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Composition and antitermite activities of essential oils from *Melaleuca* species

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Abstract The composition and antitermite activities of nine essential oils from two *Melaleuca* species, gelam and cajuputi, were compared using gas chromatography–mass spectrometry analysis and bioassays. Gelam oils were rich in compounds whose boiling points were high, and they were separated into the elemene-rich type and the γ-terpinene- and terpinolene-rich type. Cajuputi oils in this experiment were categorized into three chemotypes according to their 1,8-cineole content: high, low, none. In the termiticidal activity test, gelam oils were stronger than most cajuputi oils in the contact condition. The elemene-rich gelam lost its activity in the noncontact condition, whereas another type of gelam kept its termiticidal activity. The authentic sample of elemene showed the same result as the elemene-rich gelam, indicating that the termiticidal activity of gelam was caused by at least two types of compound: elemene and "others." 1,8-Cineole exhibited the same tendency as elemene, but it was weak. Hence the 1,8-cineole content of cajuputi was irrelevant to termiticidal activity of samples that exhibited strong activity under both conditions despite their high content. The appearances of gelam and cajuputi leaves are easily confused, a problem that has not yet been solved. Other scientific methods, such as genetic analysis, are required to identify gelam. The difference in the compositions and antitermite activities, however, were charified in this experiment.

Key words *Melaleuca cajuputi* · *Melaleuca leucadendron* · Gelam · Termiticidal activity · Repellent activity

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Introduction

Melaleuca species (Myrtaceae) are native to Australia and Southeast Asia. They consist of more than 100 species, some of which are known as essential oil-rich species. Cajuputi (*Melaleuca cajuputi*) is a species found in northern Australia, Papua New Guinea, Indonesia, Thailand, and Vietnam.[1] Cajuputi forms mixed forests with other *Melaleuca* species in wet and other land. The most important use of cajuputi is as a source of oil obtained by steam and hot water distillation of its leaves and terminal twigs. The major constituents of the essential oil are 1,8-cineole and α-terpineol.[2] Commercial categorization of essential oil depends on the 1,8-cineole content. Cajuputi oil, which has a camphor-like odor, is used as an insect repellent and as a painkiller for headache, toothache, rheumatism, and convulsions in the form of applied plaster.[3]

Another tree of the *Melaleuca* species, which locally, is called "gelam" in Indonesia, is distributed in the island of Sumatra, Indonesia. Gelam is an evergreen tree that grows to a maximum of about 1.2 m diameter and 25 m height. As gelam can grow in wetland, unlike other trees, natural forests of gelam are found on the east coast of Sumatra, where wetland is formed by rivers. Gelam is a fast-growing tree: Its trunk grows to 6–9 cm diameter and its height to 4–6 m within 2–3 years after germination. After logging, gelam trees renew themselves by beginning to bud. The appearance of the gelam leaf is similar to that of the cajuputi leaf, which is used as a commercial essential oil source. At present, however, the tree is used not for essential oil but for wood for scaffolding and the posts of small houses because of its straightness and hardness. Although the leaves of gelam have never been used commercially, they could become a source of essential oil because gelam is one of the *Melaleuca* species whose essential oils are used commercially, like those of the tea tree and cajuputi.

No previous study on gelam oil was found in the literature. Therefore, this study examined the chemical composition of gelam essential oil and its biological activity against termites through a comparison with cajuputi oil.

Materials and methods

Plant materials

Three kinds of gelam leaf samples were named GA, GB, and GC in this report for convenience. GA was collected in an experimental gelam field near Gashing River in Palembang (Indonesia) in October 1999. GA trees grew in a straight line in a marshy area. GB and GC were collected in the suburbs of Dungun (Malaysia Terengganu) at the same time.

