the reverse was the case. The species also produces camphoraceous oleo-resin.

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The uses of the wood and camphor of *D. aromatica* in both eastern and European medicines have been well documented (Burkill 1966; Perry 1980; Siang 1983; Duke & Ayensu 1985). The camphor has also been used by the Malays and the Sumatran people in the ceremonial purification of dead bodies and their preservation until burial (Burkill 1966). A mixture of the volatile oils of *D. aromatica*, *Piper longum*, *Santalum album*, *Asarum sieboldi* and *Alpinia officinarum* is said to be effective in the treatment of acute anginal attack (Guo *et al.* 1983). The methanol extract of the wood is also shown to have antifungal properties (Hong & Abdul Razak 1983; Kim *et al.* 2005).

Chemical examination on the oleo-resin of *D. aromatica* shows that it consists of 35% terpenes (including pinene), 10% alcohols (including borneol), 20% sesquiterpenes and 35% resin (Burkill 1966, p. 881). The resin consists mainly of triterpenes (Cheung & Wong 1972) and the oxygenated derivatives of asiatic acid as minor constituents (Cheung & Tokes 1968). The camphor consists of borneol, camphor, camphene, sesquiterpenes and terpineol (Perry 1980, Duke & Ayensu 1985), while the wood extracts contain largely terpenes and fatty acids (Ali & Koh 1991). However, an earlier distillation attempt on the leaves in 1910 yielded little oil and no details were provided (Burkill 1966, p. 881). A later attempt at distilling the leaves and twigs showed that no oil or borneol was obtained (Eaton 1925). Since then, there has not been any study on the essential oils in the leaves of this plant. In addition, no published work can be traced with reference to the volatile oil of its seed. The lack of information and details on both the leaf and seed oils of *D. aromatica* prompted us to undertake this investigation.

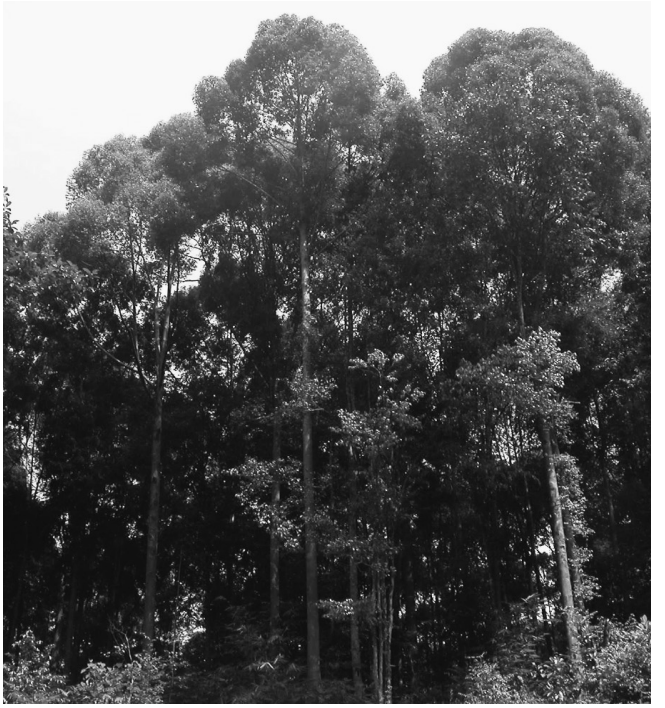

Figure 1. Dryobalanops aromatica *Gaertn. (Bornean Camphor-Tree), a popular timber species today, was once well-known for its camphor.*

Thus, the present study aims to identify and document fully for the first time the chemical constituents present in the essential oils obtained from the fresh leaves and seed of *D. aromatica*. This study may allow us to identify potential uses and better utilization of these plant parts which are usually discarded when the tree is harvested for its timber.

EXPERIMENTAL

Plant Material

Fresh leaves (*Figure 2A*) and seed (*Figure 2B*) of *D. aromatica* were collected from the Bukit Sawat forest in the Belait District of Brunei Darussalam. The species was identified by the author, Dr Kamariah Abu Salim and confirmed by Awang Ariffin Abdullah Kalat of the Brunei National Herbarium (BRUN), Sg. Liang. A voucher specimen bearing reference no. SN-B000340 was deposited at BRUN.

