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Original Research Article

Chemical composition of essential oil of exudates of Dryobalanops aromatica

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Abstract

Purpose: *To identify the chemical composition of essential oil from the exudates of Dryobalanops aromatica from Malaysia.*
Methods: *Exudate was collected from D. aromatica and subjected to fractional distillation to obtain essential oil. Gas chromatography-mass spectrometry (GC-MS) was used to characterize the composition of the isolated essential oil.*
Results: *The yield of essential oil was 7.58 %, with the highest yield (3.24 %) within the first 2 h of fractional distillation. Thirty compounds which accounted for 97.56 % of essential oil composition were identified. These include sesquiterpenes (46.87 %), monoterpenes (31.05 %), oxygenated monoterpenes (16.76 %) and oxygenated sesquiterpenes (2.13 %). Borneol accounted for 0.74 % of the essential oil.*
Conclusion: *Essential oil from the exudates of D. aromatica contains terpenoid compounds and borneol.*

Keywords: *Dryobalanops aromatica, exudate, fractional distillation, essential oil, GS-MS, borneol*

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INTRODUCTION

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

Although ancient Chinese literature reported that *D. aromatica* is the premium source of borneol, there are no scientific reports on the isolation of borneol from this plant [3,4]. Researches on *D. aromatica* have so far focused mainly on the tree bark. Moreover, 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. It is an essential ingredient in 65 traditional Chinese medicines [5]. In recent years, natural borneol has been isolated from the leaves of *Cinnamomum glanduliferum* [6], *Cinnamomum camphora* [7] and *Cinnamomum burmannii* [8]. Borneol can also be synthesized from turpentine oil or camphor as *Borneolum syntheticum* in the form of DL-borneol and iso-borneol. However, synthetic borneol has been 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 safer ingredient for medicinal purposes. However, due to high demand and high production cost, natural borneol is in short supply [10].

In view of the high distribution of *D. aromatica* in Malaysian Peninsular 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 studies were biological activities of the essential oil and the isolated pure compounds, particularly on anti-cancer properties, namely, cytotoxic and anti-tumor promoting activities.

EXPERIMENTAL

Plant material

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

Fractional distillation of exudates

Exudates were subjected to fractional distillation in double distilled water for 2 h at 80 – 90 °C. After cooling at room temperature for 2 h, the essential oil and distilled water were separated and this was 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.

GC-MS analysis

The essential oil was analyzed by Agilent 7890A/5975C GC-MS system equipped with HP5ms stationary phase column (30m x 0.25mm internal diameter x 0.25µm film thickness) composed of 5 % phenylpolysiloxane/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, held for 5 min, then increased to 140 °C at 10 °C/min, held 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 50-400*m*/*z*. Identification of compounds were achieved through the determination of retention indices (RI) with reference to a homologous series of *n*-alkanes (C_8-C_{20}) 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 decreased with increasing number of cycles of fractional distillation.

Essential oil composition

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; and the major compounds identified, in order of abundance, were *β*-caryophyllene (31.76 %), *α*-pinene (21.49 %), *α*-caryophyllene (13.50 %), terpinen-4-ol (8.58 %) and *α*-terpineol (5.89 %). In addition, 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 for treating heart disease, coma and respiratory problems; indigestion and pain relief [11]. In this study, borneol was detected in the essential oil of *D. aromatica* at 0.74 %.

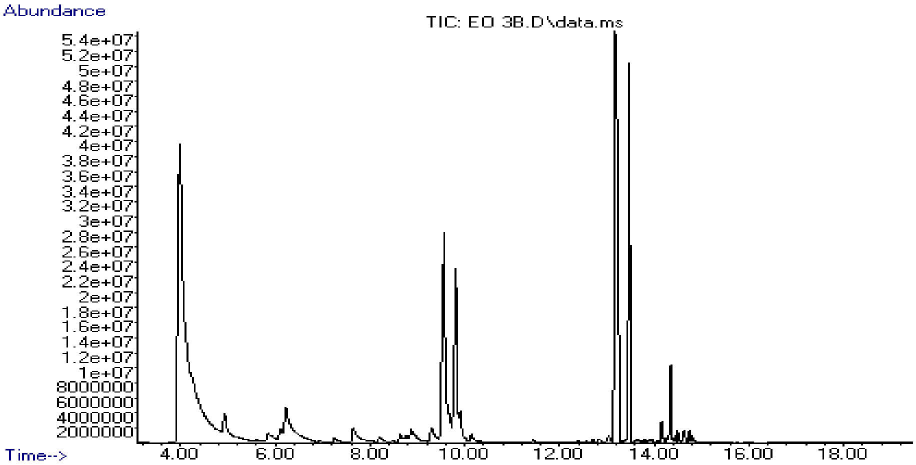

Figure 1: Total ion chromatogram of essential oil extracted from the exudates of *D. aromatica* by GC-MS analysis

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

No.	Compound	Molecular formula	RT (min)	RI [a]	Sample (n=3)			Mean ±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

The other compounds detected 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 components of essential oil from the exudates of *D. aromatica*. This study has identified some compounds not earlier reported by Huang and Lu [3]. These are *d*-borneol, *α*-caryophyllene, *β*-elemene, *β*-caryophyllene, asiatic acid, dryobalanone, erythrodiol and hydroxy-dammarenone II. The synthesis of phytochemicals 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 differences observed in the chemical composition of essential oil between the present study and that of Huang and Lu might be due to geographical variation of plant species, which led to differences in environmental conditions.

The compounds detected in this study (*d*-borneol, terpinen-4-ol, α-terpineol, α-pinene and caryophyllene) are terpenoid compounds well known for their anti-microbial, antiviral, anti-inflammatory and cytotoxic effects [14-20].

CONCLUSION

Twenty-seven compounds have been identified from the essential oil from the exudates of *D. aromatica.* These terpenoid compounds have great potential in the pharmaceutical, perfumery, aroma-therapeutic, cosmetic, detergent and food industries.

DECLARATIONS

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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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