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Original Research Article

Determination of borneol and other chemical compounds of essential oil of *Dryobalanops aromatica* exudate from Malaysia

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Abstract

Purpose: *To determine borneol and other chemical compounds of essential oil derived from the exudate of Dryobalanops aromatica in Malaysia.*
Methods: *Exudate was collected from D. aromatica and subjected to fractional distillation to obtain essential oil. Gas chromatography-mass spectrometry (GC-MS) was performed to characterize the composition of the isolated essential oil.*
Results: *Essential oil (7.58 %) was obtained with the highest yield (3.24 %) in the first 2 h of fractional distillation. Thirty compounds which accounted for 97.56 % of total essential oil composition were identified by GC-MS, and they include sesquiterpenes (46.87 %), monoterpenes (31.05 %), oxygenated monoterpenes (16.76 %) and oxygenated sesquiterpenes (2.13 %). Borneol (0.74 %) was also detected in the essential oil.*
Conclusion: *Borneol and other terpenoid compounds are present in the essential oil of the exudate of D. aromatica.*

Keywords: *Exudate, Dryobalanops Aromatica, Fractional distillation, Essential oil, Gas chromatography-mass spectrometry, Borneol*

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INTRODUCTION

D. aromatica is a large emergent tropical rainforest species which is recognised for its valuable timber, locally known as 'kapur', Borneo Camphor, Camphor Tree, or Sumatran Camphor. It can grow up to 60 m height with girth of 9 m [1]. It is distributed only in Peninsular Malaysia, Borneo and Sumatra [2]; and has been classified as critically endangered species in 2007. The genus *Dryobalanops* belongs to the Dipterocarpaceae family and is one of the two species that can be found in Peninsular Malaysia besides *D. oblongifolia* [2].

Although many ancient Chinese literatures have reported the premium source of borneol from *D. aromatica*, however, there are no scientific reports on the isolation of borneol from this plant [3,4] particularly from the sources of exudates. Research on *D. aromatica* were mainly focused on the tree bark and there is limited information on the complete chemical profiling of the extracts or essential oil isolated from the exudates of *D. aromatica.*

Borneol is an expensive compound used in Chinese and Western medicine for many years and is an ingredient used in 65 traditional Chinese medicinal formulae [5]. In recent years, natural borneol were isolated from the leaves of *Cinnamomum glanduliferum* [6], *Cinnamomum camphora* [7] and *Cinnamomum burmannii* [8]. Besides natural borneol, borneol can be synthesized from turpentine oil or camphor as *Borneolum syntheticum* in the form of dl-borneol and iso-borneol, where the content of dl-borneol is greater than 55 %. However, synthetic borneol was shown to exert some toxic effects that due to the presence of iso-borneol and camphor [9]. This makes natural borneol especially d-borneol the valuable raw ingredient for medicinal purposes. Nevertheless, due to the high demand and production cost, there is a shortage for the source of natural borneol [10].

In view of the high distribution of *D. aromatica* in Peninsular Malaysia and the potential of *D. aromatica* as an alternative source of natural borneol; this study was conducted to determine the presence of borneol and other chemical composition in the essential oil extracted from the exudates of *D. aromatica*. Further downstream studies are focused on the wide range of potential biological activities of the essential oil and the isolated pure compounds, particularly on anti-cancer properties, namely cytotoxic and anti-tumour promoting activities.

EXPERIMENTAL

Plant material

Exudates of *D. aromatica* were collected from Commonwealth Forest Reserve, Selangor, Malaysia. Plant identification was authenticated by Professor Dr Ong Hean Chooi from University of Malaya, Malaysia. Voucher specimen (UTAR/LTX01/12) was prepared and deposited at Universiti of Tunku Abdul Rahman. Exudates were obtained either by natural secretion from the tree or by mechanical incision.