Isolation of Essential Oils

Immediately, after collection, the fresh leaves and seed were subjected to hydro-distillation in a Neo-Clevenger apparatus for 4 h. The oils were collected in dark brown glass vials and stored at 4°C until further analysis. The percentage compositions of the oils were calculated based on the fresh weight of the respective plant parts.

Properties of Essential Oils

Oil density was determined by using a piknometer, refractive index by a Shimadzu Bausch and Lomb Abbe refractometer, and optical rotation by an Onel Pol S-2 polarimeter.

Gas Chromatography-mass Spectrometry (GC-MS analysis)

GC-MS analysis of the oils was carried out on a Hewlett Packard GCD system. Separation was performed in an Innowax fused silica capillary (FSC) column (60 m × 0.25 mm id; 0.25 mm film thickness). Helium at a flow rate of 1 ml min^{-1} was used as the carrier gas. The temperature of the GC oven was initially set at 60°C for 10 min, and then increased at a rate of 4°C min^{-1} to 220°C. It was held isothermally at 220°C for 10 min, programmed to 240°C at a rate of 1°C min^{-1}, and finally maintained at

Figure 2. Dryobalanops aromatica *Gaertn (A) fresh leaves and (B) fresh fruit.*

240°C for 20 min. Split injection was conducted at a flow rate of 50 ml min^{-1} with a split ratio of 50:1. The temperature of the injector was set at 250°C and the ionization energy was 70 eV. The mass range was from 35 to 425 m/z.

Determination of Essential Oil Composition

Chemical identification of the different components of the oils was based on their retention times and comparison of their mass spectra with those of the Wiley GC-MS Library and a home-made library (Baser Library of Essential Oil Constituents). The relative percentage composition of the volatile compounds was calculated from the Total Ion Chromatogramme (TIC), assuming that the relative response factor was equal to 1.

RESULTS AND DISCUSSION

Yields and General Considerations

The yields and physico-chemical properties of the leaf and seed oils of *D. aromatica* are shown in *Table 1*.

Although the density, refractive index and optical rotation of both oils appear to show very little difference, the oil yield of the seed was significantly (twenty-nine times) higher than that of the leaves. Thus, the seeds are a better source of oil than the leaves. However, the higher oil yield from the seed may not necessarily prove to be of significant importance for commercial exploitation because the trees only flower and fruit once in every 3–4 years. In addition, the conservation status of the species must be considered and given a priority in any attempt to exploit the seed for commercialization of the oil. It may be better for the discarded seed (when present) during timber extraction to be collected for propagation and re-planting purposes.

The low percentage of oil yield from the leaves seems to be in agreement with earlier work carried out when negligible (Burkill 1966, p. 881) or no yield at all was obtained (Eaton 1925). Thus, any commercial exploitation of discarded leaves for the oil during timber harvest needs to take into account this low yield, available resources and the cost of production.

Chemical Composition

The identified compounds in the oils of *D. aromatica* leaves and seed, their relative amounts and their retention indices (Kovat's Indices for Polar Column) are shown in *Table 2*.

The leaf oil contains 84 compounds representing 92 % of the total oil. α-terpineol (16%), terpinen-4-ol (15%), globulol (8%) and α-pinene (7%) were the major constituents. The seed oil contains 31 compounds representing 100% of the total oil, with the major constituents being α-pinene (41%), α-thujene and β-pinene (13% each), sabinene, limonene and bicyclogermacrene (6% each), and myrcene (5%). These compounds are probably the most significant volatile compounds that characterize the overall scent of the two oils. They are also common ingredients in perfumes, food products, cosmetics and medicines.

Table 1. Physico-chemical properties of leaf and seed oils from *Dryobalanops aromatica* Gaertn.

Properties	Leaf oil	Seed oil
Yield [% (w/w) by fresh weight]	0.065	1.893
Colour	Clear, golden yellow	Clear, colourless
Density (d_{20})	0.893	0.853
Refractive index (n_D^{20})	1.476	1.467
Optical rotation (α_D^{20})	–10.103	–11.056

Table 2. Percentage composition and retention index (RI) of the compounds identified in the essential oils of fresh leaves and seed *of Dryobalanops aromatica* Gaertn., in order of their elution in a FSC column; tr = trace (< 0.1 %).

