Essential Leaf Oils from *Melaleuca cajuputi*

J.H. Kim, K.H. Liu and Y. Yoon
School of Agricultural Biotechnology
Seoul National University
Suwon 441-744
Korea

Y. Sornnuwat, T. Kitirattrakarn and
C. Anantachoke
Forest Products R&D Div., Royal Forest Dept.
Chatuchak, Bangkok 10900
Thailand

Keywords: cajeput oil, cajuput oil, hydro-distillation, terpene, volatile oil

Abstract

Hydrodistillation of cajuput (*Melaleuca cajuputi*) leaves collected from 6 sites in Narathiwat gave different yields of cajuput oils. The maximum oil yield (0.97%) was obtained from leaves from Ban Koke Kuwae, Thambon Kosit, and Amphur Tak Bai. The oil yields from leaf samples of other sites were 0.84% from Ban Pha Ye and Thambon Sungai Padi in Amphur Sungai Padi; 0.76% from Ban Lubosama, and Thambon Pasemat, in Amphur Sungai Kolok; 0.70% from Ban Tha Se, and Thambon Kosit, in Amphur Tak Bai; 0.66% from Ban Mai, and Thambon Sungai Padi, in Amphur Sungai Padi; and 0.56% from Ban Toh Daeng, and Thambon Phuyoh, in Amphur Sungai Kolok. Cajuput oil densities from the 2 sites of Amphur Sungai Kolok and from Ban Mai, Thambon Sungai Padi, Amphur Sungai Padi were almost the same, but higher than others. Although major components were not different, the minor components varied in terms of both structure and proportion. The major compositions of both cajuput oils from Ban Toh Daeng, Thambon Phuyoh, and Amphur Sungai Kolok consisted of 49.22% monoterpenes and 46.45% sesquiterpenes, and the rest were hydrocarbons and a diterpene. Other cajuput oils obtained composed mainly of monoterpenes (more than 62%), sesquiterpenes, hydrocarbons and some unknown compounds respectively. There was no diterpene present in these oils. Since cajuput oil was locally used as insecticide, termicidal activities of all oils were also investigated.

INTRODUCTION

Although there are many species and subspecies of Cajuput (*Melaleuca* sp.; Myrtaceae, the *Eucalyptus* family), there is only one species, *M. cajuputi* Powell, in Thailand (C. Niyomdham, pers. commun., 1992). The plant is distributed in the South and East of Thailand. It has been well established that *M. cajuputi* is the source of cajuput oil. The principal production of this oil is in Indonesia and Vietnam. Although this plant species has also widely spread in Thailand, all of the oil used in local pharmaceutical industry is imported. Chemical studies of the volatile leaf oil of *M. cajuputi* in Thailand revealed low yield of the important component, 1,8-cineole (Rativanich et al., 1992; Pensook, 1995). Since the market demand and the oil price depended on the content of 1,8-cineole, there seemed no promise for commercial production of cajuput oil in Thailand.

Some reports indicated geographic variation of chemical composition of the volatile leaf oil of this species and within the same subspecies (Brophy et al., 1989; Oyen and Dung, 1999). Therefore, a study on chemical diversity of the *M. cajuputi* leaf oils from different sites was carried out.

MATERIALS AND METHODS

Materials

Fresh leaves of *M. cajuputi* were collected from 6 sites of peat swamp forest in Narathiwat Province, in April 2001 (Ban Lubosama, Thambon Pasemat, Amphur Sungai Kolok; Ban Toh Deang, Thambon Puyoh, Amphur Sungai-Kolok; Ban Mai, Thambon Sungai Padi, Amphur Sungai Padi; Ban Pha Ye, Thambon Sungai Padi, Amphur Sungai-

Padi; Ban Tha Se, Thambon Kosit, Amphur Tak Bai; Ban Koke Kuwae, Thambon Kosit, Amphur Tak Bai). Voucher specimens were deposited at Forest Product Chemistry Research and Development Laboratory, Royal Forest Department, Bangkok, Thailand for positive identification.