Three kinds of leaf sample and three essential oils of cajuputi were named CA, CB, CC, CD, CE, and CF in this report for convenience. CA was collected in a residential area at Palembang (Indonesia) in October 1999. CB was collected at the roadside of a mountain 15 km from Yogyakarta (Indonesia) in July 2000. CC was collected in a cajuputi essential oil factory in Sukun in the suburbs of Ponorogo (Indonesia) in July 2000. CD was an essential oil obtained by hot water distillation from a tree growing at the University of Gadja Mada. CE was the product of the above factory in Sukun and was obtained by steam distillation. CF was a commercial product in Indonesia. CD and CF were provided by JIFPRO (Japan International Forestry Promotion and Cooperation Center). In this experiment, all leaf samples (GA–GC and CA–CC) and the leaves distilled for essential oil samples were collected from trees that were 3–4 years old.

Essential oils

The essential oils of GA–GC and CA–CC were prepared by hot water distillation methods standardized by the Association of Official Agricultural Chemistries.[4] Fresh leaves were cut into pieces of less than 1 × 1 cm, and 150- to 200-g samples were boiled with 1000 ml of deionized water for 16 h.

Ethanol extraction

The fresh leaves of CB and CC (20–30 g) were placed in ethanol (60 ml). The capped samples were then microwaved for 25 s and were left to stand at room temperature for 3 days. After that the leaves were filtered out, and the ethanol solution was concentrated to obtain ethanol extractives.

GC-MS analysis

Oil components were identified by gas chromatography–mass spectrometry (GC-MS) analysis. The GC was equipped with a TC-FFAP column (30 m × 0.25 mm) that was held at 60°C for 30 min and then heated from 60°C to 210°C at 2°C/min using helium as the carrier gas. Mass spectra were obtained at 70 eV. Peaks were confirmed by library searches and comparisons with authentic samples.

Bioassays

Termiticidal activity

The test termites were *Reticulitermes speratus* (Kolbe) worker termites whose colonies were collected at International Christian University (Mitaka, Tokyo) and were cultured for 2–3 days in a chamber maintained at room temperature. Figure 1 shows the bioassay apparatus for the termiticidal activity test. In the bioassay for termiticidal activity, cut filter papers (diameter 25 mm) were placed in each hole of a six-hole multidish (3 rows × 2 lines, hole diameter 25 mm). Essential oils were diluted to 10.0% to 0.1% with methanol, and specimens of some high-quantity compounds in essential oils were diluted with methanol to the concentration at which they existed in the leaves. Samples of 50 μl were placed on the filter paper in each hole of one line. In the bioassay for contact termiticidal activity,