Peak no.	Compound	Peak area (%)		RI
		Leaves	Seed	
1	α-Pinene	6.9	40.8	1032
2	α-Thujene	2.0	12.9	1035
3	Camphene	0.1	0.3	1076
4	β-Pinene	2.3	12.7	1118
5	Sabinene	0.2	6.4	1132
6	Myrcene	1.1	4.9	1174
7	α-Terpinene	0.3	0.3	1188
8	Limonene	3.0	6.0	1203
9	1, 8-Cineole	–	0.2	1213
10	β-Phellandrene	0.3	0.7	1218
11	γ-Terpinene	1.1	0.9	1255
12	(*E*)-β-Ocimene	0.1	0.2	1266
13	*p*-Cymene	0.8	0.2	1280
14	Terpinolene	0.8	0.8	1290
15	6-Methyl-5-hepten-2-one	tr	–	1348
16	Hexanol	0.4	–	1360
17	(*Z*)-3-Hexenol	0.3	-	1391
18	α-Fenchone	0.1	–	1406
19	(*E*)-2-Hexenol	tr	–	1412
20	α-*p*-dimethylstyrene	0.2	–	1452
21	Fenchyl acetate	tr	–	1482
22	Bicycloelemene	–	0.1	1495
23	α-Copaene	0.3	0.1	1497
24	Camphor	0.6	–	1532
25	Isopinocamphone	0.1	–	1562
26	(*E*)-*p*-menth-2-en-1-ol	0.1	tr	1571
27	Fenchyl alcohol	0.2	–	1591
28	(*E*)-β-Bergamotene	0.1	–	1594
29	α-Guaiene	0.2	tr	1597
30	Terpinen-4-ol	15.0	–	1611
31	β-Caryophyllene	3.6	4.3	1612
32	Aromadendrene	0.2	0.1	1628
33	(*Z*)-*p*-menth-2-en-1-ol	0.1	–	1638
34	Alloaromadendrene	0.2	tr	1661
35	*p*-Mentha -1, 5-dien-8-ol	0.1	–	1678
36	α- Humulene	3.1	0.7	1687
37	*p*-Mentha-1, 8-dien-4-ol	tr	–	1700
38	γ-Muurolene	0.2	–	1704
39	α-Terpineol	15.8	0.2	1707
40	Ledene	1.0	0.1	0.1
41	Borneol	0.6	-	1719
42	Verbenone	0.3	–	1725

Table 2 (Cont.). Percentage composition and retention index (RI) of the compounds identified in the essential oils of fresh leaves and seed *of Dryobalanops aromatica* Gaertn., in order of their elution in a FSC column; tr = trace (< 0.1 %).

