Chemical Constituents and Antibacterial Activity of Volatile Oils of *Combretum latifolium* Bl. and *C. quadrangulare* Kurz Leaves

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ABSTRACT

The volatile constituents from leaves of Combretum latifolium Bl. and Combretum quadrangulare Kurz (Combretaceae) were obtained by hydrodistillation and analyzed by GC-MS. From the leaf oil, we identified six compounds accounting for 81.6% of C. latifolium and nine compounds accounting for 68.0% of C. quadrangulare. The major compounds of the volatile oils in C. latifolium and C. quadrangulare were palmitic acid (37.05%, 17.74%), hexahydrofarnesyl acetone (11.54%, 17.36%), isophytol (13.47%, 3.71%), neophytadiene (7.71%, 3.52%) and n-nonacosane (4.68% and 5.37%), respectively. Antibacterial activity of the volatile oils was evaluated by using agar disc diffusion method. The antibacterial assay showed activity of the oils from the leaves of C. latifolium and C. quadrangulare as diameter of zones of inhibition against the gram-positive bacterium, Staphylococcus aureus (8.50±0.05, 7.50±0.05 mm, respectively), and gram-negative bacterium, Escherichia coli (9.33±0.06, 8.00±0.10 mm, respectively). The extracts showed little antibacterial activity against Pseudomonas aeruginosa. The chemistry and antibacterial activity of the volatile oils of these two plants have been studied here for the first time.

Keywords: Chemical constituents, Volatile oils, Antibacterial activity, Combretaceae, *Combretum latifolium* Bl., *Combretum quadrangulare* Kurz

INTRODUCTION

Many species of *Combretum* (Combretaceae) have been used as traditional medicines for many applications, including abdominal disorders, bacterial infections, diarrhea, bilharzias, malaria, respiratory infections, pneumonia, skin and venereal diseases, fevers and sore throats, especially in rural areas (Banskota et al., 2003; Eloff et al., 2008). Some *Combretum* species have anti-infective acti-

vities, including antibacterial, antifungal and antiparasitic activities. Antibacterial activity of *Combretum* extracts for ethnomedicinal uses have been confirmed in many ethnobotanical and antibacterial studies (Clarke, 1878; Fyhrquist et al., 2002; Banskota et al., 2003; Eloff et al., 2008; Lima et al., 2012). The Combretaceae is a large family, consisting of 20 genera with at least 600 species. The genus *Combretum* comprises about 370 species of trees, woody climbers and shrubs. Several species of this family are known in tropical and southern Africa, Madagascar, tropical America, and tropical Asia, including Thailand (Nanakorn, 1986; Eloff et al., 2008). Two *Combretum* species, which are common in northern Thailand, are *Combretum latifolium* Bl. and *Combretum quadrangulare* Kurz. Several reported on antibacterial activity of *Combretum* species: *Combretum caffrum* (Eckl. & Zeyh.) Kuntze bark (Masika and Afolayan, 2002), *Combretum erythrophyllum* (Burch.) Sond. leaves (Martini and Eloff, 1998; Eloff, 1999; Martini et al., 2004), *Combretum apiculatum* Sond. (McGaw et al., 2000), *Combretum fruticosum* (Leofl.) Stuntz. (Smith et al., 2000), *Combretum imberbe* Engl. & Diels. leaves (Katerere et al., 2003), *Combretum woodii* Dümmer leaves (Eloff et al., 2005a ; 2005b), *Combretum molle* R.Br. *ex* G. Don aerial parts (Ahmed et al., 2004; Mamidou Kone et al., 2007; Steenkamp et al., 2007; Gronhaug et al., 2008), *Combretum micranthum* G. Don leaves, root bark and stem bark (Karou et al., 2005; Akeem et al., 2012; Udoh et al., 2012; Osonwa et al., 2012), *Combretum padoides* Engl. & Diels (Angeh et al., 2007), *Combretum hartmannianum* Schweinf. leaves and roots (Eldeen and van Staden, 2007), *Combretum adenogonium* Steud ex A. Rich. leaves, stem bark and roots (Maregesi et al., 2007; Mushi et al., 2012), *Combretum glandifolium* (Rahman et al., 2008), *Combretum niororense* Aubrev. *ex* keay leaves (Coulidiati et al., 2009), *Combretum pincianum* Hook (Adejuwon et al., 2011; Kanwal and Karim, 2011), *Combretum glutinosum* Perr. *ex* DC. (Yahaya et al., 2012). Studies in Thailand on roots and seeds of *C. quadrangulare* reported anthelmintic activity (Euswas et al., 1988), antibacterial activity (Nantachit et al., 2006; Wungchinda, 1979) and toxicity (Nakornchai et al., 1994). While, the leaves of *C. quadrangulare* and *C. latifolium* have been reported to have antioxidant and anticancer activities (Nopsiri et al., 2014).