Methods

1. Distillation of the Volatile Oils. Fresh leaves were hydro-distilled for 8 h using a modified Dean and Stark apparatus.

2. Determination of Physical Properties. Oil densities were determined (25°C) by a pycnometer and refractive indexes measured (20°C) with a refractometer (Atago, USA, Inc).

3. Chemical Analyses of the Oil Components. Chemical analyses of the volatile oils were done on a gas chromatograph (Shimadzu GC-17A) interfaced with a mass spectrometer (Shimadzu QC-5000 MSD). The GC was equipped with DB-5/MS column (30 m long, 0.25 mm internal diameter, and film coating thickness 0.25 μm). Oven temperature was programmed for an initial hold of 50°C for 3 min and an increase of 5°C/min until 250°C. The injector and detector temperatures were 250°C. Helium was used as carrier gas at a flow rate of 1.0 mL/min. The volatile oils were prepared as 1% solutions in hexane with injection volume of 1 μL. Mass spectra were recorded at an ionization energy of 70 eV in EI mode. Mass scan ranged from 30-400. The chemical compositions of the oils were identified by comparing the retention times and mass spectra with those of published data.

4. Bioassays for Termiticidal Activity. No choice feeding bioassay as described by Pearce (1999) was used in this experiment. The destructive subterranean termites, *Coptotermes gestroi* Wasmann were separated from laboratory colony. Fifty sound workers and five soldiers of test termites were introduced in each petri-dish (50 mm diameter, 15 mm height) containing treated filter paper moistened with distilled water (0.15 mL), then kept in the dark at room temperature (28-30°C) for 7 d. Treated filter paper (Whatman No.2, 47 mm diameter) was prepared in 2 sets. The first set was each impregnated with 2 mL 10% leaf oil solution in acetone and the second was each impregnated with 1 mL 5% oil solution in the same solvent. The filter paper was left to air-dry before placing in each petri-dish. Distilled water and acetone were used instead of oil solution in controls. Four replications were prepared for each test experiment. Termite behavior and number of survival termites were recorded everyday. The dead termites were removed everyday. Termicidal activity potential of the oil was evaluated based on percent real mortality as described by Finney (1977).

RESULTS AND DISCUSSION

Chemical Studies of the Oils

Oil yields were lower than 1.0% on the basis of oven-dried leaf weights. All were pale green, turning yellow on storage. Densities ranged from 0.8806 to 0.9259 g/mL at and refractive indices ranged from 1.5000-1.5129 (Table 1).

Chemical compositions of the oils from leaves collected in 2 sites of Amphur Sungai Kolok were different from those of Amphur Sungai Padi and Tak Bai. They contained less monoterpenes, but higher sesquiterpenes, compared to others. Besides, they both consisted of diterpenes while the others were not. Both these oils were slightly different from each other. Major components of the oil from Ban Lubosama, Thambon Pasemat were geranyl acetone (25.00%), terpinolene (7.92%), (E)-β-farnescene (7.58%), β-elemene (6.57%) and γ-terpinene (6.25%) respectively whereas the oil from Ban Toh Deang, Thambon Puyoh contained geranyl acetone (27.35%), β-elemene (14.01%), (E)-β-farnescene (7.44%) and farnesol acetone (5.84%) respectively. There were other hydrocarbons but no unknown compounds identified in both oils. However, the oil composition of leaves from Ban Lubosama, Thambon Pasemat was more complex than the other.

More than 72% of monoterpenes were present in the oils from Amphur Sungai Padi and Tak Bai. The other components were sesquiterpenes and hydrocarbons. None of the oils contained diterpenes. However, the oil from the leaves collected from Ban Mai, Thambon Sungai Padi and Ban Tha Se, Thambon Tak Bai contained significant amounts of unknown compounds, 0.38 and 1.83% respectively. The leaf oils from leaves collected from Ban Mai, Ban Pha Ye, Thambon Sungai Padi and Ban Koke Kuwae, Thambon Kosit were little different, both in terms of chemical components and proportions of the components. Composition of the leaf oil from Ban Tha Se, Thambon Tak Bai was different from the rest and the most complex among the 4 samples, though the major components were similar to other three samples. Major components of the oils were geranyl acetone, terpinolene, γ-terpinene, p-cymene, (E)-β-farnescene, terpinene-4-ol and cyclohexane carboxaldehyde, however with different proportions.