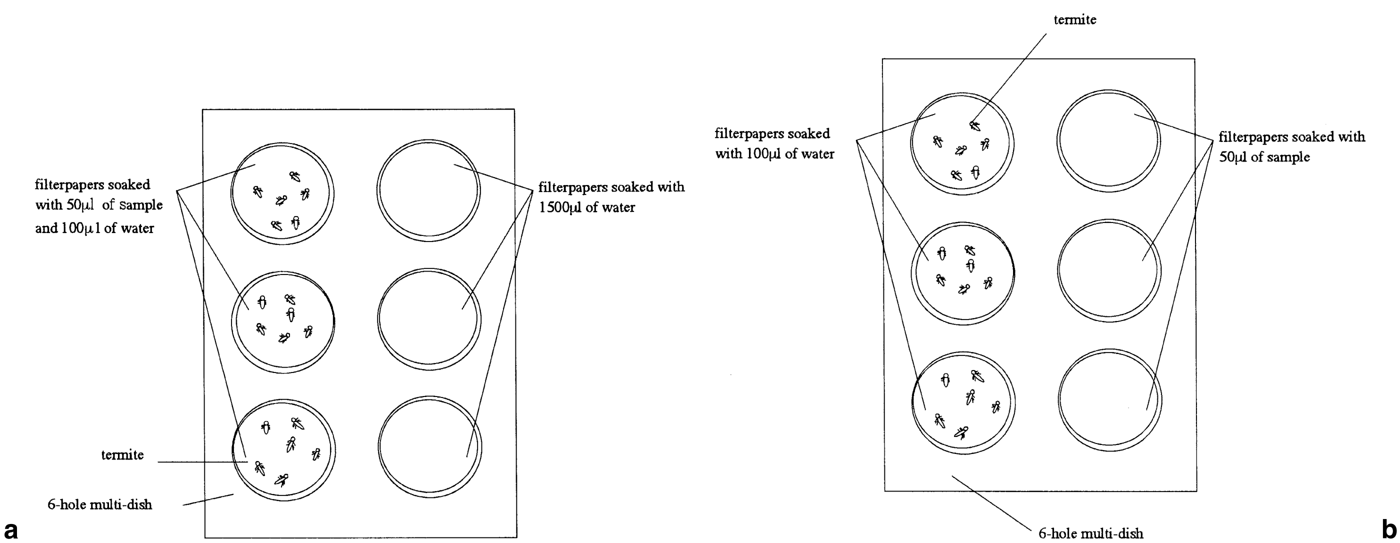

Fig. 1. **a** Bioassay apparatus for termiticidal activity tests in contact condition. **b** Bioassay apparatus for termiticidal activity tests in the noncontact condition

six termites were put on one line on which a sample was soaked on a filter paper. In the bioassay for noncontact termiticidal activity, six termites were put on the other line, which did not contain a sample. The six-hole multidishes were closed tightly and kept at 27°C in an incubator. The numbers of living termites were counted each day. The bioassay for termiticidal activity was performed nine times (3 holes/multidish × 3 times) for each condition.

Repellent activity

Essential oils were diluted 1:1000 with methanol. Figure 2 shows the bioassay apparatus for the repellent activity test. Filter paper was soaked with 10μl of a sample, and six termites were placed in a petri dish (diameter 9cm, depth 1cm). Thimble filter papers (inner diameter 2.5cm, length 10cm), which were cut at a point 2cm from the bottom, had a hole (inner diameter 2mm) at the bottom. After that the thimble bottoms were turned over, and the termites and filter paper soaked in sample were covered with the thimble. All the equipment was kept for 2h at room temperature. After 2h, the termites that emerged from inside of the thimble filter paper were counted. This bioassay for repellent activity was performed nine times.

Results and discussion

Yield of essential oils by steam distillation

Essential oils were obtained from all *Melaleuca* species in this experiment. Table 1 shows the yields of each essential oil. The oil contents of cajuputi ranged from 3.6% (CA) to 9.1% (CB). Those of gelam ranged from 1.6% (GA) to 3.6% (GB), which were somewhat lower than those of cajuputi, but these values were enough for commercial use. For example, in Japan the essential oils of sugi (*Cryptomeria japonica* D. Don), hiba (*Thujopsis dolabrata* S. and Z. var. *hondae* Makino), and hinoki (*Chamaecyparis obtusa* Endl.) are in commercial use,[5,6] and their yields are similar to those of gelam. Hence in terms of their yields alone, not only essential oils obtained from cajuputi but also those obtained from gelam could be used commercially.

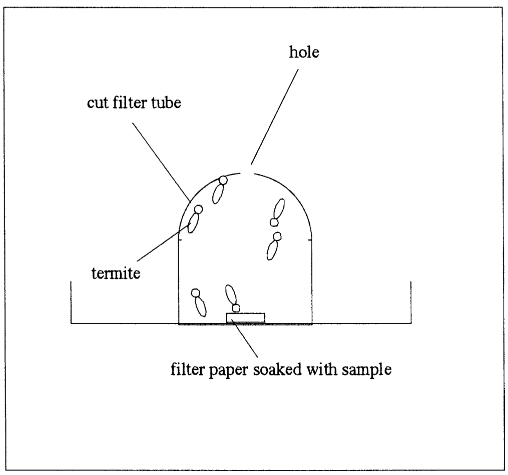

Fig. 2. Bioassay apparatus for repellent activity tests

Composition analysis with GC/MS

The essential oils of *Melaleuca* species were analyzed with GC-MS. Table 2 shows the composition of each essential oil. The retention time of β-pinene was regarded as 1.00, and the relative retention times for the other compounds were calculated by dividing their retention times by that of β-pinene. In every analysis the peaks were distributed in two parts: a relative retention time below 3.0 and that above 6.8. The major composition below 3.0 was that of monoterpenes, and that above 6.8 was that of sesquiterpenes. All samples contained β-pinene, limonene, and α-caryophyllene.

