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Chemical Constituents of Essential Oils from Resin and Bark of *Agathis borneensis*

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ABSTRACT

The chemical constituents of essential oils obtained from resin and bark of *Agathis borneensis* were analysed using capillary gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometer (GC/MS). The resin essential oil dominated by α-pinene (30.93%), δ-limonene (17.79%), β-pinene (11.28%) and terpinen-4-ol (8.35%). The main components in the bark essential oil were β-pinene (8.68%), terpinen-4-ol (8.54%), α-pinene (8.50%) and α-terpineol (8.48%).

Keywords: *Essential oil, gas chromatography, Agathis borneensis, resin, bark*

INTRODUCTION

Agathis sbp. belong to the family Araucariaceae. Araucariaceae comprises of three genera which are *Agathis, Araucaria* and *Wollemia* with over 40 species. Araucariaceae are evergreen conifers that can be found in warm temperate regions of the southern hemisphere, with the exception of Southern Africa (Brophy *et al.* 2000). Agathis species can be found in Philippines, Moluccas, Celebes, Borneo and Peninsular Malaysia. This genus is very important as high value timber and has a valuable resin which is used as varnishes and lacquers (Appanah and Weinland 1993).

Savluchinske-Feio *et al.* (2006) showed that the resin acid derivatives from this genus have shown several bioactivities such as antifungal and antibacterial. *Agathis borneensis* also known by several vernacular names such as "Bindang" in Sarawak, "Damar Minyak" in Peninsular Malaysia, "Manggilan" in Sabah and "Tulong" in Brunei. In Sarawak, it found in mixed dipterocarp and kerangas forest in areas above 610 meter. The timber is classified as softwood which belongs to a taxanomic group of gymnosperm and is light in weight (Ismail *et al.* 1999). This paper described the analysis of *A. borneensis* resin and bark oil constituents by using capillary gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometer (GC/MS).

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MATERIALS & METHODS

Plant materials

The resin and fresh bark samples of *A. borneensis* were collected from Bario Highland, Miri Division of Sarawak. The resin was ground into powder while the bark was milled into small meals.

Oil isolation

The bark and resin samples were subjected to water distillation using a Clavenger-type apparatus. Approximately 300 g of fresh bark meals and 30 g of ground resin were weighted, transferred to 2 L flat round bottom flask and mixed with 1.5 L of distilled water. The flask was assembled to the clavenger trap, connected to the condenser and heated. The hydrodistillation process was carried out for eight hours. After eight hours the oil trap in the clavenger was then cooled at room temperature. The oily layer obtained was separated and any trace water remained in the oil was adsorbed with anhydrous sodium sulfate. The percentage of the oil was calculated based on dried weight of the resin and bark samples.

Gas chromatographic analysis of the essential oil

GC analysis of the essential oil was performed on a Hewlett Packard gas chromatograph model HP-6890 equipped with flame ionization detector (FID) and an HP-5 fused capillary column (5% phenyl-methylpolysiloxane stationary phase) with film

thickness of 0.25 µm, 30 m length and 0.25 mm internal diameter. The temperature for injector and detector were programmed at 260ºC and 280ºC, respectively. The GC oven temperature was programmed from 50ºC for five minutes, then increased at 3.5ºC/min to 280ºC and hold at final temperature for five minutes. Prior to injection, 1.0 µL of essential oil was diluted with 199 µL of dichloromethane. Exactly 1 µL of diluted oil was then injected using a microsyringe to GC column in a splitless mode. Hydrogen was used as carrier gas with a flow rate of 1 mL/min. Diluted essential oil was also analyzed on a Shimadzu gas chromatography-mass spectrometer (GC-MS) model QP 2010 PLUS equipped with a quadrapole mass analyser. The GC column was BPX-5 (5% phenyl polysilphenylene-siloxane) with film thickness of 0.25 µm, 30 m length and 0.25 mm internal diameter. The injection mode and temperature program used were similar to GC-FID analysis. Helium was used as carrier gas with a flow rate of 1 mL/min. Identification of oil components was achieved based on their Kovat's indices (KI) by using authentic n-alkanes standard (C_9 to C_{32}) as references and further confirmed by library search against the mass spectral library in NIST08 database incorporated with GC-MS data system.