Geographic variation affected chemical composition of oils from leaves (Table 2 and 3). The trees spread in an interim of peat swamp forest mixed with the other species in Amphur Sungai Kolok, while they spread as a pure stand in Amphur Sungai Padi and Tak Bai. This diversity may be due to soil type (Osaki et al., 1998).

Termiticidal Acitivity of the Oils

Termiticidal activities on feeding test of all test oils were similar and effective even after the treated filter paper was left standing for 3 months (Fig. 1, 2, 3 and 4). The test oils caused 100% mortality of *C. gestroi* within 1 d. The effective lethal time of the oils at 10 and 5% concentrations were similar (Elt_{100} = 1 d).

CONCLUSION

All the leaf samples gave rather low oil yields (<1%). The chemical compositions were slightly different among the oils from leaves collected in various sites. The yielded oils contained no 1,8-cineole or contained very low quantity (< 3%) of this compound and there was no component, which could be of commercial importance (>50%). It is therefore not promising for commercial production of cajuput oils from these areas.

Termicidal activities of all the oils obtained were similar. All the test oils in this study were highly toxic to the test termites, even after being left standing for 3 months. They were very toxic to wood destructive fungi as observed in preliminary studies. Therefore they may be developed into remedial insecticide for termite control and/or wood preservatives. Previous reports on biological activities (Cuong et al., 1994; Benjapong, 1996) and the chemical composition of the oils revealed that it was possible to use the oils in the production of value added products, such as soap, shampoo, disinfectant, and insect repellant.

ACKNOWLEDGEMENTS

We acknowledge Phikul Thong Study Center for financial support and their staffs (Mr. Chawalit Urapeepattanapong, Mr. Tanit Nuyim, Mr. Manetr Boonyanant and Mr. Somchai Benjachaya) for their assistances in fieldwork. Our deep gratitude also due to Ms. Sirinan Thabthimthet for her help in refractive index determination of volatile leaf oils.

Literature Cited

Benjapong, N. 1996. Insects and medicinal plant. J. Govern. Pharm. Organ. 22:35-42.

Brophy, J.J., Boland, D.J. and Lassak, E.V. 1989. Leaf essentail oils of *Melaleuca* and *Leptospermum* species from tropical Australia. p.193-203. In: D.J. Boland (ed.), Tree for the Tropics: Growing Australia multipurpose trees and shrubs in developing countries, Monograph No. 10, Australian Centre for International Agricultural Research, Canberra.

Cuong, D.N., Xuyen, T.T., Motl, O., Stransky, K., Presslova, J., Jedlickova, Z. and Sery, V. 1994. Antibacterial properties of Vietnamese cajuput oil. J. Essent. Oil Res. 6:63-67.

Finney, D.T. 1977. Probit analysis. 3rd ed., Cambridge University Press, Cambridge.
Osaki, M., Watanabe, T., Ishizawa, T., Nilnond, C., Nuyim, T., Sittibush, C. and Tadano, T. 1998. Nutritional characteristics in leaves of native plants grown in acid sulfate, peat, sandy podzolic and saline soils distributed in Peninsular Thailand. Plant and Soil 201:175-182.
Oyen, L.P.A. and Nguyen Xuan Dung (ed.). 1999. Plant resources of south-east Asia. No.19, Essential-oil plants, Prosea Foundation, Bogor.
Pearce, M.T.J. 1999. Termites biology and pest management. CAB International, London.
Pensook, S. 1995. Utilization of *Melaleuca cajuputi* leaf for cajuput oil production. Annual Report of Phikul Thong Study Centre, S. Sombun press, Bangkok.
Rativanich, T., Caruhapattana, B., Kitirattrakarn, T., Anantachoke, C. and Pensook, S. 1992. *Melaleuca cajuputi* leaf oil from Toh Deang peat swamp forest. Proc. Royal Forest Department Annual Meeting, Bangkok, Thailand. 16-20 Nov.:361-367.