With many previous studies demonstrating antimicrobial activities of *Combretum* species, we specifically investigated the chemical constituents and antibacterial activity of the leaf volatile oils of *C. quadrangulare* and *C. latifolium*, two *Combretum* species from northern Thailand that have yet to be studied for these properties.

MATERIALS AND METHODS

Plant material

The plants were collected in northern Thailand. *Combretum latifolium* Bl. was collected in Wang Nuea District, Lampang Province in December 2009 and *Combretum quadrangulare* Kurz was collected in Doi Saket District, Chiang Mai Province in June 2009. Identifications were made by J. F. Maxwell at the CMU Herbarium, Department of Biology, Faculty of Science, Chiang Mai University,

Chiang Mai, Thailand. Voucher specimens were deposited at the Faculty of Pharmacy and the Department of Biology, Faculty of Science, Chiang Mai University.

Isolation of the volatile oils

Fresh leaves of two *Combretum* species (*C. latifolium* 5.61 kg and *C. quadrangulare* 6.19 kg) were washed with distilled water, chopped into small pieces, and subjected to hydrodistillation in a Clevenger-type apparatus for 8 h, with a water-cooled oil receiver to reduce formation of artifacts due to overheating. The volatile oils were collected over water, separated and dehydrated over anhydrous sodium sulfate, and kept at 4°C for further analysis and testing.

GC-MS analysis

The volatile oils were analyzed on a Hewlett-Packard GC-7890A gas chromatograph equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), with the temperature programmed to rise from 50°C (held first for 5 min) to 200°C at 10°C/min, ending at 250°C (held for 10 min), for a total run time of 30 min; the carrier gas was He, at a constant flow rate of 1.0 ml/min; the injector temperature was 270°C; the Hewlett-Packard 5975C mass selective detector temperature was 280°C. Samples were injected by splitting mode (1:25). The GC-MS analysis was performed on a Hewlett-Packard GC-7890A coupled with a Hewlett-Packard 5975C mass selective detector using electron impact ionization (EI) and set as follows: source temperature, 230°C; interface temperature, 270°C; ionization energy, 70 eV; mass range of 30-500 *m/z*; and quadrupole temperature, 150°C. The components of the oils were identified by comparing their retention indices (RI) relative to the *n*-alkane index on an HP-5 column and by comparing the mass spectra to reference libraries (Wiely7n.1 and NIST) using corresponding data of authentic compounds or published spectra (Mancini et al., 2009; Zito et al., 2010; Radulovic et al., 2011).

Antibacterial activity

Test bacteria. Three common bacterial strains, representing human bacterial pathogens, were used for the antibacterial activity test. The gram-positive bacterium was *Staphylococcus aureus* (ATCC 25923). Two gram-negative bacteria were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853).

Antibacterial assay. The old National Committee for Clinical Laboratory Standards (NCCLS) method for antibacterial susceptibility testing has been modified for testing volatile oils (Hammer et al., 1998; 1999; Ferraro et al., 2000), with researchers adapting different experimental protocols to better represent future applications in their particular field. The agar disc diffusion method (Gaydos and Harrington, 1982; Pelczar et al., 1993; Hammer et al., 1998; 1999; Ferraro et al., 2000; Mubashir et al., 2008; Zafar et al., 2011) was used to determine the clear zone diameter of volatile oils from two *Combretum* species against the test bacteria. Sterilized Petri dishes (9 cm diameters) with 20 ml of Mueller-Hinton agar were prepared. The standardized inoculums of the test bacteria were prepared by

suspending the pathogenic bacteria in trypticase soy broth to a turbidity of 0.5 McFarland standard. The innoculi were spread on the agar surface. The volatile oils were dissolved in methanol (1 mg/ml), then sterile paper discs (Whatman No.1) of 6 mm diameter were separately impregnated with 5 µl (5 µg/disc) of volatile oils and placed in appropriate position on the surface of the plate with quadrants marked at the back of the Petri dishes with the selected test bacteria. The Petri dishes were incubated at 37°C for 24 h. Three common antibiotics – ampicillin, gentamicin and ceftriaxone (1 mg/ml each) – and one standard volatile oil with known antibacterial activity – eugenol (1 mg/ml) – were used as positive controls. Methanol and sterile water were used as negative controls. The zones of inhibition were assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms compared to the controls. Each test was replicated three times. The average of the three independent determinations was recorded.