RESULTS & DISCUSSION

Hydrodistillation of *A. borneensis* resin obtained yellow oil with 7.3% yield (based on dry weight). Gas chromatographic analysis of the resin oil identified 51 compounds as constituents of the oil. The gas chromatograms obtained from GC-FID and GC-MS analyses of the resin oil are shown in Figures 1 and 2, respectively. The chemical constituents identified in the resin oil are listed in Table 1. Approximately 3.02% of the chemical constituents of resin oil was not identified due to poor similarity index of these components during the library search with NIST mass spectral library of GC-MS. The chromatographic analysis showed that the major constituents in the resin essential oil were α-pinene, δ-limonene, β-pinene and terpinen-4-ol. α-pinene showed the highest percentage which is 30.93%, followed by d-limonene (17.79%), β-pinene (11.28%) and terpinen-4-ol (8.35%).

Hydrodistillation of fresh *A. borneensis* bark sample yielded 0.49% yellow oil. Bark essential oil consists of 60 compounds as confirmed by GC-FID and GC-MS analytical data. The analysis showed that the major components identified in the bark essential oil were β-pinene (8.68%), terpinen-4-ol (8.54%), α-pinene (8.50%) and α-terpineol (8.48%). The gas chromatograms obtained from GC-FID and GC-MS analyses of the bark essential oil are shown in Figures 3 and 4, respectively. The compounds identified in the bark essential oil are presented in Table 2. Approximately 14.39% of chemical constituents in *A. borneensis* bark oil remained unidentified by both GC-FID and GC-MS.

The essential oils of resin and bark samples contained several similar major components such as α-pinene, β-pinene and terpinen-4-ol. However, the percentage of α-pinene in the resin oil was higher compared to the bark oil with 30.93% and 8.50%, respectively. The amount of β-pinene was also higher in the resin oil compare to the bark oil with 11.28% and 8.68%, respectively. Interestingly, the resin and bark oils consists of almost similar quantity of terpinen-4-ol with 8.35% and 8.54%, respectively.

Data on chemical composition of essential oils from genus of *Agathis* is limited. Brophy *et al.* (2000) reported the major compounds detected in the essential oil from four species of *Agathis* such as *A. microstachya, A. robusta, A. australis* and *A. atropurpurea*. The major compound identified in the essential oil from *A. microstachya* was the monoterpene α-pinene (17.9%). The main components in the essential oil of *A. robusta* were α-pinene, β-pinene, β-caryophyllene, δ-germacrene, δ-cadinene, caryophyllene oxide and spathulenol. The chemical components identified in the essential oil of *A. australis* were δ-α-pinene, δ-camphene, δ-limonene, dipentene, 1,8-cineole, δ-borneol, 1-cadinene, kaurene, tricyclene, car-3-ene, p-cymene, terpinolene, α-copaene, aromadendrene, γ-muurolene, isophyllocladene, isoatisirene, isopimara-8,15-diene, dehydroabietane, δ-germacrene, bicyclogermacrene, 16-kaurene, sclarene and 8-β-hydroxysandarocopimar-15-ene. While, the essential oils from *A. atropurpurea* contains α-pinene, α-copaene, bicyclogermacrene, δ-cadinene, phyllocladene and 16-kaurene as it major components.

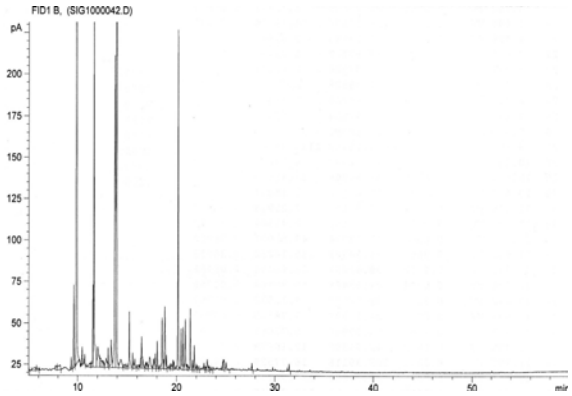

Figure 1. Gas chromatogram traced by GC-FID of *A. borneensis* resin oil.