Fractional distillation of exudates

Exudates were subjected to fractional distillation in the presence of double distilled water for 2 h. The vapour temperature was maintained at 80 – 90 °C. After cooling at room temperature for 2 h, essential oil and distilled water collected at the collection bottle were separated and followed by another cycle of fractional distillation. The process was repeated until the yield of essential oil became minimal. The essential oil was then pooled and stored at 20 °C.

Gas chromatography-mass spectrometry (GC-MS) analysis

The essential oil was analyzed by Agilent 7890A/5975C GC-MS system equipped with HP5ms stationary phase column (30 m x 0.25 mm internal diameter x 0.25 μm film thickness) which composed of 5 % phenyl 95 % dimethylpolysiloxane. Helium was used as carrier gas and programmed at a flow rate of 1.0 ml/min. The sample injection volume was 1 μL with split ratio of 1:10. The initial oven temperature was programmed at 80 °C hold for 5min, then increased to 140 °C at 10 °C/min, hold for 0.5 min and increased to 300 °C at 30 °C/min with final hold for 2 min. Electron ionization system with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 230 and 280 ºC, respectively. The MS system was operated in scan mode with a mass range of 50 – 800 m/z. Identification of compounds were achieved through the determination of retention indices (RI) with reference to a homologous series of n-alkanes (C8 - C20) and by NIST mass spectrum library matching.

RESULTS

Yield of essential oil

Through fractional distillation, different compounds were extracted based on their volatility. Less volatile compounds were separated first followed by more volatile compounds. The average essential oil obtained from the exudates of *D. aromatica* was 7.58 % over 28 h of fractional distillation. The higest yield of essential oil was at the first 2 h of fractional distillation, which was 3.24 %; and the yield was decreased with increased number of cycle of fractional distillation.

Composition of essential oil

This is the first study reported on the chemical composition of essential oil extracted from the exudates of *D. aromatica*. Thirty compounds, which accounted for 97.56 % of essential oil composition from the exudates of *D. aromatica* were identified using GC-MS analysis (Figure 1; Table 1). The essential oil consisted of monoterpenes and sesquiterpenes; where major compounds detected were β-caryophyllene (31.76 %), followed by α-pinene (21.49 %), α-caryophyllene (13.50 %), terpinen-4-ol (8.58 %) and α-terpineol (5.89 %). Besides, 0.74 % of borneol was detected.

DISCUSSION

Borneol is a bicyclic monoterpene that has been used in traditional Chinese medicine for many years as a premium ingredient to treat heart disease, coma and respiratory problems; improve digestive system; relieve pain; and promote relaxation [11]. In this study, borneol was detected in the essential oil of *D. aromatica* at 0.74 %. Besides, other compounds detected were β-caryophyllene (31.76 %), α-caryophyllene (13.50 %), α-pinene (21.49 %), terpinen-4-ol (8.58 %) and α-terpineol (5.89 %) which accounted for 81.22 % of the total essential oil. This study reported for the first time on the presence of α-pinene, terpinen-4-ol and α-terpineol as the major compounds from the exudates of *D. aromatica*. The finding of this study was different from Huang and Lu study, they reviewed that the exudates and essential oil of *D. aromatica* comprised of d-borneol, α-caryophyllene, β-elemene, β-caryophyllene, asiatic acid, dryobalanone, erythrodiol and hydroxy-dammarenone II [3]. The synthesis of phytochemicals of a plant species can be affected qualitatively or quantitatively in response to environmental conditions; geographical variation; genetic factors and evolution; or physiological variations such as type of plant material, pollinator activity cycle, organ development and mechanical or chemical injuries [12,13]. The difference observed in the chemical composition of essential oil reported from the present study and Huang and Lu study might be due to geographical variation of plant species, which lead to difference in environmental and culture conditions.

In summary, the compounds detected in this study such as d-borneol, terpinen-4-ol, α-terpineol, α-pinene and caryophyllene are terpenoid compounds, which is a large class of phytochemical well known for its anti-microbial, antiviral, anti-inflammatory and cytotoxic effects [14-20].Therefore, these terpenes compounds with potential biological activities should be subjected for further investigation.