RESULTS

The fresh leaves of *C. latifolium* and *C. quadrangulare* were subjected to hydrodistillation in a modified Clevenger-type apparatus to yield 0.0013% and 0.0005% (w/w) of oil, respectively, both light colored. The GC chromatograms of the volatile oils from *C. latifolium* Bl. and *C. quadrangulare* Kurz are shown in Figures 1 and 2, respectively. GC-MS analysis successfully detected six components of *C. latifolium* Bl. and nine components of *C. quadrangulare* Kurz, accounting for 81.6% and 68.0%, respectively, of the chromatographic components. The components identified from the volatile oils – with their retention time (RT), percentage composition (%), and retention indices (RI) – are summarized in Table 1. Palmitic acid was the major component of the *C. latifolium* (37.05%) and *C. quadrangulare* (17.74%) oils. Other components included hexahydrofarnesyl acetone (11.54% and 17.36%, respectively), isophytol (13.47% and 3.71%, respectively), neophytadiene (7.71% and 3.52%, respectively) and *n*-nonacosane (4.68% and 5.37%, respectively). In addition, phytol isomer (7.17%) was found in *C. latifolium* oil and phytol (11.52%), *n*-heptacosane (4.75%), *β*-selinene (2.46%) and tetradecanoic acid (1.54%) were found in *C. quadrangulare* oil.