In addition to compounds commonly detected among all samples, 13 others were often seen among three gelams, but the content of each was different between GA and other two gelams. The compositions of GB and GC were similar, whereas that of GA was quite different; hence gelam oils can be separated into two chemotypes. The major constituents of GA were α-caryophyllene and elemene. Although *Melaleuca symphyocarpa* is reported as a rare *Melaleuca* species with high elemene content,[7] the elemene content of GA is 28.3%, which is higher than that of *M. symphyocarpa* (17.9%). The major constituents of GB and GC were α-terpinene, γ-terpinene, terpinolene, α-phellandrene, α-caryophyllene, and an unknown compound (relative retention time 17.17); the contents of the other compounds were similar. In addition, GB and GC contained similar isomers (i.e., the position of the isopropyl group): a pair of terpinene-4-ol in GB and 4-methyl-3-(methylethyl)-3-cyclohexene-1-ol in GC and a pair of *p*-cymene in GB and *o*-cymene in GC.

p-Cymene can be derived from γ-terpinene by oxidation in air; hence if *p*-cymene were an artifact due to hot water distillation, both GB and GC should have contained *p*-cymene. Although GB and GC contain γ-terpinene, which is a possible precursor of *p*-cymene, *p*-cymene was detected only in GB. These results suggest that *p*-cymene is not an artifact derived from γ-terpinene but a metabolite in GB leaves.

Table 1. Yields of essential oils obtained from leaves of *Melaleuca* species

Oil	Yield (ml/100g)
Gelam	
GA	1.62
GB	2.25
GC	3.62
Cajuputi	
CA	3.66
CB	9.10
CC	4.32

Table 2. Results of GC-MS analysis of essential oils

Relative retention time[a]	Compound	GA	GB	GC	CA	CB	CC	CD	CE	CF
0.61	*β-trans*-Ocimene		4.06				0.54		5.76	13.15
0.67	*α*-Pinene			2.5	1.38	1.76				
0.68	3-Carene	4.9	0.27	0.37						
0.69	*α*-Phellandrene		5.42	7.79						
0.74	Camphene		0.02	0.02	0.05			0.03	0.02	
1.00	*β*-Pinene	0.30	1.14	0.38	0.86	1.84	0.66	2.21	3.63	5.59
1.21	*β*-Myrcene	0.17			0.59	0.77	1.08	0.44	1.61	2.65
1.46	*α*-Terpinene	0.06	3.55	4.29	0.18			0.55		1.44
1.47	Limonene	0.43	3.63	2.00	2.56	1.44	1.59	0.03	1.70	0.23
1.70	1,8-Cineole	1.92			31.78	55.16	62.29		69.43	
1.88	Leaf aldehyde		0.02							
1.98	*γ*-Terpinene		15.67	17.77	0.51		0.32	8.91	1.45	9.97
2.49	*o*-Cymene			4.81	0.3			0.88		
2.51	*p*-Cymene		7.46							1.44
2.65	Terpinolene	0.30	19.01	21.47	0.31			3.86	1.03	4.11
6.77	*p*-Isopropenyl toluene			0.35						
8.63	Caryophyllene homologue		4.22	4.88	6.55	9.09		20.08	1.91	11.02
9.53	Isothujol		0.03							
9.58	*α*-Caryophyllene	11.36	1.9	2.13	1.56	4.64	2.87	11.92	0.88	5.31
9.59	Isocaryophyllene						4.94	3.36		
9.71	*α*-Guaiene	0.36	0.13		1.31				0.03	0.53
9.76	Allo-aromadendrene	0.03	0.28	0.47	2.12		4.39	0.27	0.94	5.46
9.84	Elemene	28.3	1.49	1.28						
9.97	4-Methyl-3 (methylethyl)-3-cyclohexen-1-ol			3.72				2.65		1.38
10.01	*α*-Bulnesene	0.02			2.67					
10.05	Terpinene-4-ol		3.46		0.43					
10.18	Unknown				0.46			5.72		0.02
10.22	Linalyl propanoate								8.78	
10.78	*α*-Humulene				3.39					
10.89	*α*-Terpineol		1.47	1.81	11.88	8.04	16.64	0.43		27.91
11.06	Cadiene homologue							1.76		
11.17	Unknown									2.4
11.35	*β*-Eudesmene	1.72	0.37	0.32			0.26	0.15		1.57
11.66	Decahydro-4a-methyl-1-methylene-7-(2-methylethenyl)-, naphthalene							8.1		
11.66	Elemophilene							1.88		
11.73	*α*-Guaiene							4.84		
11.97	*δ*-Cadiene	0.50	0.16	0.50	0.14			1.49		0.26
12.65	1-Allo-aromadendrene homologue					1.09				
13.83	Caryophyllene oxide	1.71	0.77	0.15	0.77			0.43		
14.64	(−)-Globulol	1.69	0.8	0.75	10.38					
14.82	Ledol	0.71	0.39	0.44	0.67			3.67		
15.43	Ledene					4.92		0.05		1.19
15.74	*δ*-Cadinol	0.60	0.68	1.06	1.00			0.04		
15.94	(−)-Spathulenol	1.76	2.07	0.54	0.18					
16.02	Copaene					1.18	0.27	0.03		
16.05	Aromadendrene homologue					1.45				
17.17	Unknown	2.29	10.8	8.26						
17.18	Unknown			3.01						
17.23	1-Terpinenol		2.56							
17.35	Unknown	10.92								
18.95	Unknown	16.45	0.10	0.21						