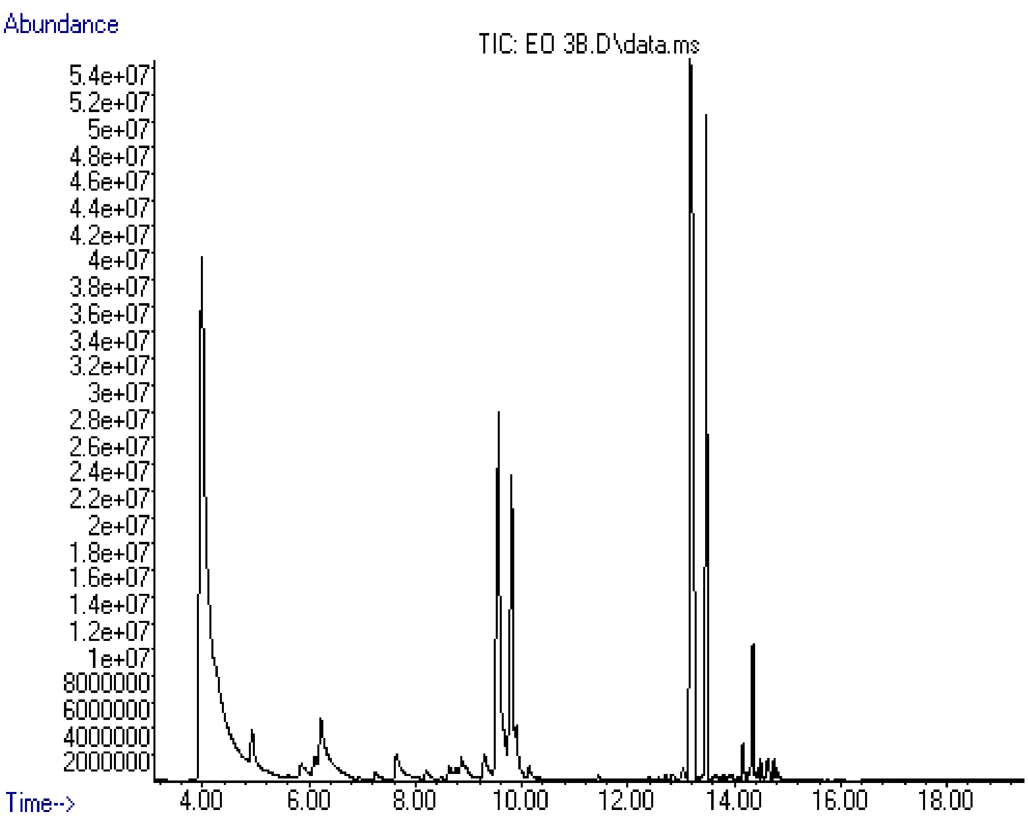

Figure 1: Total ion chromatogram of essential oil extracted from the exudates of *D. aromatica* following GC-MS analysis

Table 1: Chemical composition of essential oil extracted from the exudates of *D. aromatic*