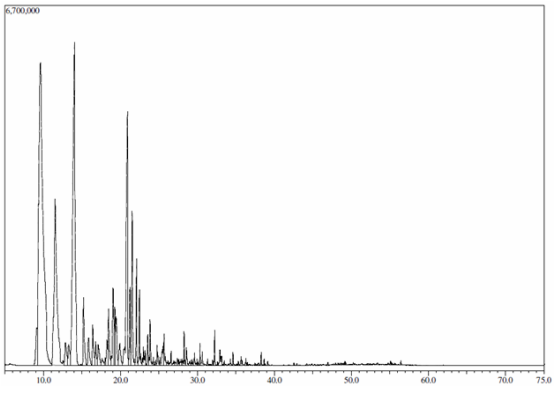

Figure 2. Gas chromatogram traced by GC-MS of *A. borneensis* resin oil

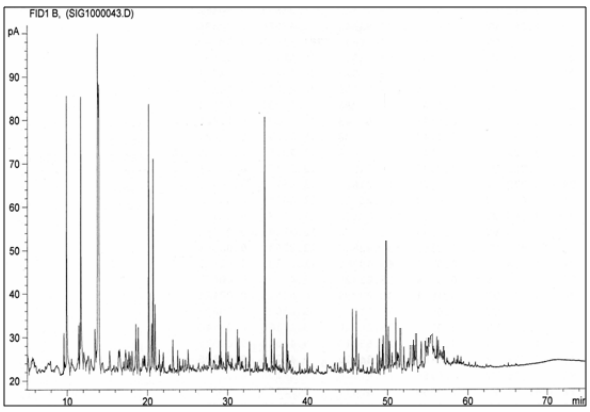

Figure 3. Gas chromatogram traced by GC-FID of *A. borneensis* bark oil.

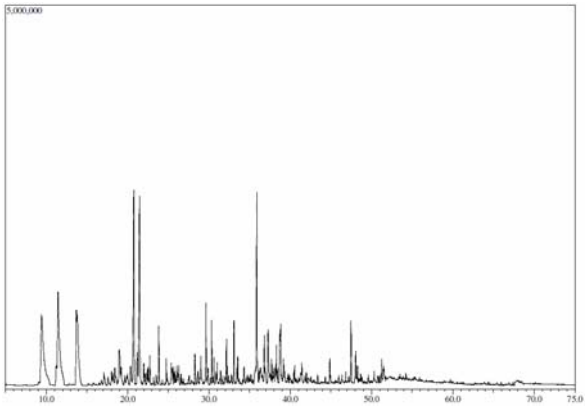

Figure 4. Gas chromatogram traced by GC-MS of *A. borneensis* bark oil

Table 1. Chemical constituents of the essential oil from resin of *A. borneensis*

Compound name	Kovat's Index	% area	Identification
α-pinene	940	30.93	a, b
β-pinene	983	11.28	a, b
3-carene	1013	0.96	a, b
α-terpinene	1023	0.80	a, b
D-limonene	1039	17.79	a, b
γ-terpinene	1065	1.80	a, b
Sabinene hydrate	1079	1.01	a, b
Terpinolene	1091	1.15	a, b
α,p-dimethylstyrene	1100	0.62	a, b
Linalool	1108	0.80	a, b
Limonene oxide	1119	0.27	a, b
Trans-dihydrocarvone	1129	0.16	a, b
Cis-β-terpineol	1134	0.72	a, b
α-campholenic aldehyde	1139	1.36	a, b
2(10)-pinen-3-ol	1152	2.34	a, b
2-pinen-4-ol	1157	1.22	a, b
Dihydro-α-terpineol	1161	1.61	a, b
2-(4-methylcyclohexyl)-1-propanol	1173	0.88	a, b