GC-MS, gas chromatography–mass spectrometry

[a] Retention time of *β*-pinene was regarded as 1.00. The relative retention time of each compound was then calculated by dividing its time by the retention time of *β*-pinene

Both *M. leucadendron* and *M. cajuputi* are likely to be used as the scientific name for gelam; however, the composition of gelam oils were different from those of cajuputi in this experiment, so gelam can be distinguished from *M. cajuputi*. Essential oils of *M. leucadendron* were categorized as the methyl eugenol-rich type, the isomethyl eugenol-rich type,[2] and the terpenoids-rich type. The former two types are used commercially, whereas the latter has not yet been used.[2] Methyl eugenol and isomethyl eugenol were not detected in gelam essential oils in this experiment. Although a more scientific analysis (e.g., a statistical approach for essential oils or a genetic approach for gelam and cajuputi) is needed for taxonomic identification of gelam, the gelam in this report could have been the terpenoids-rich type of *M. leucadendron*.

β-Myrcene was commonly detected in cajuputi oil as well as in all other samples used in this experiment. 1,8-Cineole is known as a major constituent of cajuputi, and the commercial value of cajuputi depends on its content.[3] For example, in Indonesia cajuputi essential oil whose 1,8-cineole content is higher than 55% is graded as "prime quality," and that with less than 55% is graded as "standard." In this experiment, 1,8-cineole was detected in CA–CC and CE but not in CD or CF. The content in CA was about 30%, and that in others except for CD and CF ranged from 50% to 70%. CC and CE (cultivated as commercial essential oil plants) can be categorized as "prime quality." Usually a wild plant contains a smaller amount of essential oil than does of one cultivated as a commercial source of essential oil. Between CA and CB (both wild types) CA can be categorized as "standard" and CB as "prime quality"; hence CB could be used as a commercial source of essential oil. This means that we have identified a new wild-type *Melaleuca* that can be used as an essential oil source. Cajuputi in this experiment could be categorized into three chemotypes according to the content of 1,8-cineole: high, low, none.

As the content of 1,8-cineole in CA, CD, and CF were at low levels or none, these oils were of little value in terms of their 1,8-cineole concentration. However, CA and CF contain a high content of α-terpineol, which is known to have strong biological activity.[8] Therefore, these cajuputi oils may have biological activity, and studies to identify the compounds with biological activity other than that of 1,8-cineole are required.