No.	Compound	Molecular formula	RT (min)	RI [a]	Sample (n=3)			Content ±SD (%)
					1	*2*	*3*	
1	*α*-Pinene	$C_{10}H_{16}$	3.983	934	36.73	16.96	10.78	21.49±13.56
2	*β*-Pinene	$C_{10}H_{16}$	4.928	979	1.42	0.84	0.56	0.94±0.44
3	*α*-Phellandrene	$C_{10}H_{16}$	5.597	1009	-	0.72	1.24	0.65±0.62
4	1,4-Cineole	$C_{10}H_{18}O$	5.852	1019	0.64	-	-	0.21±0.37
5	*m*-Cymene	$C_{10}H_{14}$	6.100	1029	0.55	0.27	0.26	0.36±0.16
6	D-Limonene	$C_{10}H_{16}$	6.224	1034	4.15	-	-	1.38±2.40
7	*β*-Myrcene	$C_{10}H_{16}$	6.231	1035	-	5.55	-	1.85±3.20
8	*Y*-Terpinene	$C_{10}H_{16}$	6.934	1063	-	0.79	9.73	3.51±5.40
9	(+)-4-Carene	$C_{10}H_{16}$	7.638	1092	-	0.93	0.41	0.45±0.47
10	(+)-2-Carene	$C_{10}H_{16}$	7.652	1093	1.27	-	-	0.42±0.73
11	(+)-Fenchol	$C_{10}H_{18}O$	8.203	1118	0.28	-	-	0.09±0.16
12	Terpinen-1-ol	$C_{10}H_{18}O$	8.631	1139	0.36	-	-	0.12±0.21
13	(-)-Camphor	$C_{10}H_{16}O$	8.872	1151	1.03	0.99	1.14	1.05±0.08
14	Borneol	$C_{10}H_{18}O$	9.300	1172	0.84	0.79	0.58	0.74±0.14
15	Terpinen-4-ol	$C_{10}H_{18}O$	9.562	1185	10.64	5.81	9.29	8.58±2.49
16	*α*-Terpineol	$C_{10}H_{18}O$	9.899	1202	7.96	5.89	3.81	5.89±2.08
17	Verbenone	$C_{10}H_{14}O$	10.141	1216	0.25	-	-	0.08±0.14
18	Bornyl acetate	$C_{12}H_{20}O_2$	11.444	1290	-	0.76	0.47	0.41±0.38
19	Copaene	$C_{15}H_{24}$	12.713	1388	0.1	0.15	-	0.08±0.08
20	*α*-Farnesene	$C_{15}H_{24}$	12.816	1397	0.07	-	-	0.02±0.04
21	(-)-*β*-Elemene	$C_{15}H_{24}$	12.864	1401	-	0.47	0.37	0.28±0.25
22	Isocaryophillene	$C_{15}H_{24}$	13.044	1422	0.28	-	-	0.09±0.16
23	*β*-Caryophyllene	$C_{15}H_{24}$	13.182	1439	21.23	37.96	36.09	31.76±9.17
24	*α*-caryophyllene	$C_{15}H_{24}$	13.478	1475	8.40	16.31	15.78	13.50±4.42
25	Curcumene	$C_{15}H_{22}$	13.616	1492	-	-	0.87	0.29±0.50
26	*(E)*-*β*-famesene	$C_{15}H_{24}$	13.802	1519	-	0.94	2.22	1.05±1.11
27	*α*-bergamotene	$C_{15}H_{24}$	13.864	1529	-	-	0.24	0.08±0.14
28	cis-*α*-bisabolene	$C_{15}H_{24}$	14.023	1554	-	-	0.11	0.04±0.06
29	caryophyllene oxide	$C_{15}H_{24}O$	14.354	1608	1.51	0.37	3.99	1.96±1.85
30	humulene epoxide	$C_{15}H_{24}O$	14.505	1638	-	-	0.52	0.17±0.30
Monoterpene hydrocarbons			-	-	44.12	26.06	22.98	31.05±11.42
Oxygenated monoterpenes			-	-	22.00	13.48	14.82	16.76±4.58
Sesquiterpene hydrocarbons			-	-	30.08	55.83	54.81	46.87±14.58
Oxygenated sesquiterpenes			-	-	1.51	0.37	4.51	2.13±2.14
Others			-	-	-	0.76	1.34	0.70±0.67
Total identified			**-**	**-**	***97.71***	***96.50***	***98.46***	***97.56***

RT: Retention time; [a] Retention index determined using GC-MS with HP-5ms column in comparison to a series of homologous n-alkanes; "-": Not detected; SD: Standard deviation

CONCLUSION

The terpenes-rich compounds detected in the essential oil of the exudate of *D. aromatica* are of potential benefit to pharmaceutical, perfumery, aroma-therapeutic, cosmetic, detergent and food industries. Therefore, further downstream studies are currently focused on cytotoxic and anti-tumour promoting activities.

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DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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