Tables

Table 1. Physical properties of the oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Physical properties of the oils	Amphur Sungai Kolok		Amphur Sungai Padi		Amphur Tak Bai	
	Thambon Pasemat Ban Lubosama	Thambon Puyoh Ban Toh Daeng	Thambon Sungai Padi Ban Mai	Thambon Sungai Padi Ban Pha Ye	Thambon Kosit Ban Tha Se	Thambon Kosit Ban Koke Kuwae
Moisture of Leaves (%)	60.76	59.51	54.46	57.13	49.99	56.52
Oil Yield (%)	0.76	0.56	0.66	0.84	0.70	0.97
Oil Color	Clear pale green	Clear pale green	Clear pale green	Clear Pale green	Clear Pale green	Clear pale green
Density (g/mL) at 25°C	0.9244	0.9259	0.9147	0.8775	0.8806	0.8867
Refractive index at 20°C	1.5049	1.5129	1.5000	1.5016	1.5001	1.5001

Table 2. Types of chemical composition of the oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Type of chemical and amount (%)	Amphur Sungai Kolok		Amphur Sungai Padi		Amphur Tak Bai	
	Thambon Pasemat Ban Lubosama	Thambon Puyoh Ban Toh Daeng	Thambon Sungai Padi Ban Mai	Thambon Sungai Padi Ban Pha Ye	Thambon Kosit Ban Tha Se	Thambon Kosit Ban Koke Kuwae
Monoterpenes	62.48	49.22	83.40	72.59	74.32	75.84
Sesquiterpenes	28.61	46.45	15.17	19.99	15.81	17.57
Diterpenes	0.83	2.06	-	-	-	-
Other Hydrocarbons	8.07	2.27	1.05	7.42	8.03	6.58
Unknown	-	-	0.38	-	1.83	-
Total	99.99	100.00	100.00	100.00	99.99	99.99

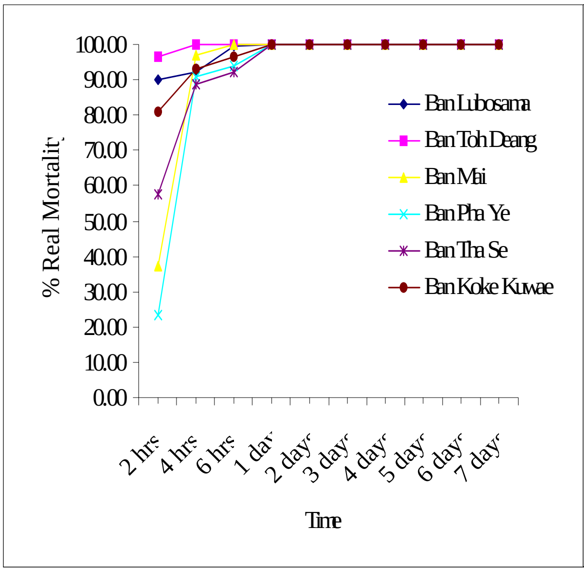
Table 3. Chemical compositions in the oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Chemical composition (%)	Amphur Sungai Kolok		Amphur Sungai Padi		Amphur Tak Bai	
	Thambon Pasemat	Thambon Puyoh	Thambon Sungai Padi	Thambon Sungai Padi	Thambon Kosit	Thambon Kosit
	Ban Lubosama	Ban Toh Daeng	Ban Mai	Ban Pha Ye	Ban Tha Se	Ban Koke Kuwae
α-Phellandrene	2.39	0.72	4.50	4.01	4.33	5.00
(E)-Ocimene	2.83	1.34	3.78	4.43	4.04	3.13
Unknown	-	-	0.38	-	0.26	-
β-Myrcene	0.67	0.82	1.50	1.20	1.72	1.85
Unknown	-	-	-	-	0.25	-
β-Phellandrene	0.39	-	-	-	0.13	-
Unknown	-	-	-	-	0.11	-
α-Terpinene	1.70	0.48	1.78	1.03	2.10	2.95
γ-Terpinene	6.25	1.95	11.19	8.44	12.23	15.09
p-Cymene	4.58	2.42	10.20	14.97	7.22	6.82
α-Limonene	0.98	2.10	2.36	2.06	2.91	1.86
1,8-Cineole	0.71	–	1.19	-	2.88	-
Unknown	-	-	-	-	0.45	-
Unknown	-	-	-	-	0.16	-
Terpinolene	7.92	2.67	14.39	11.67	15.01	18.34
Unknown	-	-	-	-	0.45	-
β-Linalool	0.39	1.37	1.15		1.53	0.65
Unknown	-	-	-	-	0.15	-
1-Carboxaldehyde-3-cyclohexene	-	-	1.05	1.94	0.54	0.32
Terpinen-4-ol	3.01	1.62	5.34	5.60	5.17	6.22
p-Cymene-8-ol	0.54	0.52	1.80	3.00	0.95	0.85
α-Terpineol	1.25	0.68	2.40	2.13	2.34	2.22
Cadina-1,4-diene	0.21	-	-	-	-	-
Eugenol Acetate	0.35	-	-	-	-	-
α-Copaene	0.43	0.96	-	-	0.23	-
β-Elemene	6.57	14.01	2.62	2.41	1.87	1.64
(E)-β-Farnescene	7.58	7.44	6.54	7.24	7.53	7.65
α-Humulene	1.22	-	-	-	-	-
α-Caryophyllene	3.31	3.94	2.81	3.29	3.42	3.74
1-Decanol	1.93	-	-	-	-	-
1-Dodecene	0.73	-	-	-	-	-
Germacrene D	1.33	2.08	0.55	-	0.52	-
β- & γ-Caryophyllene	0.43	3.58	0.39	-	0.27	-
Germacrene B	1.30	-	-	-	0.60	-