Terpinen-4-ol	1195	8.35	a, b
p-cymen-8-ol	1203	1.60	a, b
α-terpineol	1210	3.18	a, b
2-pinen-4-one	1224	1.86	a, b
Cis-carveol	1234	1.21	a, b
Carveol	1246	0.60	a, b
Epoxy linalooloxide	1268	0.89	a, b
Linalool formate	1278	0.10	a, b
3,3,5-trimethylcyclohexyl acetate	1290	0.42	a, b
p-cymene-7-ol	1308	0.62	a, b
6-methylspiro[4.5]decan-6-ol	1314	0.72	a, b
Carvone hydrate	1359	0.17	a, b
α-cubebene	1382	0.44	a, b
α-bergamotene	1438	0.24	a, b
β-sesquiphellandrene	1446	0.22	a, b
α-santalol	1465	0.10	a, b
β-farnesene	1491	0.39	a, b
o-menth-8-ene-4-methanol	1560	0.17	a, b
Unidentified components	-	3.02	

Notes: a - Kovat's Index ; b - GC-MS library.

Table 2. Chemical constituents of the essential oil from bark of *A. borneensis*

Compound name	Kovat's Index	% area	Identification
α-pinene	935	8.50	a, b
β-terpinene	976	0.72	a, b
β-pinene	981	8.68	a, b
o-cymene	1032	3.51	a, b
α-limonene	1034	5.73	a, b
Fenchol	1129	0.59	a, b
α-campholenic aldehyde	1138	0.44	a, b
2(10)-pinen-3-ol	1151	2.03	a, b
Verbenol	1156	0.49	a, b
Borneol	1183	0.67	a, b
Terpinen-4-ol	1192	8.54	a, b
ρ-cymen-8-ol	1202	1.84	a, b
α-terpineol	1209	8.48	a, b
2-pinen-4-one	1222	0.84	a, b
Cis-carveol	1232	0.47	a, b
2-hydroxycineole	1240	0.92	a, b
Carvone	1259	0.47	a, b
Epoxy linalooloxide	1□67	2.15	a, b
2,3-pinenediol	1324	0.51	a, b
α-cubebene	1381	1.08	a, b
Cedr-8-ene	1391	0.68	a, b
α-longipinene	1418	1.96	a, b
α-bergamotene	1438	1.56	a, b
β-sesquiphellandrene	1446	0.69	a, b

β-farnesene	1457	0.68	a, b
Curcumene	1488	1.09	a, b
Farnesene epoxide	1552	0.80	a, b
Spathulenol	1593	0.49	a, b
(-)-isolongifolol	1598	6.86	a, b
(-)-globulol	1600	0.68	a, b
Guaiol	1610	1.19	a, b
Unidentified	1620	0.51	-
1,2-epoxide-humulene	1626	1.46	a, b
Cubenol	1629	0.72	a, b
3,5,6,7,8,8α-hexahydro-4,8α-dimethyl-6-(1-methylethenyl)-2(1H)naphthalenone	1640	2.48	a, b
Guai-1(10)-en-11-ol	1654	0.64	a, b
γ-muurolene	1662	0.48	a, b
α-bisabolol oxide	1668	0.53	a, b
1-tetradecanol	1686	2.59	a, b
α-bisabolol	1699	0.64	a, b
Unidentified	1744	0.45	-
1,5,5,8-tetramethyl-3,7-cycloundecadien-1-ol	1769	0.59	a, b
1-nonadecene	1890	0.67	a, b
8-epimanoyl oxide	2011	0.51	a, b
Unidentified Components	-	14.39	-

Notes: a - Kovat's Index ; b – GC-MS library.

CONCLUSION

The chemical constituents in the resin and bark oil of *A. borneensis* have been determined. The major components identified in the resin essential oil were α-pinene, δ-limonene, β-pinene and terpinen-4-ol. On the other hand, β-pinene , terpinen-4-ol , α-pinene and α-terpineol were detected as major components in the bark essential oil.

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