43	Germacrene D	0.2	0.3	1726
44	Carvenone	0.1	–	1737
45	(*E*)-*p*-menth-2-en-1, 8-diol	0.2	–	1740
46	α-Muurolene	0.3	–	1740
47	α-Selinene	0.1	–	1740
48	Piperitone	0.1	–	1748
49	Bicyclogermacrene	1.7	5.9	1755
50	(*E,E*)-α-Farnesene	0.1	–	1758
51	δ-Cadinene	0.2	–	1776
52	γ-Cadinene	0.1	–	1776
53	(*Z*)-*p*-menth-2-en-1, 8-diol	0.1	–	1797
54	Myrtenol	0.1	–	1804
55	p-Mentha-1, 3-dien-7-al	0.1	–	1811
56	(*E*)-Carveol	0.1	–	1845
57	(*Z*)-Calamenene	0.1	–	1853
58	*p*-Cymen-8-ol	0.6	–	1864
59	Hexanoic acid	0.1	–	1871
60	α-Calacorene-1	0.2	–	1941
61	Palustrol	0.7	–	1953
62	(*E*)-12-Norcaryophyll-5-en-2-one	0.1	–	1984
63	Caryophylleene oxide	0.6	–	2008
64	Epiglobulol	0.5	–	2033
65	Humulen epoxide - I	0.1	–	2045
66	(*E*)-Nerolidol	0.1	–	2050
67	Ledol	0.6	–	2057
68	Humulen epoxide-II	0.4	–	2071
69	Unknown-I	4.0	–	2077
70	Cubenol	0.1	–	2080
71	1-Epi-cubenol	0.2	–	2088
72	Globulol	8.2	0.2	2098
73	Viridiflorol	3.8	0.1	2104
74	Spathulenol	1.0	0.1	2144
75	Neointermedeol	0.3	–	2153
76	β-Bisabolol	0.9	–	2170
77	T-Cadinol	0.4	–	2187
78	T-Muurolol	0.5	–	2209
79	δ-Cadinol	0.3	–	2219
80	Isospathulenol	0.1	–	2228
81	(*E*)-α-bergamotol	0.9	tr	2247
82	α-Cadinol	0.8	–	2255
83	Selin-11-en-4-α-ol	0.2	tr	2273
84	Caryophylla-2(12), 6(13)-dien-5-α-ol	0.2	–	2324
85	Caryophylla-2(12), 6-dien-5-β-ol	0.1	–	2392
86	Hexadecanoic acid	0.6	–	2931

α-thujene, β-pinene, limonene, γ-terpinene, β-caryophyllene, α-humulene, ledene, bicyclogermacrene, viridiflorol, spathulenol and an unknown compound were also present but in quantities ≤ 4% each in the leaves, whilst only β-caryophyllene was present in similar quantity in the seed of the plants. The earlier attempt (Eaton 1925) at distillation of the leaves of this species reported that no borneol was obtained, but this study showed its presence in the leaf oil although in a low quantity (0.6%). Compounds such as camphene, terpineol and pinene which have been reported to be present in the camphor and oleo-resin of the plant (Perry 1980; Duke & Ayensu 1985) were also found in this study.

The essential oils from the seed and leaves of *D. aromatica* showed important similarities because out of the 85 identified compounds, 29 (1–8, 10–14, 23, 26, 29, 31, 32, 34, 36, 39, 40, 43, 49, 72–74, 81 and 83, see *Table 2*) were common in both leaves and seed although in different quantities. However, some specific compounds allowed for the differentiation of the two essential oils. Indeed, 55 compounds (15–21, 24, 25, 27, 28, 30, 33, 35, 37, 38, 41, 42, 44–48, 50–71, 75–80, 82, and 84–86) including an unknown were found only in the leaves and not in the seed, while 2 compounds (9 and 22) were found only in the seed and not in the leaves. This pattern of findings has been similarly obtained in many studies of plant species involving different organs (Rehder *et al.* 2006; Ghasempour *et al.* 2007; Bhuiyan *et al*. 2009; Chowdhury *et al*. 2009). Thus, common volatile compounds were found to be non-uniformly distributed in different organs of *D. aromatic*, whilst the different volatile compounds accumulated could be the result of various metabolic processes in the specific cells or vessels of these organs.

Chemical Classification

Table 3 shows the identified volatile compounds listed by chemical class, which to some degree reflects their biosynthetic origin. Out of the 85 identified compounds, 5 were fatty acids and their derivatives, 79 isoprenoids and 1 benzenoid. The presence of fatty acids and isoprenoids, in particular terpenes and sesquiterpenes, had been recorded in the oleo-resin, camphor, resin and wood extract of this species (Burkill 1966; Cheung & Wong 1972; Perry 1980; Duke & Ayensu 1985; Ali & Koh 1991). In this study, the fatty acids and their derivatives were found in the leaf oil only, and were represented by C6 compounds, mainly acids and alcohols, which made up 1.4% of the oil. In plants, fatty acids are synthesized in chloroplasts from acetyl-CoA and malonyl-CoA in repetitive reactions that result in longer molecules (Cseke *et al*. 2006). The alcohols which give the characteristic `green' note or odour of the leaves are biosynthesised from α-linolenic and linoleic acids *via* their respective hydroperoxides (Stone *et al*. 1975; Hatanaka *et al*. 1987; Hatanaka 1993).