Termiticidal activity test

Figure 3 shows the contact termiticidal activity of essential oil against the termite *R. speratus*. About 80%–100% of termites in the control had survived at the end of the contact termiticidal activity test in the control area. All of the 10-fold diluted samples exhibited strong termiticidal activity, and all termites died within 1 day from the start of the experiment. A difference in termiticidal activity between gelam and cajuputi was observed in the 100-fold diluted samples (Fig. 3a). All gelam samples exhibited strong activity even after being diluted 10-fold, whereas among the cajuputi samples different termiticidal activity was seen between CA and CB–CF. The 10-fold diluted solution of CA exhibited strong activity, whereas the termiticidal activity of CB–CF declined gradually during the test period. For example, for CB, 30%–50% of termites survived at the end of the experiment and for CC–CF 70%–80% of termites survived. A difference in termiticidal activity between gelams was observed at a 1000-fold dilution (Fig. 3b). GA killed about 70% of termites within 1 day from the start of the experiment; but after that point the termiticidal activity declined gradually, and 15%–30% of termites survived at the end of the experiment. Termiticidal activities of GB and GC were weaker than that of GA, with 70%–80% of the termites surviving at the end of the experiment. These results were similar to those of 1% solutions of CC, CD, CE, and CF. With 0.1% solutions of CC, CD, CE, and CF, 70%–80% of termites survived at the end of the experiment, similar to the control preparation.

Gelams exhibited the same or stronger termiticidal activity than cajuputi species. Therefore, compounds that contribute to termiticidal activity in gelam were examined from two points of view. The first is that the content in gelam is higher than that in cajuputi. Gelam showed stronger termiticidal activity than cajuputi; therefore, if it was caused by one compound, its content in gelam should be higher than that in cajuputi. The second is that the content in GA, which had the strongest termiticidal activity among the gelam preparations, is higher than those in GB or GC. GA showed the strongest termiticidal activity among the gelam preparations. If this activity was caused by one compound,

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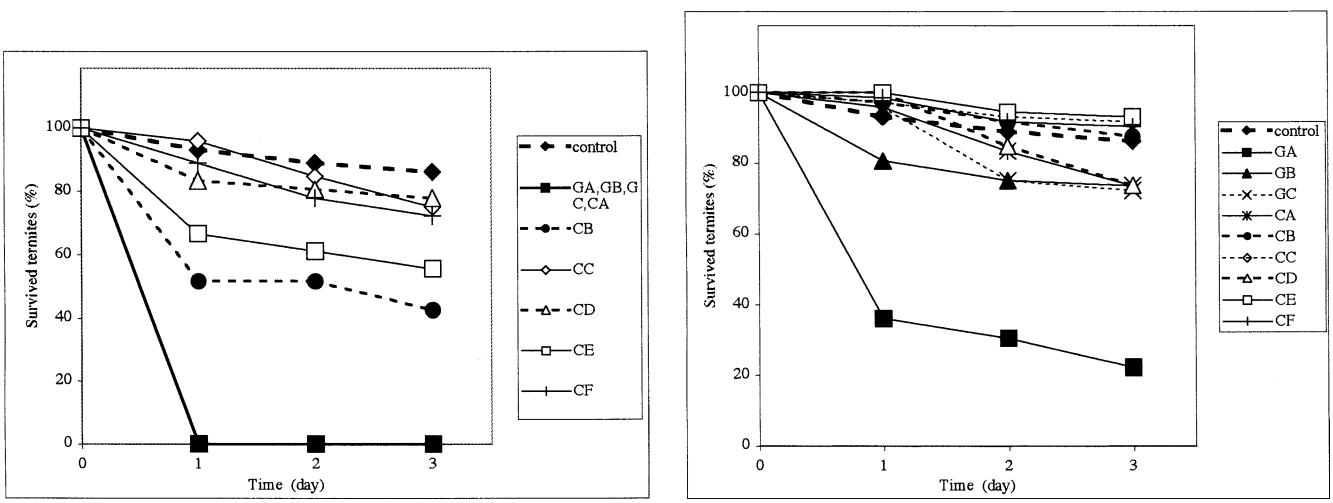