Table 3. (Continued) Chemical compositions in the oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Chemical composition (%)	Amphur Sungai Kolok		Amphur Sungai Padi		Amphur Tak Bai	
	Thambon Pasemat Ban Lubosama	Thambon Puyoh Ban Toh Daeng	Thambon Sungai Padi Ban Mai	Thambon Sungai Padi Ban Pha Ye	Thambon Kosit Ban Tha Se	Thambon Kosit Ban Koke Kuwae
p-tert-Butyl-benzoic acid	0.19	-	-	-	-	-
(+)-δ-Cadinene	0.37	0.87	-	-	0.17	-
(-)-Spathulenol	2.88	4.63	2.26	2.17	1.20	0.59
3-Thujanol	0.63	-	-	-	-	-
p-Menth-8-en-2-ol acetate	2.54	3.85	2.93		2.23	1.46
(±)-trans-Nerolidol	0.66	1.52	-	4.88	-	-
Guaiol	-	-	-	-	-	2.46
Geranyl acetone	25.00	27.35	18.89	14.05	9.53	9.40
Kauran-18-al	0.83	2.06	-	-	-	-
Palmitic acid	1.10	-	-	-	-	-
Limonene dioxide	0.35	-	-	-	-	-
δ-Cadinol	1.89	-	-	-	-	-
α-Bisabolol	0.42	-	-	-	-	1.49
Farnesol acetone	-	5.84	-	-	-	-
1-Dodecanol	1.56	-	-	-	-	-
Bulnesol	-	-	-	-	-	0.72
Cyclohexane carboxaldehyde	-	1.60	-	5.48	7.49	5.54
Farnesol	1.90	1.02	-	-	-	-
2-Methyl-tridecane	0.69	0.67	-	-	-	-
2,6,10,14-Tetramethyl-heptadecane	1.06	-	-	-	-	-
1,3-Dimethoxy butane	0.81	-	-	-	-	-
Total	**99.99**	**100.00**	**100.00**	**100.00**	**99.99**	**99.99**