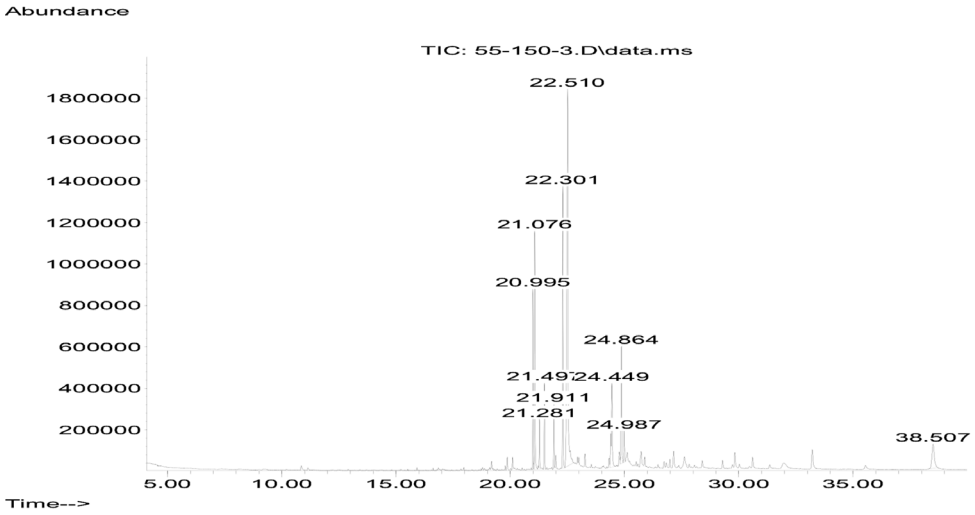


Figure 1. GC chromatogram of leaf volatile oils from *C. latifolium.*

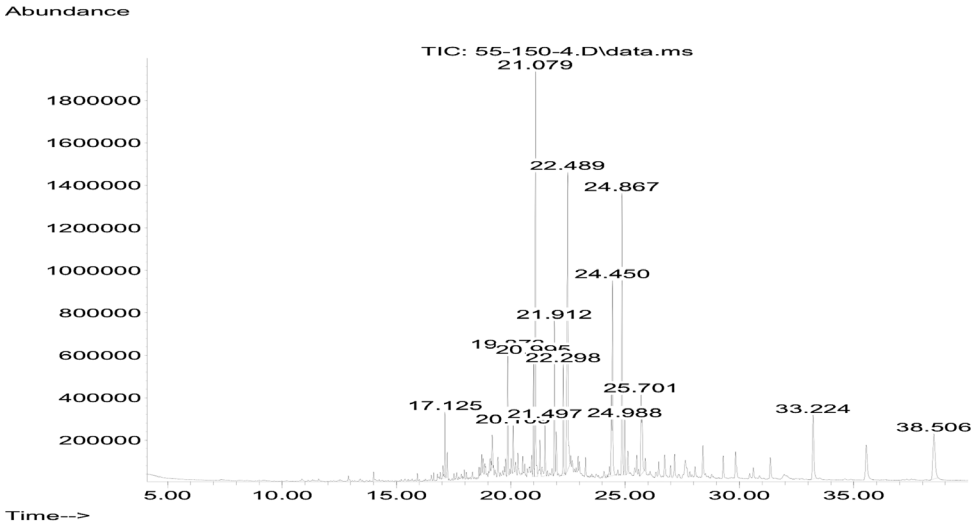


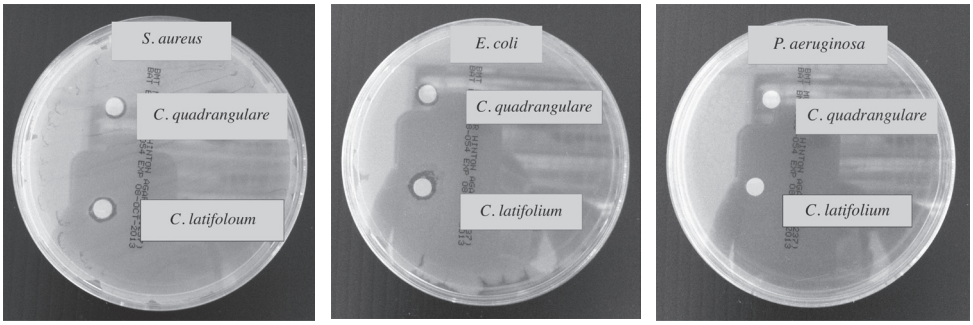
Figure 2. GC chromatogram of leaf volatile oils from *C. quadrangulare.*

Table 1. Chemical constituents of leaf volatile oils from *C. latifolium* and *C. guadrangulare*.

Plants	Compounds	RA[a]	RT	RI[c]	RI[d]	Identification	References
C. latifolium	Neophytadiene	7.71	20.99	1812	1841	RI, MS	Radulovic et al. (2011)
	Hexahydrofarnesyl acetone	11.54	21.08	1819	1845	RI, MS	Zito et al. (2010)
	Isophytol	13.47	22.30	1921	1951	RI, MS	Radulovic et al. (2011)
	Palmitic acid	37.05	22.51	1936	1969	RI, MS	Mancini et al. (2009)
	Phytol isomer	7.17	24.45	1947	2112	RI, MS	Zito et al. (2010)
	n-Nonacosane	4.68	38.51	2850	2900	RI, MS	Zito et al. (2010)
	Total	**81.62**					
	Group components						
	Oxygenated diterpenes	28.35					
	Ketone	11.54					
	Carboxylic acid	37.05					
	Hydrocarbon	4.68					
C. quadrangulare	*β*-Selinene	2.46	17.13	1479	1475	RI, MS	Mancini et al. (2009)
	Tetradecanoic acid	1.54	20.11	1733	1766	RI, MS	Zito et al. (2010)
	Neophytadiene	3.52	20.99	1812	1841	RI, MS	Radulovic et al. (2011)
	Hexahydrofarnesyl acetone	17.36	21.08	1819	1849	RI, MS	Zito et al. (2010)
	Isophytol	3.71	22.30	1920	1951	RI, MS	Radulovic et al. (2011)
	Palmitic acid	17.74	22.49	1935	1957	RI, MS	Mancini et al. (2009)
	Phytol	11.52	24.45	2091	2122	RI, MS	Mancini et al. (2009)
	n-Heptacosane	4.75	33.22	2411	2500	RI, MS	Zito et al. (2010)
	n-Nonacosane	5.37	38.51	2850	2014	RI, MS	Zito et al. (2010)
	Total	**67.97**					
	Group components						
	Oxygenated sesquiterpene	2.46					
	Oxygenated diterpenes	18.75					
	Ketone	17.36					
	Carboxylic acids	19.28					
	Hydrocarbon	10.12					

Note: [a]Relative area in % (peak area relative to total peak area). [b]Retention Time (min). [c]Retention Indices determined in this study. [d]Retention Indices of Kovat index. RI, MS: comparison of the mass spectrum with MS libraries and RI of literature.