The isoprenoids in both leaf and seed oils were mainly monoterpenes and sesquiterpenes, and their derivatives. The amount of monoterpenes and their derivatives was higher in the seed oil (87.5%) than in the leaf oil (53.4%). However, the sesquiterpenoid fractions were higher in the leaf oil (33%) than in the seed oil (12%). Previous work on the oleo-resin of this plant recorded the presence of 20% sesquiterpenes, an amount which lies in between the contents of leaf and seed oils in this study. In the leaf oil, oxygenated monocyclic monoterpenes (32%) and oxygenated bicyclic sesquiterpenes (20%) dominated whilst bicyclic monoterpenes (73%) and bicyclic sesquiterpenes (11%) formed the major isoprenoids in the seed. Most isoprenoids can be traced back to geranyl- or farnesyl- pyrophosphates (Croteau & Karp 1991). The isoprenoids are synthesized in cytosol from acetyl-CoA *via* the mevalonic pathway as well as in plastids from pyruvic acid and glyceraldehydes-3-phosphate *via* 1-deoxy-D-xyluluose-5-phosphate (DOXP) and

2-C-methyl-D-erythritol-4-phosphate (MEP) (Eisenreich *et al*. 1998; Kuzuyama 2002; Dubey *et al*. 2003; Eisenreich *et al*. 2004).

The irregular terpene, 6-methyl-5-heptene-2-one, occurred in a negligible amount. Similarly, the only benzenoid, α-*p*-dimethylstyrene, was also present in a very small quantity.

CONCLUSION

This study provides the complete chemical profiles of the essential oils obtained from the fresh leaves and seed of *D. aromatica*. The essential oils of both leaves and seed of *D. aromatica* were particularly rich in monoterpenes. Sesquiterpenes were present in both oils in lower amounts whilst fatty acids and benzenoid were present in minute

Table 3. Classes of volatile compounds identified from fresh leaves and seed of *D. aromatica* Gaertn.; tr = trace (< 0.1 %)

Class of compounds	Peak area (%)	
	Leaves	Seed
Fatty acids and derivatives		
Acids (59 & 86)	0.7	–
Alcohol (16, 17, 19)	0.7	–
Total %	1.4	–
Isoprenoids		
Irregular terpene (15)	tr	–
Monoterpenes and derivatives		
Acyclic monoterpenes (6, 12)	1.2	5.1
Monocyclic monoterpenes (7, 8, 10, 11, 13, 14)	6.3	8.9
Oxygenated monocyclic monoterpenes (9, 26,30, 33, 35, 37, 39, 44, 45, 48, 53, 55, 56, 58)	32.4	0.4
Bicyclic monoterpenes (1, 2, 3, 4, 5)	11.5	73.1
Oxygenated bicyclic monoterpenes (18, 21, 24, 25, 27, 41, 42, 54)	2.0	–
Total %	53.4	87.5
Sesquiterpenes and derivatives		
Acyclic sesquiterpenes (50)	0.1	–
Oxygenated acyclic sesquiterpenes (66)	0.1	–
Monocyclic sesquiterpenes (36, 43)	3.3	1.0
Oxygenated monocyclic sesquiterpenes (65, 68, 76)	1.4	–
Bicyclic sesquiterpenes (22, 23, 28, 29, 31, 32, 34, 38, 40, 46, 47, 49, 51, 52, 57, 60)	8.5	10.6
Oxygenated bicyclic sesquiterpenes (61, 62, 63, 64, 67, 70, 71, 72, 73, 74, 75, 77, 78, 79, 80,		
81, 82, 83, 84, 85)	19.6	0.4
Total %	33.0	12.0
Benzenoid		
(20)	0.2	–
Unknown		
(69)	4.0	–

concentrations in the leaf oil only. The study concluded that *D. aromatica* was a good source of aromatic oil which contained important chemical components well-known in the flavour, fragrance, food, cosmetics and pharmaceutical industries. However, the commercial extraction of either the essential oil or favoured component(s) of these oils might not be viable due to the low yield of the leaf oil, and the infrequent fruiting and seed production pattern of the species.

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