Figures



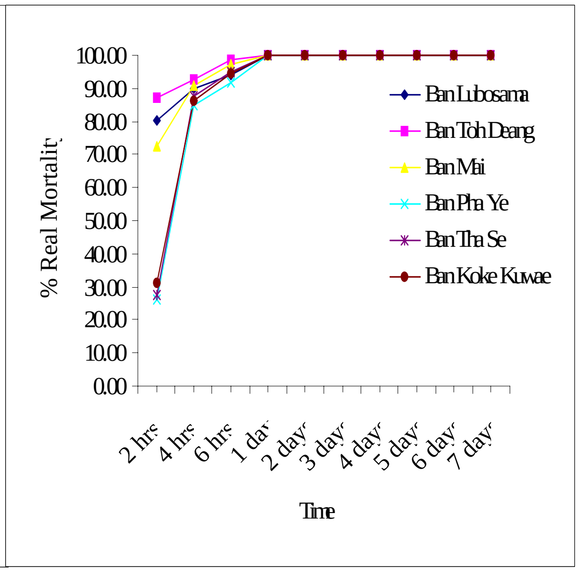
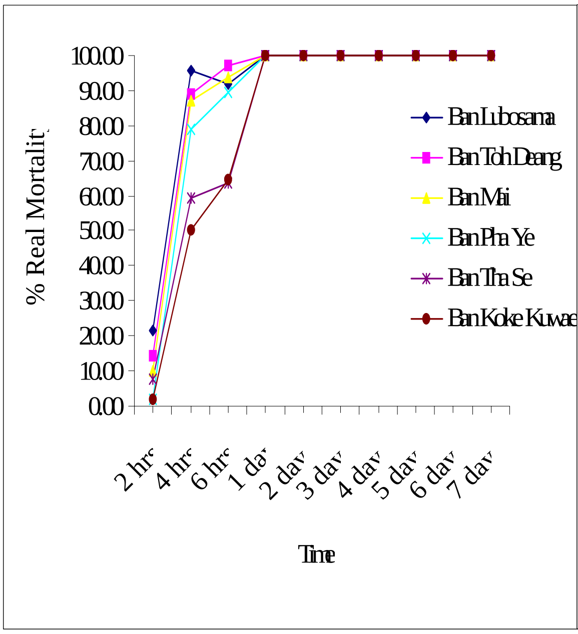


Fig. 1. Mortality of *C. gestroi* after exposure to filter paper treated with 10% oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Fig. 2. Mortality of *C. gestroi* after exposure to filter paper treated with 10% oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand after being left standing for 3 months.



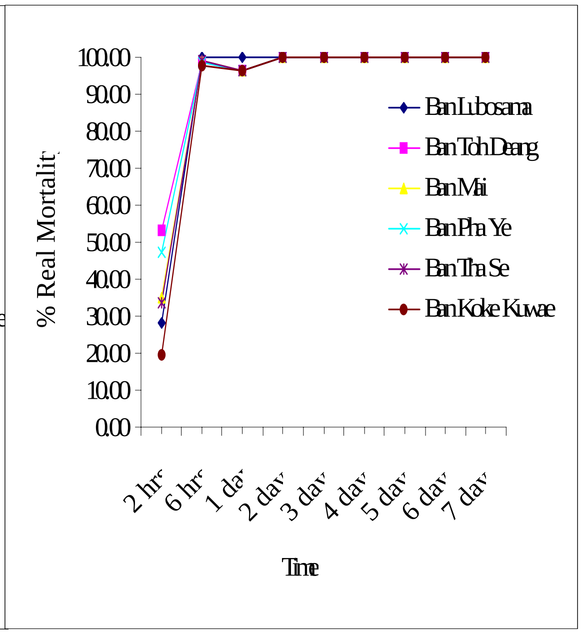


Fig. 3. Mortality of *C. gestroi* after exposure to filter paper treated with 5% oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand.

Fig. 4. Mortality of *C. gestroi* after exposure to filter paper treated with 5% oils from cajuput (*M. cajuputi*) leaves collected from 6 sites in Thailand after being left standing for 3 months.