The antibacterial test results are shown in Table 2. The oils showed antibacterial activity against both gram-positive and gram-negative bacteria. The leaf volatile oils of *C. latifolium* and *C. guadrangulare* exhibited antibacterial activity against gram-positive bacterium (*S. aureus*) with inhibition zones of 8.50±0.05 and 7.50±0.05 mm, respectively, and also exhibited activity against gram-negative bacterium (*E. coli*) with inhibition zones of 9.33±0.06 and 8.00±0.10 mm, respectively. Figure 3 and Table 2 showed antibacterial activity of the *C. latifolium* with larger inhibition zones (8.50±0.05 and 9.33±0.06 mm) against selected strains of bacteria (*S. aureus* and *E. coli,* respectively). And both volatile oils showed only weak antibacterial activity against *P. aeruginosa.* The clear zones of the methanolic blank against the three selected strains of bacteria were all 6.00±0.05 mm.



(A) (B) (C)

Figure 3. Antibacterial activity test of leaf volatile oils from *C. latifolium* and C. quadrangulare by disc diffusion method on *S. aureus* (A), *E. coli* (B) and *P. aeruginosa* (C).

Table 2. Antibacterial activity of leaf volatile oils from *C. latifolium* and *C. quadrangulare.*

Sample	Zone of inhibition in (mm)[a]		
	Pathogen		
	S. aureus	*E. coli*	*P. aeruginosa*
C. latifolium	8.50±0.05	9.33±0.06	6.33±0.03
C. quadrangulare	7.50±0.05	8.00±0.10	6.33±0.03
Eugenol[b]	12.60±0.10	10.00±0.07	14.30±0.10
Ampicillin[c]	56.67±0.15	25.50±0.05	6.50±0.05
Gentamicin[d]	30.50±0.05	37.00±0.10	27.83±0.08
Ceftriaxone[e]	34.50±0.05	35.50±0.05	26.00±0.00

Note: [a]Diameter of inhibition zone (mm) values, including the diameter of disc (6 mm), are given as mean ± SD of triplicate experiments. Tested volume = 5 µg/disc. [b]Volatile oil and [c,d,e]antibiotics used as positive controls.

DISCUSSION

This is the first report that describes the chemical constituents and antibacterial activity of the leaf volatile oils of *C. latifolium* and *C. quadrangulare*. The leaf volatile oils of *C. latifolium* and *C. quadrangulare* showed antibacterial activities against gram-positive bacterium (*S. aureus*) and gram-negative bacterium (*E. coli*). The oil of *C. latifolium* showed stronger antibacterial activity against selected strains of bacteria (*S. aureus* and *E. coli*) than *C. quadrangulare.* Most of the components of the volatile oils were carboxylic acids (palmitic acid and tetradecanoic acid), oxygenated diterpene (phytol and isophytol) and ketones (hexahydrofarnesyl acetone). Palmitic acid seems to be a major fatty acid. Similar results were reported in some mangroves from the Pichavaram mangrove forest (Chandrasekaran et al., 2011). Palmitic acid isolated from *Schotia brachypetala* Sond., *Pelargonium* spp. and *Pentanisia prunelloides* (Klotzsch ex Eckl. & Zeyh.)

Walp. have been shown to have antibacterial activity (Agoramoorthy et al., 2007). Hexahydrofarnesyl acetone, a major volatile oil compound, has also been suggested as a possible antimicrobial agent (Radulovic et al., 2011). Neophytadiene, identified as a strong bactericidal compound in the red alga *Centroceras clavulatum* (*C. agardh*) Montagne, has also been reported in several plants that have been used as antipyretics, analgesics and vermifugics, including atopical application for sores and inflammation (Venkata et al., 2012). Tetradecanoic acid, which presented in the leaf volatile oil, has been reported as an antimicrobial compound (Zito et al., 2010). Phytol and isophytol are acyclic terpenoids. Phytol is a diterpene that shows antimicrobial activity and thus has become an important plant component in cosmetics, shampoos, toilet soaps and household cleaners. Interestingly, phytol shows high antimicrobial against foodborne pathogens (Venkata et al., 2012). The antibacterial activity of the oil constituents of the two *Combretum* species studied here seem to be in agreement with previous reports. These results suggest that the volatile oils of the leaves of *C. latifolium* and *C. quadrangulare* offer potential in treating infectious diseases caused by *S. aureus* and *E. coli.* Further research is needed on the other biological activities of these oils.

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