b

Fig. 3. **a** Termiticidal activity of essential oils diluted 100-fold in the contact condition. **b** Termiticidal activity of essential oils diluted 1000-fold in the contact condition Error bars show the standard deviations

its content should be highest in GA. Compounds that met these criteria were 3-carene, elemene, and unknown compounds (relative retention times were 17.35 and 18.95). Elemene and 3-carene have been reported as defensive substances against termites but not as termiticides.

It was reported that essential oils of *Melaleuca bracteata* and *M. dealbata*, which contain compounds with long retention times, have strong miticidal activity.[7] Hence the strong termiticidal activity of gelam might be caused by unknown compounds with a relative retention time above 17.

Figure 4 shows the results of the noncontact termiticidal activity test. Termites in the control did not die throughout this experiment. GB and three cajuputi samples (CA, CE, CF) kept their termiticidal activity, whereas GA lost its activity, exhibiting the same activity as that of the control. These results suggest that the termiticidal activities of GB and the three cajuputi samples in the noncontact termiticidal activity test were due to volatile compounds, and that of GA, whose activity was lost in the noncontact condition, was caused by compounds with low volatility.

Figure 5 shows the results of the termiticidal test with compounds determined to be present in high content in each of the essential oil samples. Most termites survived in the control preparation. Elemene showed a different tendency in the contact and noncontact termiticidal activity tests. In the contact termiticidal activity test, elemene showed termiticidal activity, whereas it lost its activity in the noncontact termiticidal test. This tendency supports the GA results. Elemene had a high GA content, so elemene contributed to the termiticidal activity of GA. However, other compounds also caused termiticidal activity, as elemene did not kill all the termites, as shown in Fig. 5a. α-Terpineol exhibited strong termiticidal activity in both the contact and noncontact termiticidal tests. α-Terpineol had high contents of CA and CF, which exhibited strong termiticidal activity in both experiments; hence α-terpineol possibly produces the strong termiticidal activity in CA and CF. Terpinene-4-ol exhibited strong termiticidal activities in both conditions. The terpinene-4-ol content was higher than that of α-terpineol in GB; hence this compound may produce termiticidal activity in GB. 1,8-Cineole and α-caryophyllene exhibited different termiticidal activities in the experiments, but they were both weak and could not have contributed much to the termiticidal activity of GB or three cajuputi samples (CA, CE, CF), which exhibited strong activity in both experiments despite their high contents. γ-Terpinene and α-terpinene showed no termiticidal activity in either experiment.

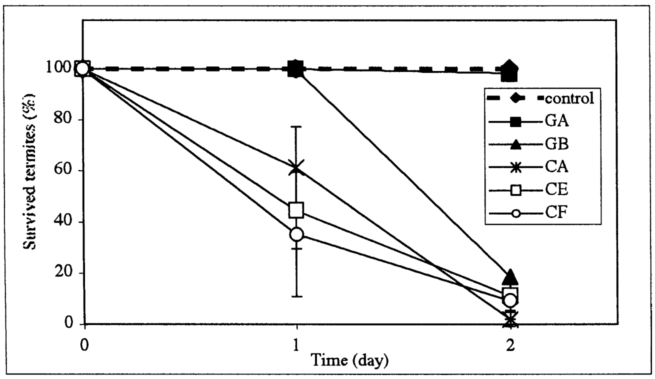

Fig. 4. Termiticidal activity of essential oils diluted 100-fold in the noncontact condition Error bar shows the standard deviation

Repellent activity test

Figure 6 shows the results of the repellent activity test. In the control test about 30% of termites, on average, went outside the thimble filter paper. Termites in other samples also went outside the thimble filter paper, but the number of termites that did so in essential oil samples was somewhat higher than that of the control. These results indicate that essential oils have low repellent activity. The repellent activity of gelam was higher than that of cajuputi, in the order GA > GB > CF > CA > CE. Among terpenoids, *d*-citronellol extracted from *Thujopsis dolabrata*, chamaecynone and isochamaecynone extracted from the wood of *Chamaecyparis pisifera*, and α-cadinol and T-

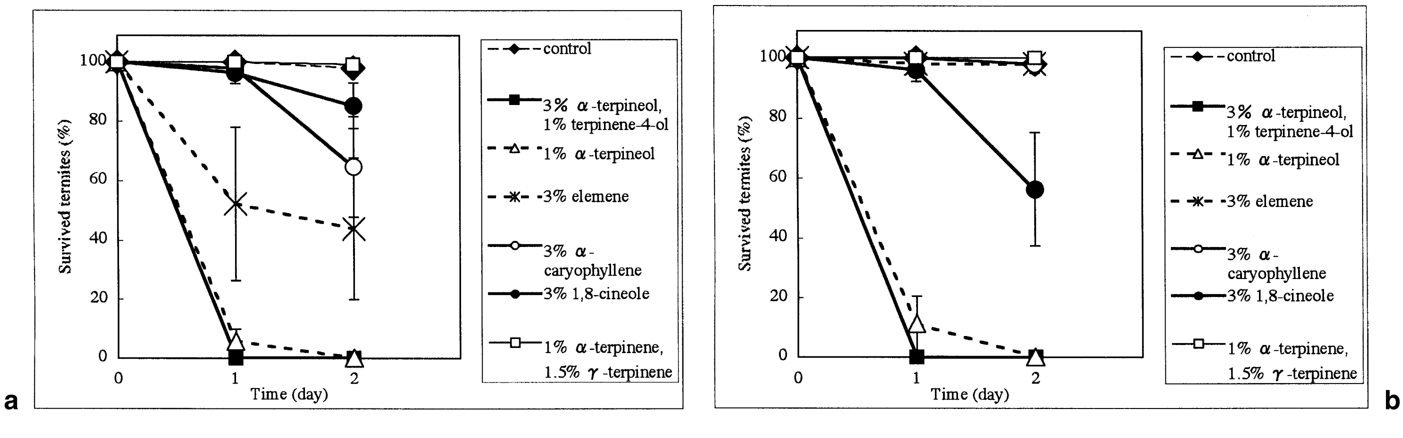

Fig. 5. **a** Termiticidal activity of the major compounds in *Melaleuca* species in the contact condition. **b** Termitical activity test of the major compound of *Melaleuca* species in the noncontact condition. Error bars show the standard deviations

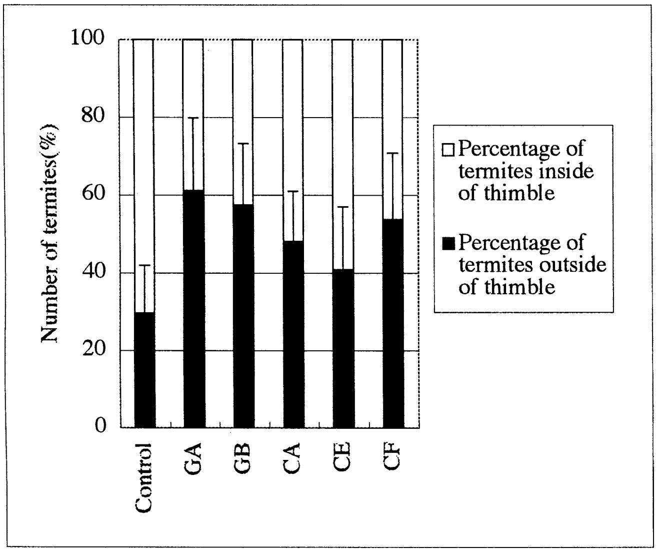

Fig. 6. Repellent activities of essential oils diluted 1000-fold. Error bars show the standard deviations

muurolol extracted from the wood of *C. obutusa* were reported as repellents.[9] However, these compounds were not detected in this experiment, so other repellents must have been present.

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